

Anti-CD3 antibodies, anti-CD123 antibodies and bispecific antibodies specifically binding to CD3 and/or CD123

The present invention concerns antibody-like binding protein specifically binding to CD3 and binding specifically to at least one further antigen, for example CD123. The invention further concerns anti-CD3 antibodies and anti-CD123 antibodies. The present invention also concerns antibody-like binding protein specifically binding to CD123 and binding specifically to at least one further antigen. The invention also relates to pharmaceutical compositions comprising the antibody-like binding protein, anti-CD3 antibodies or anti-CD123 antibodies of the invention, and their use to treat cancer. The invention further relates to isolated nucleic acids, vectors and host cells comprising a sequence encoding said antibody-like binding protein, anti-CD3 or anti-CD123 antibody and the use of said anti-CD123 antibody as a diagnostic tool.

The first generation of bispecific antibodies was developed over 20 years ago. Since then a number of clinical studies have tested bispecific antibodies engineered to target cancer cell surface antigens. This group of anti-cancer fusion proteins contains two or more functional domains that localize immunological effector cells in the proximity of targeted cancer cells to achieve anti-cancer activity.

As bispecific antibody technology developed, a different group of fusion proteins named bispecific T-cell engagers (BiTE) were generated by connecting two antibody single chain variable regions (scFv) only (no Fc amino acid segments were included) with a flexible linker, one scFv binds targeted cells and the other binds CD3 on T cell surface. One BiTE, blinatumomab, with CD19xCD3 bi-specific binding activities showed promising results in Phase II clinical trials for patients with minimal residual disease in B-lineage acute lymphoblastic.

CD123 (the interleukin-3 receptor alpha chain IL-3R α) is a tumor antigen over-expressed in a variety of hematological neoplasms. The majority of AML blasts express surface CD123 and this expression does not vary by subtype of AML. Higher expression of CD123 on AML at diagnosis has been reported to be associated with poorer prognosis. It has been reported that CD123 is expressed on leukemic stem cells (LSCs). There is growing evidence to suggest that AML arises from these leukemic stem cells

(LSCs) which have been shown to be quiescent and relatively resistant to DNA damaging chemotherapy.

The increased expression of CD123 on LSCs compared with hematopoietic stem cells (HSCs) presents thus an opportunity for therapeutic targeting of AML-LSCs.

5 The monoclonal antibody (MAb) 7G3, raised against CD123, has previously been shown to inhibit IL-3 mediated proliferation and activation of both leukemic cell lines and primary cells (US Patent No. 6,177,078). However, it has remained unclear whether targeting CD123 can functionally impair AML-LSCs.

10 The use of CD123xCD3 antibody-like binding protein leads to tumor cell killing, as herein shown by the inventors.

The idea of producing a bispecific antibody-like binding protein with CD123xCD3 bi-specific binding activities has already been proposed and described in the international patent application WO2013/173820.

15 Furthermore, a CD123 x CD3 Dual Affinity Re-Targeting (DART) Bi-Specific Antibody Based Molecule from MacroGenics entered phase I clinical trials in 2014.

20 However, as shown by the inventors, the CD123xCD3 Dual Affinity Re-Targeting (DART) Bi-Specific Antibody Based Molecule from MacroGenics, for example, has an activation of 82% of CD4+ expressing T-cells and 83% of CD8+ expressing T-cells in the absence of target cells. The inappropriate activation of T-cells may lead to severe side effects, such as the cytokine release syndrome. The cytokine release syndrome refers to the release of cytokines by the activated T cells producing a type of systemic inflammatory response similar to that found in severe infections and characterized by hypotension, pyrexia and rigors. Deaths due to cytokine release syndrome have been reported for example for OKT3.

25 Therefore, in spite of these advancements in bispecific antibody technology, there remains a need for additional cancer therapeutics, particularly those that efficiently target and kill cancer cells, either directly or indirectly.

30 The inventors have succeeded in generating, screening and selecting specific rat anti-CD3 antibodies displaying high affinity for both human and *Macaca fascicularis* CD3 protein.

The inventors developed antibody-like binding proteins having biological and immunological specificity to the antigen CD3 and at least one further target antigen. In one example, to demonstrate the use of these anti-CD3 antibodies in the generation of bispecific antibody-like binding proteins the inventors generated anti-CD3/anti-CD123

antibody-like binding proteins and demonstrated the therapeutic use thereof. Those bispecific anti-CD3/anti-CD123 antibody-like binding proteins have a low T-cell activation, as it has been observed for the anti-CD3 antibody alone. However, once the CD123 expressing target cells, such as THP-1 cells, are present the bispecific anti-
5 CD3/anti-CD123 antibody-like binding protein shows a high activation of T-cells. Accordingly, the anti-CD3 antibody of the invention defined above is particularly useful for the preparation of antibody-like binding proteins of the invention.

Definitions

Throughout the instant application, the term "and/or" is a grammatical conjunction
10 that is to be interpreted as encompassing that one or more of the cases it connects may occur. For example, the wording "such native sequence proteins can be prepared using standard recombinant and/or synthetic methods" indicates that native sequence proteins can be prepared using standard recombinant and synthetic methods or native sequence proteins can be prepared using standard recombinant methods or native sequence
15 proteins can be prepared using synthetic methods.

Furthermore, throughout the instant application, the term "comprising" is to be interpreted as encompassing all specifically mentioned features as well optional, additional, unspecified ones. As used herein, the use of the term "comprising" also discloses the embodiment wherein no features other than the specifically mentioned
20 features are present (*i.e.* "consisting of"). Furthermore the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

The term "gene" means a DNA sequence that codes for, or corresponds to, a
25 particular sequence of amino acids which comprises all or part of one or more proteins or enzymes, and may or may not include regulatory DNA sequences, such as promoter sequences, which determine for example the conditions under which the gene is expressed. Some genes, which are not structural genes, may be transcribed from DNA to RNA, but are not translated into an amino acid sequence. Other genes may function
30 as regulators of structural genes or as regulators of DNA transcription. In particular, the term gene may be intended for the genomic sequence encoding a protein, *i.e.* a sequence comprising regulator, promoter, intron and exon sequences.

A sequence “at least 85% identical to a reference sequence” is a sequence having, on its entire length, 85%, or more, in particular 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the entire length of the reference sequence.

5 In the context of the present application, the “percentage of identity” is calculated using a global pairwise alignment (*i.e.* the two sequences are compared over their entire length). Methods for comparing the identity of two or more sequences are well known in the art. The « needle » program, which uses the Needleman-Wunsch global alignment algorithm (Needleman and Wunsch, 1970 J. Mol. Biol. 48:443-453) to find the optimum
10 alignment (including gaps) of two sequences when considering their entire length, may for example be used. The needle program is for example available on the ebi.ac.uk World Wide Web site. The percentage of identity between two polypeptides, in accordance with the invention, is calculated using the EMBOSS: needle (global) program with a “Gap Open” parameter equal to 10.0, a “Gap Extend” parameter equal to
15 0.5, and a Blosom62 matrix.

Proteins consisting of an amino acid sequence “at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical” to a reference sequence may comprise mutations such as deletions, insertions and/or substitutions compared to the reference sequence. In case of substitutions, the protein consisting of an amino acid sequence at least 80%,
20 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference sequence may correspond to a homologous sequence derived from another species than the reference sequence.

“Amino acid substitutions” may be conservative or non-conservative. Preferably, substitutions are conservative substitutions, in which one amino acid is substituted for
25 another amino acid with similar structural and/or chemical properties. The substitution preferably corresponds to a conservative substitution as indicated in the table below.

Conservative substitutions	Type of Amino Acid
Ala, Val, Leu, Ile, Met, Pro, Phe, Trp	Amino acids with aliphatic hydrophobic side chains
Ser, Tyr, Asn, Gln, Cys	Amino acids with uncharged but polar side chains
Asp, Glu	Amino acids with acidic side chains
Lys, Arg, His	Amino acids with basic side chains
Gly	Neutral side chain

An “antibody” also called “immunoglobulin” may be a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains or regions, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences that together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated CDR1-L, CDR2-L, CDR3-L and CDR1-H, CDR2-H, CDR3-H, respectively. A conventional antibody antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

In context of the invention, the antibody or immunoglobulin is an IgM, IgD, IgG, IgA and IgE.

“Framework Regions” (FRs) refer to amino acid sequences interposed between CDRs, i.e. to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species. The light and heavy chains of an immunoglobulin each have four FRs, designated FR1-L, FR2-L, FR3-L, FR4-L, and FR1-H, FR2-H, FR3-H, FR4-H, respectively. Accordingly, the light chain variable domain may thus be designated as (FR1-L)-(CDR1-L)-(FR2-L)-(CDR2-L)-(FR3-L)-(CDR3-L)-(FR4-L) and the heavy chain variable domain may thus be designated as (FR1-H)-(CDR1-H)-(FR2-H)-(CDR2-H)-(FR3-H)-(CDR3-H)-(FR4-H).

Knowing the amino acid sequence of the CDRs one skilled in the art can easily determine the framework regions FR1-L, FR2-L, FR3-L, FR4-L and/or FR1-H, FR2-H, FR3-H, FR4-H.

As used herein, a “human framework region” is a framework region that is substantially identical (about 85%, or more, in particular 90%, 95%, 97%, 99% or 100%) to the framework region of a naturally occurring human antibody.

In the context of the invention, CDR/FR definition in an immunoglobulin light or heavy chain is to be determined based on IMGT definition (Lefranc et al. Dev. Comp. Immunol., 2003, 27(1):55-77; www.imgt.org).

As used herein, the term “antibody” denotes conventional antibodies and fragments thereof, as well as single domain antibodies and fragments thereof, in particular variable heavy chain of single domain antibodies, and chimeric, humanized, bispecific or multispecific antibodies.

As used herein, antibody or immunoglobulin also includes “single domain antibodies” which have been more recently described and which are antibodies whose complementary determining regions are part of a single domain polypeptide. Examples of single domain antibodies include heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional four-chain antibodies, engineered single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, goat, rabbit, bovine. Single domain antibodies may be naturally occurring single domain antibodies known as heavy chain antibody devoid of light chains. In particular, *Camelidae* species, for example camel, dromedary, llama, alpaca and guanaco,

produce heavy chain antibodies naturally devoid of light chain. Camelid heavy chain antibodies also lack the CH1 domain.

The variable heavy chain of these single domain antibodies devoid of light chains are known in the art as "VHH" or "nanobody". Similar to conventional VH domains, VHHs contain four FRs and three CDRs. Nanobodies have advantages over conventional antibodies: they are about ten times smaller than IgG molecules, and as a consequence properly folded functional nanobodies can be produced by in vitro expression while achieving high yield. Furthermore, nanobodies are very stable, and resistant to the action of proteases. The properties and production of nanobodies have been reviewed by Harmsen and De Haard HJ (Appl. Microbiol. Biotechnol. 2007 Nov;77(1):13-22).

The term "monoclonal antibody" or "mAb" as used herein refers to an antibody molecule of a single amino acid composition that is directed against a specific antigen, and is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be produced by a single clone of B cells or hybridoma, but may also be recombinant, i.e. produced by protein engineering.

The term "chimeric antibody" refers to an engineered antibody which in its broadest sense contains one or more regions from one antibody and one or more regions from one or more other antibody(ies). In particular a chimeric antibody comprises a VH domain and a VL domain of an antibody derived from a non-human animal, in association with a CH domain and a CL domain of another antibody, in particular a human antibody. As the non-human animal, any animal such as mouse, rat, hamster, rabbit or the like can be used. A chimeric antibody may also denote a multispecific antibody having specificity for at least two different antigens.

The term "humanized antibody" refers to an antibody which is wholly or partially of non-human origin and which has been modified to replace certain amino acids, in particular in the framework regions of the heavy and light chains, in order to avoid or minimize an immune response in humans. The constant domains of a humanized antibody are most of the time human CH and CL domains.

Numerous methods for humanization of an antibody sequence are known in the art; see e.g. the review by Almagro & Fransson (2008) Front Biosci. 13: 1619-1633. One commonly used method is CDR grafting, or antibody reshaping, which involves grafting of the CDR sequences of a donor antibody, generally a mouse antibody, into the framework scaffold of a human antibody of different specificity. Since CDR grafting may

reduce the binding specificity and affinity, and thus the biological activity, of a CDR grafted non-human antibody, back mutations may be introduced at selected positions of the CDR grafted antibody in order to retain the binding specificity and affinity of the parent antibody. Identification of positions for possible back mutations can be performed using information available in the literature and in antibody databases. Amino acid residues that are candidates for back mutations are typically those that are located at the surface of an antibody molecule, while residues that are buried or that have a low degree of surface exposure will not normally be altered. An alternative humanization technique to CDR grafting and back mutation is resurfacing, in which non-surface exposed residues of non-human origin are retained, while surface residues are altered to human residues. Another alternative technique is known as “guided selection” (Jespers et al. (1994) *Biotechnology* 12, 899) and can be used to derive from for example a murine or rat antibody a fully human antibody conserving the epitope and binding characteristics of the parental antibody. A further method of humanization is the so-called 4D humanization. The 4D humanization protocol is described in the patent application US20110027266 A1 (WO2009032661A1) and is exemplified in the following applying the 4D humanization to humanize the rat antibody variable light (VL) and heavy (VH) domains. In one example, a rat antibody homology model was done with typically MOE software (v. 2011.10- Chemical Computing Group, Quebec, Canada) using PDB structures (Berman et al., *Nucleic Acids Research*, 2000, 28:235-242) as templates and was subsequently energy minimized using the standard procedures implemented in MOE. A molecular dynamics (MD) simulation was then performed on the minimized 3D homology model (done with MOE software) of rat antibody and compared to the, for example, 49 human models derived from the seven representative light chains (vk1, vk2, vk3, vk4, vlamba1, vlamba2, vlamba3) and the seven representative heavy chains (vh1a, vh1b, vh2, vh3, vh4, vh5, vh6) designed by LGCR/SDI and available within MOE. For instance, one model of chains couple (V_{kx}-V_{hx}) with the best both hydrophobic, electrostatic components and sequence identity outside CDR has been selected for the “4D humanization”. For the pairwise association between the rat antibody variable domain and the selected model, the sequences were aligned based typically on the optimal 3D superposition of the alpha carbons of the corresponding homology models. The unwanted motifs were then considered and mutated. Finally, the resulting humanized sequences were blasted for sequence similarity against, for instance, the IEDB database (<http://www.immuneepitope.org>; version 2012/01/30

accessible locally) to ensure that none of the sequences contain any known B- or T-cell epitope listed in.

For chimeric antibodies, humanization typically involves modification of the framework regions of the variable region sequences.

5 Amino acid residues that are part of a CDR will typically not be altered in connection with humanization, although in certain cases it may be desirable to alter individual CDR amino acid residues, for example to remove a glycosylation site, a deamidation site or an undesired cysteine residue. N-linked glycosylation occurs by attachment of an oligosaccharide chain to an asparagine residue in the tripeptide
10 sequence Asn-X-Ser or Asn-X-Thr, where X may be any amino acid except Pro. Removal of an N-glycosylation site may be achieved by mutating either the Asn or the Ser/Thr residue to a different residue, in particular by way of conservative substitution. Deamidation of asparagine and glutamine residues can occur depending on factors such as pH and surface exposure. Asparagine residues are particularly susceptible to
15 deamidation, primarily when present in the sequence Asn-Gly, and to a lesser extent in other dipeptide sequences such as Asn-Ala. When such a deamidation site, in particular Asn-Gly, is present in a CDR sequence, it may therefore be desirable to remove the site, typically by conservative substitution to remove one of the implicated residues. Substitution in a CDR sequence to remove one of the implicated residues is also
20 intended to be encompassed by the present invention.

"Fragments" of (conventional) antibodies comprise a portion of an intact antibody, in particular the antigen binding region or variable region of the intact antibody. Examples of antibody fragments include Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, diabodies, bispecific and multispecific antibodies formed from antibody
25 fragments. A fragment of a conventional antibody may also be a single domain antibody, such as a heavy chain antibody or VHH.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papaine, are bound together through a disulfide bond.
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The term "F(ab')₂" refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

The term "Fab'" refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')₂.

5 A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. The human scFv fragment of the invention includes CDRs that are held in appropriate conformation, in particular by using gene recombination techniques. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling
10 monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂. "dsFv" is a VH::VL heterodimer stabilised by a disulphide bond. "(dsFv)₂" denotes two dsFv coupled by a peptide linker.

The term "bispecific antibody" or "BsAb" typically denotes an antibody, which combines the antigen-binding sites of two antibodies within a single molecule. Thus,
15 BsAbs are able to bind two different antigens simultaneously. Genetic engineering has been used with increasing frequency to design, modify, and produce antibodies or antibody derivatives with a desired set of binding properties and effector functions as described for instance in EP 2 050 764 A1.

The term "multispecific antibody" denotes an antibody that combines the antigen-
20 binding sites of two or more antibodies within a single molecule.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the
25 domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

The term "hybridoma" denotes a cell, which is obtained by subjecting a B cell prepared by immunizing a non-human mammal with an antigen to cell fusion with a myeloma cell derived from a mouse or the like which produces a desired monoclonal
30 antibody having an antigen specificity.

By "purified" and "isolated" it is meant, when referring to a polypeptide (i.e. the antibody of the invention) or a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein in particular means at least 75%, 85%, 95%, or 98%

by weight, of biological macromolecules of the same type are present. An “isolated” nucleic acid molecule that encodes a particular polypeptide refers to a nucleic acid molecule that is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or
 5 moieties, which do not deleteriously affect the basic characteristics of the composition.

The term “antigen” or “target antigen” as used herein refers to a molecule or a portion of a molecule that is capable of being bound by an antibody or an antibody-like binding protein. The term further refers to a molecule or a portion of a molecule that is capable of being used in an animal to produce antibodies that are capable of binding to
 10 an epitope of that antigen. A target antigen may have one or more epitopes. With respect to each target antigen recognized by an antibody or by an antibody-like binding protein, the antibody-like binding protein is capable of competing with an intact antibody that recognizes the target antigen.

“Affinity” is defined, in theory, by the equilibrium association between the whole
 15 antibody and the antigen. Affinity may be expressed for example in half-maximal effective concentration (EC_{50}) or the equilibrium dissociation constant (K_D).

“Half maximal effective concentration” also called “ EC_{50} ” refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. EC_{50} and affinity are
 20 inversely related, the lower the EC_{50} value the higher the affinity of the antibody.

“ K_D ” is the equilibrium dissociation constant, a ratio of k_{off}/k_{on} , between the antibody and its antigen. K_D and affinity are inversely related. The K_D value relates to the concentration of antibody and the lower the K_D value and the higher the affinity of the antibody. Affinity can be experimentally assessed by a variety of known methods, such
 25 as measuring association and dissociation rates with surface Plasmon resonance or measuring the EC_{50} in an immunochemical assay (ELISA, FACS). Enzyme-linked immunosorbent assay (ELISA) is a biochemistry assay that uses a solid-phase enzyme immunoassay to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample. Antigens from the sample are attached to a surface. Then, a
 30 further specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate. Fluorescence-activated cell sorting (FACS) provides a method for sorting a heterogeneous mixture of biological cells into

two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. In these assays, the EC_{50} is the concentration of the antibody which induces a response halfway between the baseline and maximum after some specified exposure time on a defined concentration of antigen by ELISA (enzyme-linked immuno-sorbent assay) or cell expressing the antigen by FACS (Fluorescence Activated Cell Sorting). Surface plasmon resonance is a label free method wherein the binding of a molecule in the soluble phase (the “analyte”) is directly measured to a “ligand” molecule immobilized on a sensor surface. In the sensor device the binding of the ligand is monitored by an optical phenomenon termed surface plasmon. In particular, when the “analyte” molecule dissociates from the “ligand” molecule, a decrease in SPR signal (expressed in resonance units, RU) is observed. Association (‘on rate’, k_a) and Dissociation rates (‘off rate’, k_d) are obtained from the signal obtained during the association and dissociation and the equilibrium dissociation constant (‘binding constant’, K_D) can be calculated therefrom. The signal given in resonance units (RU) depends on the size of the ligand present in the analyte, however in case the experimental conditions are the same, i.e. the ligand is the same molecule at the same condition the obtained RU can indicate affinity, wherein the higher the obtained signal in RU the higher the binding.

A monoclonal antibody binding to antigen 1(Ag1) is “cross-reactive” to antigen 2 (Ag2) when the EC_{50} s are in a similar range for both antigens. In the present application, a monoclonal antibody binding to Ag1 is cross-reactive to Ag2 when the ratio of affinity of Ag2 to affinity of Ag1 is equal or less than 10 (in particular 5, 2, 1 or 0.5), affinities being measured with the same method for both antigens.

A monoclonal antibody binding to Ag1 is “not significantly cross-reactive” to Ag2 when the affinities are very different for the two antigens. Affinity for Ag2 may not be measurable if the binding response is too low. In the present application, a monoclonal antibody binding to Ag1 is not significantly cross-reactive to Ag2, when the binding response of the monoclonal antibody to Ag2 is less than 5% of the binding response of the same monoclonal antibody to Ag1 in the same experimental setting and at the same antibody concentration. In practice, the antibody concentration used can be the EC_{50} or the concentration required to reach the saturation plateau obtained with Ag1.

As used herein “specificity” denotes the capacity of an antibody to discriminate the target peptide sequence to which it binds (“epitope”) from closely related, highly homologous, peptide sequences.

A monoclonal antibody “binds specifically” to Ag1 when it is not significantly cross-reactive to Ag2.

A “domain” may be any region of a protein, generally defined on the basis of sequence homologies and often related to a specific structural or functional entity.

5 A “recombinant” molecule is one that has been prepared, expressed, created, or isolated by recombinant means.

As used herein, the term “subject” denotes a mammal, such as a rodent, a feline, a canine, and a primate. In particular, a subject according to the invention is a human.

Anti CD3 antibodies

10 “CD3” denotes an antigen that is expressed on T-cells as part of the multimolecular T-cell receptor complex and that consists of at least three different chains CD3 ϵ , CD3 δ and CD3 γ . CD3 δ and CD3 γ have a low sequence identity and/or similarity to human CD3 ϵ (similarity and identity is less than 20%). CD3 ϵ and CDR3 δ can form together a complex, herein called “CD3 ϵ / δ -complex”. CD3 ϵ also forms a
15 complex with CDR3 γ , the so-called “CD3 ϵ / γ -complex” Clustering of CD3 on T-cells, e.g., by immobilized anti-CD3-antibodies, leads to T-cell activation similar to the engagement of the T-cell receptor but independent from its clone typical specificity. “CD3 ϵ ” comprises three domains, an intracellular domain, a transmembrane domain and an extracellular domain.

20 Most prior art anti-CD3-antibodies recognize the CD3 ϵ -chain. One of such prior art anti-CD3-antibodies is OKT3. Prior art has exemplified T cell activation events employing antibody molecules for example by employing the antibody molecule OKT3. The anti-CD3 antibody and variant thereof have been described in the prior art (US 4,361,549 ; US 4,361 ,549 ; US 5,885,573 ; US 5,929,212 ; and WO 98/52975 or US
25 5,955,358). OKT3 has been further used as potent immunosuppressive agent in clinical transplantation to treat allograft rejection (Thistlethwaite 1984, Transplantation 38, 695-701; Woodle 1991, Transplantation 51, 1207-1212; Choi 2001, Eur. J. Immunol. 31(1), 94-106).

30 Major drawbacks of this therapy are T cell activation manifested in cytokine release due to cross-linking between T cells and Fc γ R-bearing cells and the human anti-mouse antibody (HAMA) response. Several publications have described alterations such as humanization of OKT3 to reduce these side effects: US 5,929,212; US 5,885,573 and others. On the other hand, OKT3 or other anti-CD3-antibodies can be

used as immunopotentiating agents to stimulate T cell activation and proliferation (US 6,406,696 Bluestone; US 6,143,297 Bluestone; US 6,113,901 Bluestone; Yannelly 1990, J. Immunol. Meth. 1, 91-100). Anti-CD3-anti bodies have also been described as agents used in combination with anti-CD28-antibodies to induce T cell proliferation (US 6,352,694). OKT3 has further been used by itself or as a component of a bispecific antibody to target cytotoxic T cells to tumor cells or virus infected cells (Nitta 1990, Lancet 335, 368-376; Sanna 1995, Bio/Technology 13, 1221-1224; WO 99/54440).

Approaches up to now using antibodies as agents for recruiting T-cells have been hampered by several findings. First, natural or engineered antibodies having a high binding affinity to T-cells often do not activate the T-cells to which they are bound. Second, natural or engineered antibodies having a low binding affinity to T-cells are also often ineffective with respect to their ability to trigger T-cell mediated cell lysis.

A reference sequence of full-length human CD3 ϵ protein, including the signal peptide, is available from the Uniprot database under accession number P07766 and herein enclosed under SEQ ID NO: 1 (as available on December 12, 2014).

A reference sequence of full-length *Macaca fascicularis* CD3 ϵ protein, including the signal peptide, is available from the Uniprot database under accession number Q95LI5 and herein enclosed under SEQ ID NO: 2 (as available on December 12, 2014).

A sequence of mature human CD3 ϵ His-tagged Fc-fusion proteins, cloned by the inventors from genomic DNA, is disclosed under SEQ ID NO: 3. Said mature human CD3 ϵ His-tagged Fc-fusion protein comprises amino acids 23 to 126 of the full-length human CD3 ϵ protein and thus comprises the extracellular domain of human CD3 ϵ .

A sequence of mature *Macaca fascicularis* CD3 ϵ Fc-fusion protein, cloned by the inventors from genomic DNA, is disclosed under SEQ ID NO: 4. Said mature *Macaca fascicularis* CD3 ϵ Fc-fusion protein comprises amino acids 23 to 117 of the full-length *Macaca fascicularis* CD3 ϵ protein and thus comprises the extracellular domain of human or *Macaca fascicularis* CD3 ϵ , containing one Alanine to Valine exchange at the amino acid position 35 in comparison to amino acid position 57 of the wild-type sequence.

Domain organization of human and *Macaca fascicularis* CD3 ϵ is as it follows (based on Uniprot P07766 sequence (human) and Uniprot Q95LI5 sequence (*Macaca fascicularis*)):

CD3ε domains	Positions on SEQ ID NO :1 (human)	Positions on SEQ ID NO : 2 (<i>Macaca fascicularis</i>)
Extracellular	23 – 126	22 – 117
Transmembrane domain	127 - 152	118 - 138
Cytoplasmic	153 - 207	139 - 198

Accordingly, the extracellular domain of human CD3ε consists of amino acids at positions 23 – 126 of SEQ ID NO: 1 and the extracellular domain of *Macaca fascicularis* CD3ε consists of amino acids at positions 22 – 117 of SEQ ID NO: 2.

5

The inventors have succeeded in generating, screening and selecting specific mouse and rat anti-CD3 antibodies. These anti-CD3 antibodies display high affinity for both human and *Macaca fascicularis* CD3 protein, and have however a low T-cell activation in the absence of target cells.

10

The inventors have determined the sequence of variable heavy and light chains of such monoclonal antibodies, the so-called anti-CD3 antibodies “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “12D2-E5”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “18H11-F10”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “3G5-E10”, “9D7-F3”, “8C2-F7”, “20E5-F10”, “20B5-F10”, “6C9-C9”, “3E8-G1”, “3H6-D2” and “8H2”.

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The so-called “20G6-F3” anti-CD3 antibody comprises:

- a heavy chain variable domain of consisting of sequence

20

EVQLVETGGSLVQPGKSLKLTCA**TS****GFTFTKAW**MHWVRQSPEKQLEWVAQ**IKDKSNS**
YATYYAESVKGRFTISRDDSKSTIYLQMNSLKEEDTAIYYC**RGVYYALSPFDY**WGQGVM
 VTVSS (SEQ ID NO: 5, with CDRs shown in bold characters and underlined) comprising
 CDR1-H of sequence SEQ ID NO: 6, a CDR2-H of sequence SEQ ID NO: 7, and a
 CDR3-H of sequence SEQ ID NO: 8, and

25

- a light chain variable domain consisting of sequence
 DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQSLIY**KVSN**
 RFSGFSDRFSGSGSGTDFTLKISRVDPPDLGVYYC**GQGTQYPFT**FGSGTKLEIK (SEQ

ID NO: 9, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

5 The so-called "4B4-D7" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGRLVQPGRSLKLTCA**TS****GFTFSNAWM**HWVRQSPEKQLEWVAQ**IKARSNN****YAT**YYAESVKGRFTISRDDSKSTIYLQMNSLKEEDTAIYYC**RGTTYASKPFDY**WGQGVMTVTSS (SEQ ID NO: 12, with CDRs shown in bold characters and underlined) comprising
10 CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 14, and a CDR3-H of sequence SEQ ID NO: 15, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHDNGNTY**LSWSLQRPQGSPQVLIY**KVSN**RFSGTSDRFTGSGSGTDFTLKISRVEPDDLGVYYC**GQGTQYPFT**FGSGTKLEIK (SEQ
15 ID NO: 16, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 17, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called "4E7-C9" anti-CD3 antibody comprises:

20 - a heavy chain variable domain consisting of sequence EVQVVETGGSLVQPGKSLKLTCA**TS****GFTFSNAWM**HWVRQSPEKQLEWVAQ**IKDKSNN****YAT**YYAESLKGRFTISRDDPKRSIYLQMNSLREEDTAIYYC**RYVHYGIGYAMD**AWGQGT SVTVSS (SEQ ID NO: 18, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO:
25 19, and a CDR3-H of sequence SEQ ID NO: 20, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLHNNGNTY**LSWYLQKPGQSPQPLIY**KVSN**RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGTQYPFT**FGPGTKLELK (SEQ
30 ID NO: 21, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 22, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called "18F5-H10" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQVVETGGSLVQPGKSLKLTCA**TS****GFTFTNAW**MHWVRRSPEKQLEWVAQ**IKDKSNN****YATYYAESVKGRFTISRDDSKSSIY**LQMNSLKEEDTAIYYC**RYVHYRFAYALDAWGRGT**SVSVSS (SEQ ID NO: 23, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 24, a CDR2-H of sequence SEQ ID NO: 19, and a CDR3-H of sequence SEQ ID NO: 25, and
- a light chain variable domain consisting of sequence DVLMTQTPVSLSVSLGGQVSISCRSS**QSLVHTNGNTY**LSWYLQKPGQSPQLLIY**KVSNR**LSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGTHYPFT**FGAGTKLELK (SEQ ID NO: 26, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 27, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 28.

The so-called "12D2-E5" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVKLVESGGGLVQPGRSLRLSCAAS**GFNFYAYWM**GWVRQAPGKGLEWIGE**IKKDGTI**NYTPSLKDRFTISRDNAQNTLYLQMTKLGS EDTALYYC**AREERDGYFDY**WGQGVMTVSS (SEQ ID NO: 29, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 30, a CDR2-H of sequence SEQ ID NO: 31, and a CDR3-H of sequence SEQ ID NO: 32, and
- a light chain variable domain consisting of sequence QFVLTQPNVSTNLGSTVKLSCKR**STGNIGSNY**VNWYQQHEGRSPTTMIY**RDD**KRPDGVPDFRSGSIDRSSNSALLTINNVTED EADYFC**QSYSSGIV**FGGGTKLTVL (SEQ ID NO: 33, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 34, a CDR2-L consisting of sequence 'RDD', and a CDR3-L of sequence SEQ ID NO: 35.

The so-called "11D7-C3" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQFVETGGSLVQPGKSLKLTCA**TS****GFTFSNAW**MHWVRQSPEKQLEWVAQ**IKAKSNN****YATYYAESVKGRFTISRDDSKSSVY**LQMNSLKEEDTATYYC**RGLYYGLSPSDY**WGQGVMTVSS (SEQ ID NO: 36, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 38, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQLLIY**KVSN** RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGT****HYPFT**FGSGTKLEIK (SEQ ID NO: 39, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 28.

The so-called "11H3-E5" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGSLVQPGKSLKLTCA**TSGFTFSNAWM**HWVRQSPKQLEWVAQ**IKAKSNN** **YAT**YYAESVKGRFTISRDDSKSSVYLQMNSLKEEDTAIYYC**RGTY****AYKPF****DY**WGQGV MVTVSS (SEQ ID NO: 40, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 41, and
- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHDNNGNTY**LSWSLQKPGQSPQVLIY**KVSN** RFSGTSDRFTGSGSGTDFTLKISRVEPDDLGVYYC**GQGT****QYPFT**FGSGTKLEIK (SEQ ID NO: 42, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 17, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called "13H2-C2" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EEELVETGGSLVQPGKSLKLTCA**TSGFTFSNAWM**HWVRQSPDKQLEWVAQ**IKAKSNN** **YAT**YYAESVKGRFTISRDDSKSSVYLQMNNLKEEDTAIYYC**RYVHYGLAPMDA**WGQGT SVTVSS (SEQ ID NO: 43, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 44, and
- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKAGQSPQLLIY**KVSN** RFSGISDRFSGSGSGTDFILKISRVEPDDLGVFYC**GQGT****QYPFT**FGAGTKLELK (SEQ ID NO: 45, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called “13C1-F6” anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGTLVQPGKSLKLTCA**TS****GFTFSNAWM**HWVRQSPEKQLEWVAQ**IKAKSNN**
5 **YATYYAESVKGRFTISRDDSKTSVYLQ**LN**SLREEDTAIYYC****RGTQYGYNPFDYWGQGV**
MVTVSS (SEQ ID NO: 46, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO:
37, and a CDR3-H of sequence SEQ ID NO: 47, and

- a light chain variable domain consisting of sequence
10 DVVMTQSPVSLSVSLGGQV**SISCRSSQSLVHNNGNTY**LSWYLQRSGQSPQLLIY**KVSN**
RLSGISDRFSGSGSGTDFTLKISRIEPDDLGVYYC**GQGTQYPFT**FGSGTRLEIK (SEQ ID
NO: 48, with CDRs shown in bold characters and underlined) comprising CDR1-L of
sequence SEQ ID NO: 10, a CDR2-L consisting of sequence ‘KVS’, and a CDR3-L of
sequence SEQ ID NO: 11.

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The so-called “18H11-F10” anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVESGGGLVQPGKSLK**LSCAASGFIFSDYY**MAWVRQAPKKGLEWVAT**ISISGSRTY**
YPDSVKGRFTVSRDNAKSSLYLQMNSLKSEDTATYYC**ATNNPGGW**FFVYWGQGT**LVTV**
20 SS (SEQ ID NO: 49, with CDRs shown in bold characters and underlined) comprising
CDR1-H of sequence SEQ ID NO: 50, a CDR2-H of sequence SEQ ID NO: 51, and a
CDR3-H of sequence SEQ ID NO: 52, and

- a light chain variable domain consisting of sequence
NIQMTQSPSLLSASVGDRVTL**SCKAGQNINND**LAWYQQKLGEAPRLLIY**NAN**SLQTGIPS
25 RFSGSGSGADFTLTIS**SLQPEDVATYFCQQYSSGDT**FGAGTKLELK (SEQ ID NO: 53,
with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence
SEQ ID NO: 54, a CDR2-L consisting of sequence ‘NAN’, and a CDR3-L of sequence
SEQ ID NO: 55.

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The so-called “1E6-C9” anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGSLVQPGKSLKLTCA**TS****GFTFSYAWM**HWVRQSPDKQLQWVAQ**IKAKSNN**
YATYYAESVEGRFTISRDDSKSSVYLQMNSLK**KEEDTAIYYC****RGVYYGLLGLDAWGQGT**
SVTVSS (SEQ ID NO: 56, with CDRs shown in bold characters and underlined)

comprising CDR1-H of sequence SEQ ID NO: 57, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 58, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLSVRLGGQVSISCRSS**QSLVHNNGNTY**LSWFLQKPGQSPQLLIY**KVSN**
- 5 RFSGISDRFSGSASGTDFTLKISRVEPDDLGVYYC**GQGTHYPFT**FGSGTKLEIK (SEQ ID NO: 59, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 28.

10 The so-called "10F4-C10" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGSLVQPGKSLKITCATS**GFTFSNAWM**HWVRQSPEKQLEWVAQ**IKAKSNNY**
ATYYAESVKGRFTISRDDSKSSIYLQMNSLKEEDTAIYYC**RAVNYGNYPLDY**WGQGVMTVSS (SEQ ID NO: 60, with CDRs shown in bold characters and underlined) comprising
- 15 CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 61, and
- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWCLQKPGQSPQLLIY**KVSN**
- 20 RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGTQYPFT**FGAGTKLELK (SEQ ID NO: 62, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called "10E6-G6" anti-CD3 antibody comprises:

- 25 - a heavy chain variable domain consisting of sequence EVQLVETGGGLVQSGKSLKLTCAATS**GFTVTNAWM**HWVRQSPEKQLEWVAQ**IKAKSNN**
YETYYAESVKGRFTISRDDSKSSVYLQMNSLKEEDTAIYYC**RGTYGYNPFDY**WGQGMVTVSS (SEQ ID NO: 63, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 64, a CDR2-H of sequence SEQ ID NO: 65, and a CDR3-L of sequence SEQ ID NO: 47, and
- 30 - a light chain variable domain consisting of sequence DVVMTQSPVSLSVSLGGQVSISCRSS**QSLVHNNGYTY**LSWYLQKPGQSPQVFIY**KVSN**
- RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGTHYPFT**FGSGTKLEIK (SEQ ID NO: 66, with CDRs shown in bold characters and underlined) comprising CDR1-L of

sequence SEQ ID NO: 67, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 28.

The so-called "18G9-H11" anti-CD3 antibody comprises:

- 5 - a heavy chain variable domain consisting of sequence
EVQLVETGGSLVQPGKSLKLTCA**TS****GFTFSNAW**IQWVRQSPEKQLEWVAQ**IKAKSNNY**
ATYYAESVKGRFTISRDDSKSSVYLQMNSLKEEDTALYYCTWRHYYSSHTMDAWGQG
TLTVSS (SEQ ID NO: 68, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO:
10 37, and a CDR3-H of sequence SEQ ID NO: 69, and
- a light chain variable domain consisting of sequence
DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQLLIY**KVSN**
RFSGISDRFSGSGSGTDFTLKISRVA**PTDLGVYYC****GQGSQYPFT**FGAGTKLELK (SEQ
ID NO: 70, with CDRs shown in bold characters and underlined) comprising CDR1-L of
15 sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of
sequence SEQ ID NO: 71.

The so-called "11F3-B9" anti-CD3 antibody comprises:

- 20 - a heavy chain variable domain consisting of sequence
EVQLVETGGSLVQPGKSLTLTCA**TS****GFTFSNAWM**HWVRQSPEKQLEWVAQ**IKAKSNN**
YATYYAESVKGRFTISRDDSKRSVYLQMNSLKEEDTAIYYCRYVNYGLAPMDVWGQGT
SVTVSS (SEQ ID NO: 72, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO:
37, and a CDR3-H of sequence SEQ ID NO: 84, and
25 - a light chain variable domain consisting of sequence
DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHDNGNTY**LSWYLQKPGQSPQLLIY**KVSN**
RFSGFSDRFSGSGSGTDFTLKISRVE**PDDLGVYYC****GQGTQYPFT**FGAGTKLELK (SEQ
ID NO: 73, with CDRs shown in bold characters and underlined) comprising CDR1-L of
sequence SEQ ID NO: 17, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of
30 sequence SEQ ID NO: 11.

The so-called "12G3-E8" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence
EVRVETGGSLVQPGKSLKLTCA**TS****GFTFSLAWM**HWVRQSPEKKLEWVAQ**IKDKANN**

YATYYAESVKGRFTISRDDSKRSVYLQMNRLKEEDTAIYYC**RGVYYGFSMTPFDYWGQ**
GVMVTVSS (SEQ ID NO: 74, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 75, a CDR2-H of sequence SEQ ID NO:
76, and a CDR3-H of sequence SEQ ID NO: 77, and

- 5 - a light chain variable domain consisting of sequence
DVAMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQVLIY**KVSN**
RFGISDRFSGSGSGADFTLKISRVEPDDLGVYYC**GQGTQYPFT**FGAGTKLELK (SEQ
ID NO: 78, with CDRs shown in bold characters and underlined) comprising CDR1-L of
sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of
10 sequence SEQ ID NO: 11.

The so-called "5B1-G2" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence
EVQVVETGGSLVQPGKSLKLTCA**TSGFSFNAWMHWVRQSPEKQLEWVAQIKDKANN**
15 **YATYYAESVKGRFTISRDDSKGSIYLMNSLKEEDTAVYYC****RGLYYGLFPSDY**WGQGV
MVTVSS (SEQ ID NO: 79, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 80, a CDR2-H of sequence SEQ ID NO:
76, and a CDR3-H of sequence SEQ ID NO: 81, and
- a light chain variable domain consisting of sequence
20 DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLLKPGQSPQLLIY**KVSNR**
FSGISDRFSGGGSGTDFTLKISRLEPDDLGIYYC**GQGTQYPFT**FGSGTKLEIK (SEQ ID
NO: 82, with CDRs shown in bold characters and underlined) comprising CDR1-L of
sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of
sequence SEQ ID NO: 11.

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The so-called "16F8-A7" anti-CD3 antibody comprises:

- a heavy chain variable domain comprising the sequence
VETGGNLVQPGKSLKLTCA**TSGFTFSNAWMHWVRQSPEKQLEWVAQIKAKSNNYATY**
YAESVKGRFTISRDDSKSSVYLQMNSLKEEDTAIYYC**RYVNYGLAPMDV**WGQGTSVTV
30 SS (SEQ ID NO: 83, with CDRs shown in bold characters and underlined) comprising
CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a
CDR3-H of sequence SEQ ID NO: 84, and
- a light chain variable domain consisting of sequence
DVVMTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQLLIY**KVSN**

RFSGFSDRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGTQYPFT**FGAGTKLELK (SEQ ID NO: 85, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

5

The so-called "11F9-F8" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGTLVQPGKSLKLTCAATSG**GFTFSNAWM**MHWVRQSPEKQLEWVAQI**KAKSNN**
YATYYAESVKGRFTISRDDSKTSVYLQLNSLREEDTAIYYC**RGTQYGYNPFDY**WGQGV

10 MVTVSS (SEQ ID NO: 46, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 47, and

- a light chain variable domain consisting of sequence DVVLTQTPVSLSVSLGGQVSISCRSS**QSLVHNNGNTY**LSWSLQKPGQSPQVLIY**KVSNR**

15 FSGISNRFSGSGSGTDFTLKISRVEPDDLGVYYC**GQGAHYPFT**FGSGTKLEIK (SEQ ID NO: 87, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 88.

20 The so-called "3G5-E10" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVKLVESGGGLVQPGRSLKLSCAAS**GFNFNVYW**MGWVRQAPGKGLEWIGE**IKKDSNSI**
NYTPSLKEKFTISRDNAAQNTLYLQVNKLGS EDTAIYYC**AREERDGYFDY**WGQGVMVTVS

25 S (SEQ ID NO: 89, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 90, a CDR2-H of sequence SEQ ID NO: 91, and a CDR3-H of sequence SEQ ID NO: 32, and

- a light chain variable domain consisting of sequence NIVMTQSPKSMMSVGERVTLTCKAS**ENVVTY**VSWYQQKPEQSPKLLIY**GAS**NRYTGV
PDRFTGSGSATDFTLTISVQAEDLADYHC**GQGYSPYT**FGGGTKLEIK (SEQ ID NO:

30 92, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 93, a CDR2-L consisting of sequence 'GAS', and a CDR3-L of sequence SEQ ID NO: 94.

The so-called "9D7-F3" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence
AVQLVESGGGLVQPKESLKISCAAS**GFTFSNAA**MYWVRQAPGKGLEWVAR**IRTKPN****NY**
ATYYADSVTGRFIISRDDSRSMVYLQMDNLQTEDTAMYYCTALISTAMAAWGQGTSVT
VSS (SEQ ID NO: 95, with CDRs shown in bold characters and underlined) comprising
5 CDR1-H of sequence SEQ ID NO: 96, a CDR2-H of sequence SEQ ID NO: 97, and a
CDR3-H of sequence SEQ ID NO: 98, and

- a light chain variable domain consisting of sequence
DIQMTQSPSFLSASVGDRVTINCKAS**QNINKY**LNWYHQMLGEAPKLVIS**NTN**NLQAGIPS
RFSGSGSGTDFTLTISLQPEDVATYFC**LQHRSGYT**FGLGTKLELK (SEQ ID NO: 99,
10 with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence
SEQ ID NO: 100, a CDR2-L consisting of sequence 'NTN', and a CDR3-L of sequence
SEQ ID NO: 101.

The so-called "8C2-F7" anti-CD3 antibody comprises:

15 - a heavy chain variable domain consisting of sequence
QIQLVQSGPELKKPGESVKISCKAS**GYTFTDFAM**NWVKQAPGNGLKWMGW**INTQTGK**
PTYADGFKQRFVFSLETSASTIYLQINNLIEDTATYFC**TRGALAS**VGQGVLTVSS
(SEQ ID NO: 102, with CDRs shown in bold characters and underlined) comprising
CDR1-H of sequence SEQ ID NO: 103, a CDR2-H of sequence SEQ ID NO: 104, and a
20 CDR3-H of sequence SEQ ID NO: 105, and
- a light chain variable domain consisting of sequence
DVVMTQTPVSLSVSLGSHVISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQPLIY**KVS**N
RFSGISDRFSGSGSGTDFTLEINRVEPDDLGVYYC**GQGAQYPFT**FGSGTKLEIK (SEQ
ID NO: 106, with CDRs shown in bold characters and underlined) comprising CDR1-L of
25 sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of
sequence SEQ ID NO: 11.

The so-called "20E5-F10" anti-CD3 antibody comprises:

30 - a heavy chain variable domain consisting of sequence
EVQLVETGENLVQPGKSLRLTCATS**GFSFSNAW**MHWIRQSPEKQLEWVA**IKDKS****NNY**
ATYYAESVNGRFTISRDDSKSSIYLHMDNLKEEDSAIYYC**RYVHYGV****RFFYTMDV**WGQG
TSVTVSS (SEQ ID NO: 107, with CDRs shown in bold characters and underlined)
comprising CDR1-H of sequence SEQ ID NO: 80, a CDR2-H of sequence SEQ ID NO:
19, and a CDR3-H of sequence SEQ ID NO: 108, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGDQVSI SCRPS **QSLVHNNGNTY**LSWYLQKPGQSPHPLIY **KVSN** RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC **GQGTQYPFT**FGSGTKLEIK (SEQ ID NO: 109, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 10, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 11.

The so-called "20B5-F10" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGSLVQPGKSLKLTCA TS **GFTFSNAWM**MHWVRQSPEKQLEWVAQI **KA KSNN** **YAT**YYAESVKGRFTISRDDSISSVYLQMNNLKEEDTAIYYC **RGVYYGFLGMDA**WGQGT SVTVSS (SEQ ID NO: 110, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 13, a CDR2-H of sequence SEQ ID NO: 37, and a CDR3-H of sequence SEQ ID NO: 111, and
- a light chain variable domain consisting of sequence DVVMTQTPVSLSVSLGGQVSI SCRSS **QRLVHNNGNTY**LSWYLQKPGQSPQLLVY **KVSN** RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC **GQGTEYPFT**FGSGTKLEIK (SEQ ID NO: 112, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 113, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 114.

The so-called "6C9-C9" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence AVQLVESGGGLVRPKESLKISCAAS **GFTFRNAAM**YWVRQAPGKGLEWVAR **IRTQPNNY** **AK**YYADSVKDRFTISRDDSKSMVYLQMDNLKTEDTAMYYC **TGLVVTAMDA**WGQGTSTV TVSS (SEQ ID NO: 115, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 116, a CDR2-H of sequence SEQ ID NO: 117, and a CDR3-H of sequence SEQ ID NO: 118, and
- a light chain variable domain consisting of sequence DIQMTQSPSFLSASVGDRV TINCKAS **QNINKY**LNWYQQKLGEAPKLLIY **VTN**NLQTGIPS RFSGSGSGTDYTLTISSLQPEDVATYFC **LQHRSMYT**FGTGTKLELK (SEQ ID NO: 119, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 100, a CDR2-L consisting of sequence 'VTN', and a CDR3-L of sequence SEQ ID NO: 120.

The so-called "3E8-G1" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQVVESGGGLVQPGRSLKLSCAAS**GFTFSNYY**MDWVRQAPKKGLEWVAT**ITASGSRI**
 5 YYPDSVKGRFTISRDNALSSLYLLMNSLKSEDTATYYC**ARERTDAYFDY**WGQGVMTVSS (SEQ ID NO: 121, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 122, a CDR2-H of sequence SEQ ID NO: 123, and a CDR3-H of sequence SEQ ID NO: 124, and

- a light chain variable domain consisting of sequence
 10 QFILTQPNVSVSTILGSTVKLSCKRST**TGNIGTNY**VSQYQHHEGRSPTTMIY**RDD**KRPDGV
 PDRFSGSIDRSSNSALLTINNVTQTEDEADYFC**QSYISGLNPV**FGGGSKLTVL (SEQ ID NO: 125, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 126, a CDR2-L consisting of sequence 'RDD', and a CDR3-L of sequence SEQ ID NO: 127.

15

The so-called "3H6-D2" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVETGGRLVQPGKSLKLTCA**TSGFTFSNAWM**HWWVRQSPEKQLEWVAQ**IKDKSNN**
 20 **YATYYAESVKGRFTISRDDSKSIYLLQMNSLKEEDTAIYYC****RALTYGYKRDAMDG**WGH
 GTSVTVSS (SEQ ID NO: 128, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 129, a CDR2-H of sequence SEQ ID NO: 130, and a CDR3-H of sequence SEQ ID NO: 131, and

- a light chain variable domain consisting of sequence DVVMTQTPVSLVSLGGQVSISCRSS**QSLVHNNGNTY**LSWYLQKPGQSPQLLIY**KVSN**
 25 **RFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYC****QGGTQYPFT**FGAGTKLELK (SEQ ID NO: 132, with CDRs shown in bold characters and underlined) comprising CDR1-L of sequence SEQ ID NO: 133, a CDR2-L consisting of sequence 'KVS', and a CDR3-L of sequence SEQ ID NO: 134.

30

The so-called "8H2" anti-CD3 antibody comprises:

- a heavy chain variable domain consisting of sequence QIQLVQSGPELKKPGESVKISCKAS**GYTFTDFAMN**WVKQAPGNGLKWMGW**INTQTGK**
PTYADGFKQRFVFSLETSASTIYLQINNLIEDTATYFC**TRGALAS**VGQGVMTVSS

(SEQ ID NO: 131, with CDRs shown in bold characters and underlined) comprising CDR1-H of sequence SEQ ID NO: 103, a CDR2-H of sequence SEQ ID NO: 104, and a CDR3-H of sequence SEQ ID NO: 105, and

- a light chain variable domain consisting of sequence
 5 DVVMTQTPVSLSVAIGQPASISCKSS**QSLVGTSGKTY**LNWLLQRPQGQSPKRLIY**LVS**KLD
 SGIPDRFSGSGSETDFTLKISRVEDDLGVYYC**LQGSHPFLT**FGSGTKLEIK (SEQ ID
 NO: 132, with CDRs shown in bold characters and underlined) comprising CDR1-L of
 sequence SEQ ID NO: 133, a CDR2-L consisting of sequence 'LVS', and a CDR3-L of
 sequence SEQ ID NO: 134.

10

In an embodiment, the anti-CD3 antibody of the invention binds to human CD3. In another embodiment, the anti-CD3 antibody of the invention further binds to *Macaca fascicularis* CD3. In particular, the anti-CD3 antibody of the invention binds to the extracellular domain of human CD3, or of both human and *Macaca fascicularis* CD3.

- 15 More specifically, the antibody binds to CD3 ϵ . More specifically, the anti-CD3 antibody binds to the human and *Macaca fascicularis* extracellular domain of CD3 ϵ . The anti-CD3 antibody binds to CD3 ϵ when present in the form of a complex, such as a CD3 ϵ / δ complex, or when present as single protein, indifferently whether expressed in isolated form, or present in a soluble extracellular domain or full-length membrane-anchored
 20 CD3 ϵ as present in for example in T-cells.

The anti-CD3 antibody according to the invention is specific for the surface human CD3 protein, or of both human and *Macaca fascicularis* CD3 proteins, in particular to CD3 ϵ .

- 25 In an embodiment, the anti-CD3 antibody according to the invention has a ratio of affinity for *Macaca fascicularis* CD3 on affinity for human CD3 ($KD(\textit{Macaca fascicularis})/KD(\textit{human})$) which is ≤ 10 , in particular ≤ 6 , ≤ 5 , ≤ 4 , ≤ 3 , for example ≤ 2 , ≤ 1 or ≤ 0.5 . Such a polypeptide according to the invention may be used in toxicological studies performed in monkeys the toxicity profile observed in monkeys relevant to anticipate potential
 30 adverse effects in humans

In particular, the anti-CD3 antibody of the invention does not bind to, or does not significantly cross-react with CD3 γ and/or CD3 δ protein(s).

In particular, the antibody does not bind to, or does not significantly cross-react with the extracellular domain of the aforementioned human and *Macaca fascicularis* CD3 γ and/or CD3 δ protein(s).

5 The sequence of full-length human CD3 δ protein is available in Uniprot database under accession number P04234 (SEQ ID NO: 86, as available on December 14, 2014). The extracellular domain of human CD3 δ consists of amino acids at positions 22–105 of SEQ ID NO: 86.

10 The sequence of full-length human CD3 γ protein is available in Uniprot database under accession number P09693 (SEQ ID NO: 185, as available on December 14, 2014). The extracellular domain of human CD3 γ consists of amino acids at positions 23–116 of SEQ ID NO: 185.

15 Furthermore, the anti-CD3 antibody according to the invention has an affinity (KD) for human CD3 or *Macaca fascicularis* CD3, or both, which is $\leq 90\text{nM}$, $\leq 50\text{nM}$, or $\leq 30\text{nM}$, for instance $\leq 20\text{nM}$, $\leq 10\text{nM}$, $\leq 8\text{nM}$, $\leq 6\text{nM}$, $\leq 4\text{nM}$ or $\leq 2\text{nM}$, for instance an affinity of 0.1 nM to 10 nM, in particular of 0.1 nM to 8 nM, or of 0.1 nM to 4 nM.

Affinity for human CD3 or for *Macaca fascicularis* CD3 is determined as the KD value with surface plasmon resonance using soluble recombinant CD3 ϵ/δ complex from human and *Macaca fascicularis* as capture antigen.

20 In one example, binding affinities of an anti-CD3 antibody is measured by surface plasmon resonance (SPR) using for instance a Biacore3000 instrument (GE Healthcare). Assay buffer is for example HBS-EP (BR-1001-88, GE Healthcare). As antigen may be used, for example, the human CD3 ϵ and human CD3 δ subunit extracellular domain constructs, including the signal peptide, in form of Fc-fusion proteins as described in the examples. Alternatively, use may be made, as antigen, of
25 the *Macaca fascicularis* CD3 ϵ and *Macaca fascicularis* CD3 δ subunit extracellular domain constructs, including the signal peptide, in form of Fc-fusion proteins as described in the examples. Capture of human or *Macaca fascicularis* CD3 ϵ/δ -Fc fusion proteins is achieved using, for example, the human antibody capture kit (GE
30 Healthcare). For example, the capture antibody may be coupled to CM5 chips (BR-1001-88, GE Healthcare) to for instance approx. 12.000 RU using for example the amine coupling kit (BR-100-50, GE Healthcare). The CD3 ϵ/δ -Fc fusions proteins are captured at 10 $\mu\text{l}/\text{min}$ to approx. 70 RU to yield R_{max} values of 30 RU. Binding kinetics for an anti-CD3 antibody may be measured at, for example, 30 $\mu\text{l}/\text{min}$ for 240 s and 600

s. for association and dissociation phase, respectively. For example, two fold dilutions of an anti-CD3 antibody from 3 to 400 nM in assay buffer may be used. Regeneration of the capture surface may be performed with for example a 1 min injection of for instance 3M MgCl₂ solution at 30 µl/min. For data analysis for example the BIAevaluation software v.4.1 (GE Healthcare) may be used. Data may be fit globally using a 1:1 Langmuir model with mass transfer.

In an embodiment, the anti-CD3 antibody of the invention also has an apparent EC₅₀ as, for example, determined by FACS analysis on human T-cells, which is ≤60nM, for instance ≤50nM, ≤40nM, ≤30nM, ≤20nM or ≤15nM. Typically, the apparent EC₅₀ is within the range 1 to 60 nM, in particular 1 to 30nM, for example 1 to 20nM.

In one embodiment, the anti-CD3 antibody of the invention has a T-cell activation that is less than 10%, less than 8%, less than 6%, less than 4%, less than 2%, less than 1%, for example less than 0.5% in the absence of target cells.

The term "activation of T-cells" herein refers to triggering CD3 signaling involving cytotoxic granule fusion, transient cytokine release, and proliferation. The antibody-like binding protein and the anti-CD3 antibody of the invention target CD3ε and activate T-cells in the presence of target cells; this activity is also referred to as a "T-cell engaging effect". The T-cell engaging effect induces cytotoxicity in the target cell.

As known by the skilled in the art, activation of T-cells induces the expression of surface marker such as CD69 and CD25. The activation of T-cells can thus be measured by detecting and measuring the expression of CD4+/CD25+, CD4+/CD69+, CD8+/CD25+, or CD8+/CD69+ T cells. Methods to measure T-cell activation are known to the skilled in the art.

A method to measure T-cell activation is further disclosed in the example section (Example 3.3). Accordingly, in context of the invention T-cell activation is measured either as the percentage of cells expressing CD69 in % of the total number of cells, or as the percentage of cells expressing CD4 and CD69 in % of total number of cells, or as the percentage of cells expressing CD8 and CD69 in % of the total number of cells.

"Low T-cell activation" in context with the anti-CD3 antibody of the invention refers to less than 10%, less than 8%, less than 6%, less than 4%, less than 2%, less than 1%, for example less than 0.5% T-cell activation in the absence of target cells.

Alignments of the sequences of the VH and VL regions of the "20G6-F3", "4B4-D7", "4E7-C9", "18F5-H10", "12D2-E5", "11D7-C3", "11H3-E5", "13H2-C2", "13C1-F6",

“18H11-F10”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “3G5-E10”, “9D7-F3”, “8C2-F7”, “20E5-F10”, “20B5-F10”, “6C9-C9”, “3E8-G1”, “3H6-D2” and “8H2” anti-CD3 antibodies were performed. The comparison of the CDR-H and CDR-L sequences tends to indicate that, structurally,
 5 “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10” and “3H6-D2” are closely related, said antibodies probably binding to the same epitope. The comparison of the CDR-H and CDR-L sequences of said related antibodies is presented in Figures 1 and 2,
 10 respectively. CDR positions were identified that are strictly conserved between the antibodies and which are thus assumed to be important for specificity, whereas other positions could support substitution.

Accordingly, the antibody according to the invention comprises:

- a heavy chain variable domain comprising a CDR1-H consisting of sequence
 15 GFX₁X₂X₃X₄AW (SEQ ID NO: 331) wherein X₁ is T or S, X₂ is F or V, X₃ is S or T and X₄ is N, K, L or Y, or any combination thereof; and
 a CDR2-H consisting of sequence IKX₁X₂X₃NX₄YX₅T (SEQ ID NO: 332) wherein X₁ is A or D, X₂ is K or R, X₃ is S or A, X₄ is N or S and X₅ is A or E, or any combination thereof; and
 20 a CDR3-H consisting of sequence TWRHYSSHTMDA (SEQ ID NO: 69) or RALTYGYKRDAMDG (SEQ ID NO: 129) or RX₁X₂X₃YX₄X₅X₆X₇X₈X₉X₁₀X₁₁DX₁₂ (SEQ ID NO: 333) wherein X₁ is Y, G or A, X₂ is V, T or L, X₃ is H, N, Y or Q, X₄ is G, R or A, X₅ is F or V or no amino acid, X₆ is R or no amino acid, X₇ is F, S or I or no amino acid, X₈ is F, L, N, M, Y, S, A or G, X₉ is Y, A, K, S, N, T, F or L, X₁₀ is A,
 25 P, G or T, X₁₁ is M, L, F or S and X₁₂ is A, V or Y, or any combination thereof, and
- a light chain variable domain comprising a CDR1-L consisting of sequence QX₁LX₂HX₃NGX₄TY (SEQ ID NO: 334) wherein X₁ is R or S, X₂ is V or E, X₃ is N, D or T, and X₄ is N or Y, or any combination thereof; and
 a CDR2-L consisting of sequence ‘KVS’; and
 30 a CDR3-L consisting of sequence GQGX₁X₂YPFT (SEQ ID NO: 335) wherein X₁ is T, A or S and X₂ is H, E or Q, or any combination thereof.

According to an embodiment, the anti-CD3 antibody according to the invention comprises the CDR sequences of the heavy and/or light chains of one of 28 so-called

“20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “12D2-E5”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “18H11-F10”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “3G5-E10”, “9D7-F3”, “8C2-F7”, “20E5-F10”, “20B5-F10”, “6C9-C9”, “3E8-G1”, “3H6-D2” and “8H2” anti-CD3 antibodies listed above.

Therefore, the invention relates to an anti-CD3 antibody, which comprises:

- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6 or a sequence differing from SEQ ID NO: 6 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 7 or a sequence differing from SEQ ID NO: 7 by one or more amino acid substitution; CDR3-H of sequence SEQ ID NO: 8 or a sequence differing from SEQ ID NO: 8 by one amino acid substitution; and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or SEQ ID NO: 142 or a sequence differing from SEQ ID NO: 10 or SEQ ID NO: 142 by one amino acid substitution; CDR2-L of sequence ‘KVS’ or a sequence differing from the sequence ‘KVS’ by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence differing from SEQ ID NO: 11 by one amino acid substitution; or
- b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13 or a sequence differing from SEQ ID NO: 13 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 14 or a sequence differing from SEQ ID NO: 14 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 15 or a sequence differing from SEQ ID NO: 15 by one amino acid substitution; and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or SEQ ID NO: 184 or a sequence differing from SEQ ID NO: 17 or SEQ ID NO: 184 by one amino acid substitution; CDR2-L of sequence ‘KVS’ or a sequence differing from the sequence ‘KVS’ by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence differing from SEQ ID NO: 11 by one amino acid substitution; or
- c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13 or a sequence differing from SEQ ID NO: 13 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 19 or a sequence differing from SEQ ID NO: 19 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

- 20 or a sequence differing from SEQ ID NO: 20 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 22 or
a sequence differing from SEQ ID NO: 22 by one amino acid substitution; CDR2-
5 L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or
- d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 24
or a sequence differing from SEQ ID NO: 24 by one amino acid substitution;
10 CDR2-H of sequence SEQ ID NO: 19 or a sequence differing from SEQ ID NO:
19 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
25 or a sequence differing from SEQ ID NO: 25 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 27 or
15 a sequence differing from SEQ ID NO: 27 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 28 or a sequence
differing from SEQ ID NO: 28 by one amino acid substitution; or
- e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 30
20 or a sequence differing from SEQ ID NO: 30 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 31 or a sequence differing from SEQ ID NO:
31 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
32 or a sequence differing from SEQ ID NO: 32 by one amino acid substitution;
and
25 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 34 or
a sequence differing from SEQ ID NO: 34 by one amino acid substitution; CDR2-
L of sequence 'RDD' or a sequence differing from the sequence 'RDD' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 35 or a sequence
differing from SEQ ID NO: 35 by one amino acid substitution; or
- 30 f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

38 or a sequence differing from SEQ ID NO: 38 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 28 or a sequence
differing from SEQ ID NO: 28 by one amino acid substitution; or

g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
41 or a sequence differing from SEQ ID NO: 41 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or
a sequence differing from SEQ ID NO: 17 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

h) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
44 or a sequence differing from SEQ ID NO: 44 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

i) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

47 or a sequence differing from SEQ ID NO: 47 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

j) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 50
or a sequence differing from SEQ ID NO: 50 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 51 or a sequence differing from SEQ ID NO:
51 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
52 or a sequence differing from SEQ ID NO: 52 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 54 or
a sequence differing from SEQ ID NO: 54 by one amino acid substitution; CDR2-
L of sequence 'NAN' or a sequence differing from the sequence 'NAN' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 55 or a sequence
differing from SEQ ID NO: 55 by one amino acid substitution; or

k) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 57
or a sequence differing from SEQ ID NO: 57 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
58 or a sequence differing from SEQ ID NO: 58 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 28 or a sequence
differing from SEQ ID NO: 28 by one amino acid substitution; or

l) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

- 61 or a sequence differing from SEQ ID NO: 61 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
5 L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or
- m) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 64
or a sequence differing from SEQ ID NO: 64 by one amino acid substitution;
10 CDR2-H of sequence SEQ ID NO: 65 or a sequence differing from SEQ ID NO:
65 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
47 or a sequence differing from SEQ ID NO: 47 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 67 or
15 a sequence differing from SEQ ID NO: 67 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 28 or a sequence
differing from SEQ ID NO: 28 by one amino acid substitution; or
- n) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
20 or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
69 or a sequence differing from SEQ ID NO: 69 by one amino acid substitution;
and
25 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 71 or a sequence
differing from SEQ ID NO: 71 by one amino acid substitution; or
- 30 o) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

84 or a sequence differing from SEQ ID NO: 84 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or
a sequence differing from SEQ ID NO: 17 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

p) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 75
or a sequence differing from SEQ ID NO: 75 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 76 or a sequence differing from SEQ ID NO:
76 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
77 or a sequence differing from SEQ ID NO: 77 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

q) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80
or a sequence differing from SEQ ID NO: 80 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 76 or a sequence differing from SEQ ID NO:
76 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
81 or a sequence differing from SEQ ID NO: 81 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

r) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

- 84 or a sequence differing from SEQ ID NO: 84 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
5 L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or
- s) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
10 CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
47 or a sequence differing from SEQ ID NO: 47 by one amino acid substitution;
and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
15 a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 88 or a sequence
differing from SEQ ID NO: 88 by one amino acid substitution; or
- t) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 90
20 or a sequence differing from SEQ ID NO: 90 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 91 or a sequence differing from SEQ ID NO:
91 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
32 or a sequence differing from SEQ ID NO: 32 by one amino acid substitution;
and
25 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 93 or
a sequence differing from SEQ ID NO: 93 by one amino acid substitution; CDR2-
L of sequence 'GAS' or a sequence differing from the sequence 'GAS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 94 or a sequence
differing from SEQ ID NO: 94 by one amino acid substitution; or
- 30 u) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 96
or a sequence differing from SEQ ID NO: 96 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 97 or a sequence differing from SEQ ID NO:
97 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

98 or a sequence differing from SEQ ID NO: 98 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100
or a sequence differing from SEQ ID NO: 100 by one amino acid substitution;
5 CDR2-L of sequence 'NTN' or a sequence differing from the sequence 'NTN' by
one amino acid substitution and CDR3-L of sequence SEQ ID NO: 101 or a
sequence differing from SEQ ID NO: 101 by one amino acid substitution; or

v) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO:
103 or a sequence differing from SEQ ID NO: 103 by one amino acid
10 substitution; CDR2-H of sequence SEQ ID NO: 104 or a sequence differing from
SEQ ID NO: 104 by one or more amino acid substitutions; CDR3-H of sequence
SEQ ID NO: 105 or a sequence differing from SEQ ID NO: 105 by one amino
acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
15 a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

w) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80
20 or a sequence differing from SEQ ID NO: 80 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 19 or a sequence differing from SEQ ID NO:
19 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
108 or a sequence differing from SEQ ID NO: 108 by one amino acid
substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
25 a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-
L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one
amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence
differing from SEQ ID NO: 11 by one amino acid substitution; or

x) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13
30 or a sequence differing from SEQ ID NO: 13 by one amino acid substitution;
CDR2-H of sequence SEQ ID NO: 37 or a sequence differing from SEQ ID NO:
37 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

111 or a sequence differing from SEQ ID NO: 111 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 113 or a sequence differing from SEQ ID NO: 113 by one amino acid substitution; CDR2-L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 114 or a sequence differing from SEQ ID NO: 114 by one amino acid substitution; or

y) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 116 or a sequence differing from SEQ ID NO: 116 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 117 or a sequence differing from SEQ ID NO: 117 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 118 or a sequence differing from SEQ ID NO: 118 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100 or a sequence differing from SEQ ID NO: 100 by one amino acid substitution; CDR2-L of sequence 'VTN' or a sequence differing from the sequence 'VTN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 120 or a sequence differing from SEQ ID NO: 120 by one amino acid substitution; or

z) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 122 or a sequence differing from SEQ ID NO: 122 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 123 or a sequence differing from SEQ ID NO: 123 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 124 or a sequence differing from SEQ ID NO: 124 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 126 or a sequence differing from SEQ ID NO: 126 by one amino acid substitution; CDR2-L of sequence 'RDD' or a sequence differing from the sequence 'RDD' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 127 or a sequence differing from SEQ ID NO: 127 by one amino acid substitution; or

aa) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13 or a sequence differing from SEQ ID NO: 13 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 19 or a sequence differing from SEQ ID NO: 19 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

129 or a sequence differing from SEQ ID NO: 129 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-L of sequence 'KVS' or a sequence differing from the sequence 'KVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence differing from SEQ ID NO: 11 by one amino acid substitution; or

bb) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103 or a sequence differing from SEQ ID NO: 103 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 104 or a sequence differing from SEQ ID NO: 104 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 105 or a sequence differing from SEQ ID NO: 105 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 133 or a sequence differing from SEQ ID NO: 133 by one amino acid substitution; CDR2-L of sequence 'LVS' or a sequence differing from the sequence 'LVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 134 or a sequence differing from SEQ ID NO: 134 by one amino acid substitution.

One or more individual amino acids may be altered by substitution, in particular by conservative substitution, in one or more of the above CDR sequences. Such an alteration may be intended for example to remove a glycosylation site or a deamidation site, in connection with humanization of the antibody.

Based on the alignments of the sequences of the VH and VL regions of the "20G6-F3", "4B4-D7", "4E7-C9", "18F5-H10", "11D7-C3", "11H3-E5", "13H2-C2", "13C1-F6", "1E6-C9", "10F4-C10", "10E6-G6", "18G9-H11", "11F3-B9", "12G3-E8", "5B1-G2", "16F8-A7", "11F9-F8", "20E5-F10", "20B5-F10" and "3H6-D2" different amino acid substitutions were identified. Therefore, in one embodiment, an amino acid is substituted:

- in CDR1-H at one or more of positions 3 to 6, for instance at position 3, 5 or 6 of CDR1-H of sequence GFTFSNAW (SEQ ID NO: 13) or for instance at position 4 and 5 of CDR1-H of sequence GFTFSNAW (SEQ ID NO: 13); and/or

- in CDR2-H, at one or more of positions 3 to 5, 7 and 9, for instance at positions 3, 4, 5, 7 or 9 of CDR2-H of sequence IKAksNNYAT (SEQ ID NO: 37), or for instance at positions 3 and 5 or 3 and 7 of CDR2-H of sequence IKAksNNYAT (SEQ ID

NO: 37); and/or

- in CDR3-H, at one or more of positions 2 to 4, 7 to 10 and 12, for instance at positions 2, 3, 4, 6, 7, 8, 9 and 10 or at positions 2, 4, 6, 8, 10 and 12 or at positions 2, 3, 7 and 8 of CDR3-H of sequence RGVYYALSPFDY (SEQ ID NO:8), or at position 7 and 8 of CDR3-H of sequence RGLYYGLSPSDY (SEQ ID NO: 38), or at position at positions 2, 3, 4, 6, 7, 8, 9 and 10 of CDR3-H of sequence RGLYYGLSPSDY (SEQ ID NO: 38); and/or

- in CDR1-L, at one or more of positions 2, 4, 6, and 9, for example at position 6 of CDR1-L of sequence QSLVHDNGNTY (SEQ ID NO: 17) or QSLVHTNGNTY (SEQ ID NO: 27) or at position 2 of CDR1-L of sequence QSLVHNNGNTY (SEQ ID NO : 10) or QRLVHNNGNTY (SEQ ID NO : 113) or at position 4, 6 and 9 of CDR1-L of sequence QSLVHNNGNTY (SEQ ID NO : 10); and/or

- in CDR3-L, at one or more of positions 4 and 5, for instance at position 4 of CDR3-L of sequence GQGSQYPFT (SEQ ID NO: 71) or GQGTQYPFT (SEQ ID NO: 11), or at position 4 and 5 of CDR3-L of sequence GQGAHYPFT (SEQ ID NO: 88) or position 5 of CDR3-L of sequence GQGTHYPFT (SEQ ID NO: 28) or GQGTEYPFT (SEQ ID NO: 114).

The anti-CD3 antibody according to the invention is in particular a conventional antibody, in particular a conventional monoclonal antibody, or an antibody fragment, a bispecific or multispecific antibody.

The anti-CD3 antibody according to the invention in particular comprises or consists of an IgG, or a fragment thereof.

According to a further embodiment, the invention relates to an anti-CD3 antibody, which comprises:

- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6, CDR2-H of sequence SEQ ID NO: 7, CDR3-H of sequence SEQ ID NO: 8 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or SEQ ID NO: 142, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 14, CDR3-H of sequence SEQ ID NO: 15 and

- a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or SEQ ID NO: 184, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 5 c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 20 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 22, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 10 d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 24, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 25 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 27, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- 15 e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 30, CDR2-H of sequence SEQ ID NO: 31, CDR3-H of sequence SEQ ID NO: 32 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 34, CDR2-L of sequence 'RDD' and CDR3-L of sequence SEQ ID NO: 35; or
- 20 f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 38 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- 25 g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 41 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 30 h) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 44 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or

- i) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 47 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 5 j) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 50, CDR2-H of sequence SEQ ID NO: 51, CDR3-H of sequence SEQ ID NO: 52 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 54, CDR2-L of sequence 'NAN' and CDR3-L of sequence SEQ ID NO: 55; or
- 10 k) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 57, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 58 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- 15 l) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 61 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- m) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 64, CDR2-H of sequence SEQ ID NO: 65, CDR3-H of sequence SEQ ID NO: 47 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 20 67, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- n) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 69 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 71; or
- 25 o) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 84 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 30 p) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 75, CDR2-H of sequence SEQ ID NO: 76, CDR3-H of sequence SEQ ID NO: 77 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- q) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80, CDR2-H of sequence SEQ ID NO: 76, CDR3-H of sequence SEQ ID NO: 81

- and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 5 r) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 84 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 10 s) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 47 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 88; or
- t) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 90, CDR2-H of sequence SEQ ID NO: 91, CDR3-H of sequence SEQ ID NO: 32 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 93, CDR2-L of sequence 'GAS' and CDR3-L of sequence SEQ ID NO: 94; or
- 15 u) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 96, CDR2-H of sequence SEQ ID NO: 97, CDR3-H of sequence SEQ ID NO: 98 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100, CDR2-L of sequence 'NTN' and CDR3-L of sequence SEQ ID NO: 101; or
- 20 v) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103, CDR2-H of sequence SEQ ID NO: 104, CDR3-H of sequence SEQ ID NO: 105 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 25 w) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 108 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 30 x) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 111 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 113, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 114; or
- y) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 116, CDR2-H of sequence SEQ ID NO: 117, CDR3-H of sequence SEQ ID NO: 118 and a light chain variable domain comprising CDR1-L of sequence SEQ ID

NO: 100, CDR2-L of sequence 'VTN' and CDR3-L of sequence SEQ ID NO: 120; or

5 z) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 122, CDR2-H of sequence SEQ ID NO: 123, CDR3-H of sequence SEQ ID NO: 124 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 126, CDR2-L of sequence 'RDD' and CDR3-L of sequence SEQ ID NO: 127; or

10 aa) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 129 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or

15 bb) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103, CDR2-H of sequence SEQ ID NO: 104, CDR3-H of sequence SEQ ID NO: 105; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 133, CDR2-L of sequence 'LVS' and CDR3-L of sequence SEQ ID NO: 134.

20 The invention also provides an anti-CD3 antibody comprising at least the heavy chain variable domain and/or the light chain variable domain of one of the so-called anti-CD3 antibodies listed above.

Thus, the invention relates in particular to an anti-CD3 antibody, which comprises:

25 a) a heavy chain variable domain of sequence SEQ ID NO: 5 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 9, or a sequence at least 85% identical thereto; or

b) a heavy chain variable domain of sequence SEQ ID NO: 12 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 16, or a sequence at least 85% identical thereto; or

30 c) a heavy chain variable domain of sequence SEQ ID NO: 18 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 21, or a sequence at least 85% identical thereto; or

d) a heavy chain variable domain of sequence SEQ ID NO: 23 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 26, or a sequence at least 85% identical thereto; or

- e) a heavy chain variable domain of sequence SEQ ID NO: 29 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 33, or a sequence at least 85% identical thereto; or
- 5 f) a heavy chain variable domain of sequence SEQ ID NO: 36 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 39, or a sequence at least 85% identical thereto; or
- g) a heavy chain variable domain of sequence SEQ ID NO: 40 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 42, or a sequence at least 85% identical thereto; or
- 10 h) a heavy chain variable domain of sequence SEQ ID NO: 43 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 45, or a sequence at least 85% identical thereto; or
- i) a heavy chain variable domain of sequence SEQ ID NO: 46 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence
15 SEQ ID NO: 48, or a sequence at least 85% identical thereto; or
- j) a heavy chain variable domain of sequence SEQ ID NO: 49 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 53, or a sequence at least 85% identical thereto; or
- k) a heavy chain variable domain of sequence SEQ ID NO: 56 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence
20 SEQ ID NO: 59, or a sequence at least 85% identical thereto; or
- l) a heavy chain variable domain of sequence SEQ ID NO: 60 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 62, or a sequence at least 85% identical thereto; or
- 25 m) a heavy chain variable domain of sequence SEQ ID NO: 63 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 66, or a sequence at least 85% identical thereto; or
- n) a heavy chain variable domain of sequence SEQ ID NO: 68 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence
30 SEQ ID NO: 70, or a sequence at least 85% identical thereto; or
- o) a heavy chain variable domain of sequence SEQ ID NO: 72 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 73, or a sequence at least 85% identical thereto; or

- p) a heavy chain variable domain of sequence SEQ ID NO: 74 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 78, or a sequence at least 85% identical thereto; or
- 5 q) a heavy chain variable domain of sequence SEQ ID NO: 79 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 82, or a sequence at least 85% identical thereto; or
- r) a heavy chain variable domain comprising sequence SEQ ID NO: 83 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 85, or a sequence at least 85% identical thereto; or
- 10 s) a heavy chain variable domain of sequence SEQ ID NO: 46 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 87, or a sequence at least 85% identical thereto; or
- t) a heavy chain variable domain of sequence SEQ ID NO: 89 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence
- 15 SEQ ID NO: 92, or a sequence at least 85% identical thereto; or
- u) a heavy chain variable domain of sequence SEQ ID NO: 95 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 99, or a sequence at least 85% identical thereto; or
- v) a heavy chain variable domain of sequence SEQ ID NO: 102 or a sequence at
- 20 least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 106, or a sequence at least 85% identical thereto; or
- w) a heavy chain variable domain of sequence SEQ ID NO: 107 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 109, or a sequence at least 85% identical thereto; or
- 25 x) a heavy chain variable domain of sequence SEQ ID NO: 110 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 112, or a sequence at least 85% identical thereto; or
- y) a heavy chain variable domain of sequence SEQ ID NO: 115 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence
- 30 SEQ ID NO: 119, or a sequence at least 85% identical thereto; or
- z) a heavy chain variable domain of sequence SEQ ID NO: 121 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 125, or a sequence at least 85% identical thereto; or

aa) a heavy chain variable domain of sequence SEQ ID NO: 128 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 130, or a sequence at least 85% identical thereto; or

5 bb) a heavy chain variable domain of sequence SEQ ID NO: 131 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 132, or a sequence at least 85% identical thereto.

For instance, the sequence of the variable domain of heavy or light chain may differ from the reference sequence SEQ ID NO: 5, 9, 12, 16, 18, 21, 23, 26, 29, 33, 36, 39, 40, 42, 43, 45, 46, 48, 49, 53, 56, 59, 60, 62, 63, 66, 68, 70, 72, 73, 74, 78, 79, 82, 83, 10 85, 87, 89, 92, 95, 99, 102, 106, 107, 109, 110, 112, 115, 119, 121, 125, 128, 130, 131, 132, as appropriate, by one or more amino acid substitution(s), in particular by one or more conservative amino acid substitution(s) and/or substitution(s) with canonical residues. In particular, the sequence of the variable domain of heavy or light chain may differ from the reference sequence SEQ ID NO: 5, 9, 12, 16, 18, 21, 23, 26, 29, 33, 36, 15 39, 40, 42, 43, 45, 46, 48, 49, 53, 56, 59, 60, 62, 63, 66, 68, 70, 72, 73, 74, 78, 79, 82, 83, 85, 87, 89, 92, 95, 99, 102, 106, 107, 109, 110, 112, 115, 119, 121, 125, 128, 130, 131, 132,, by conservative amino acid substitution(s), only.

The sequence alterations as compared with sequence SEQ ID NO: 5, 9, 12, 16, 18, 21, 23, 26, 29, 33, 36, 39, 40, 42, 43, 45, 46, 48, 49, 53, 56, 59, 60, 62, 63, 66, 68, 20 70, 72, 73, 74, 78, 79, 82, 83, 85, 87, 89, 92, 95, 99, 102, 106, 107, 109, 110, 112, 115, 119, 121, 125, 128, 130, 131, 132 will in particular be present essentially in one or more of the framework regions, FR1-L, FR2-L, FR3-L, FR4-L and/or FR1-H, FR2-H, FR3-H, FR4-H.

However, amino acid substitutions in one or more CDRs are also possible. In 25 particular, the sequence of the light chain variable domain may differ from sequence SEQ ID NO: 9 at least by a S to R substitution at position 28 of SEQ ID NO: 9 (in CDR1-L), and/or at least by a V to E substitution at position 30 of SEQ ID NO: 9 (in CDR1-L), and/or at least by a N to D or T substitution at position 33 of SEQ ID NO: 9 (in CDR1-L), and/or at least by a N to Y substitution at position 35 of SEQ ID NO: 9 (in CDR1-L) 30 and/or the sequence of the light chain variable domain may differ from sequence SEQ ID NO: 9 at least by a T to S or A substitution at position 97 of SEQ ID NO: 9 (in CDR3-L), and/or at least by a Q to H or E substitution at position 98 of SEQ ID NO: 9 (in CDR3-L) and/or the heavy chain variable domain may differ from sequence SEQ ID NO: 5 at least by a T to N or S substitution at position 28 of SEQ ID NO: 5 (in CDR1-H),

and/or at least by a F to V substitution at position 29 of SEQ ID NO: 5 (in CDR1-H), or at least by a T to N or Y substitution at position 30 of SEQ ID NO: 5 (in CDR1-H), or at least by a K to L or Y substitution at position 31 of SEQ ID NO: 5 (in CDR1-H) and/or the heavy chain variable domain may differ from sequence SEQ ID NO: 5 at least by a D to A substitution at position 53 of SEQ ID NO: 5 (in CDR2-H), or at least by a K to R substitution at position 54 of SEQ ID NO: 5 (in CDR2-H), or at least by a S to A substitution at position 55 of SEQ ID NO: 5 (in CDR2-H), or at least by a S to N substitution at position 57 of SEQ ID NO: 5 (in CDR2-H), or at least by a A to E substitution at position 59 of SEQ ID NO: 5 (in CDR2-H) and/or the heavy chain variable domain may differ from sequence SEQ ID NO: 5 at least by a G to A substitution at position 100 of SEQ ID NO: 5 (in CDR3-H) and/or at least by a T to V substitution at position 101 of SEQ ID NO: 5 (in CDR3-H) at least by a Q to Y substitution at position 102 of SEQ ID NO: 5 (in CDR3-H).

In one embodiment, the anti-CD3 antibody of the invention and a fragment thereof is, respectively, a rat antibody and a fragment of a rat antibody.

In one aspect of the invention, the anti-CD3 antibody of the invention may also be a chimeric antibody, and in particular a rat/human antibody, e.g. an antibody comprising rat variable domains of heavy and light chains and a CH domain and a CL domain from a human antibody.

In a further aspect of the invention, the anti-CD3 antibody may also be a humanized antibody or a fragment of a humanized antibody obtained, for example, by CDR-grafting or by the 4D method (US20110027266).

Accordingly, in one embodiment, the anti-CD3 antibody of the invention is a humanized antibody comprising

- a) a heavy chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 152, SEQ ID NO: 153, and
- a light chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, or

- b) a heavy chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 183, and
- a light chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 182.

According to a further embodiment, the anti-CD3 antibody of the invention is a humanized antibody comprising:

- a) a heavy chain variable domain of sequence SEQ ID NO: 138 and/or a light chain variable domain of sequence SEQ ID NO: 143; or
- b) a heavy chain variable domain of sequence SEQ ID NO: 171 and/or a light chain variable domain of sequence SEQ ID NO: 158; or
- c) a heavy chain variable domain of sequence SEQ ID NO: 176 and/or a light chain variable domain of sequence SEQ ID NO: 164.

In one embodiment, the anti-CD3 antibody according to the invention comprises the three CDR sequences or the variable domain of the heavy chain, or the six CDR sequences or variable domains of the heavy and light chains of one of so-called anti-CD3 antibodies listed above.

The invention further refers to a fragment of the humanized anti-CD3 antibody as defined above. In one embodiment, the humanized anti-CD3 antibody described above is a chimeric antibody.

The anti-CD3 antibody according to the invention may also be a single domain antibody or a fragment thereof. In particular, a single domain antibody fragment may consist of a variable heavy chain (VHH) which comprises the CDR1-H, CDR2-H and CDR3-H of one of the antibodies described above. The anti-CD3 antibody may also be a heavy chain antibody, i.e. an antibody devoid of light chain, which may or may not contain a CH1 domain.

The single domain antibody or a fragment thereof may also comprise the framework regions of a camelid single domain antibody, and optionally the constant domain of a camelid single domain antibody.

5 The anti-CD3 antibody according to the invention may also be an antibody fragment, in particular a humanized antibody fragment, selected from the group consisting of Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, and diabodies.

Accordingly, the anti-CD3 antibody of the invention is a Fab comprising, or consisting of

- 10 a) the heavy chain amino acid sequence SEQ ID NO: 186 and/or the light chain amino acid sequence SEQ ID NO: 187; or
- b) the heavy chain amino acid sequence SEQ ID NO: 188 and/or the light chain amino acid sequence SEQ ID NO: 189; or
- c) the heavy chain amino acid sequence SEQ ID NO: 190 and/or the light chain amino acid sequence SEQ ID NO: 191; or
- 15 d) the heavy chain amino acid sequence SEQ ID NO: 192 and/or the light chain amino acid sequence SEQ ID NO: 193.

20 In an embodiment, the CD3-antibody is a bispecific or multispecific antibody formed from at least one antibody fragment or at least one variable domain of the anti-CD3 antibodies of the invention. Multispecific antibodies are polyvalent protein complexes as described for instance in EP 2 050 764 A1 or US 2005/0003403 A1.

The bispecific or multispecific CD3-antibodies according to the invention can have specificity for (a) extracellular domain of human or human and *Macaca fascicularis* CD3, targeted by one of the above described anti-CD3 antibodies and (b) at least one other
25 antigen.

In one embodiment, the other antigen is CD123 and accordingly the resulting bispecific antibody is a CD3/CD123 bispecific antibody. Conventional bi-specific antibodies can be produced by techniques that are known to the skilled in the art.

30 Antibodies and fragments of anti-CD3 antibodies according to the invention can be used in an isolated (e.g., purified) form or contained in a vector, such as a membrane or lipid vesicle (e.g. a liposome).

In one further embodiment, the anti-CD3 antibody of the invention is for use for the preparation of antibody-like binding protein of the invention further defined in the section "antibody-like binding protein".

Any combination of the above embodiments makes part of the invention.

Anti CD123 antibodies

“CD123” (Cluster of Differentiation 123) is also known as “Interleukin 3 receptor, alpha (IL3RA)” or “IL3R”, “IL3RX”, “IL3RY”, “IL3RAY”, “hIL-3Ra” and denotes an interleukin 3 specific subunit of a heterodimeric cytokine receptor. The functional interleukin 3 receptor is a heterodimer that comprises a specific alpha chain (IL-3A; CD123) and the IL-3 receptor beta chain (β_0 ; CD 131) that is shared with the receptors for granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin 5 (IL-5). CD123 is a type I integral transmembrane protein with a deduced Molecular Weight of about 43kDa containing an extracellular domain involved in IL-3 binding, a transmembrane domain and a short cytoplasmic tail of about 50 amino acids. The extracellular domain is composed of two regions: an N-terminal region of about 100 amino acids, the sequence of which exhibits similarity to equivalent regions of the GM-CSF and IL-5 receptor alpha-chains; and a region proximal to the transmembrane domain that contains four conserved cysteine residues and a WSXWS motif, common to other members of this cytokine receptor family. The IL-3 binding domain comprises about 200 amino acid residue cytokine receptor motifs (CRMs) made up of two Ig-like folding domains. The extracellular domain of CD123 is highly glycosylated, with N-glycosylation necessary for both ligand binding and receptor signaling. The protein family gathers three members: IL3RA (CD123A), CSF2RA and IL5RA. The overall structure is well conserved between the three members but sequence homologies are very low. One 300 amino-acid long isoform of CD123 has been discovered so far, but only on the RNA level which is accessible on the Getentry database under the accession number ACM24116.1.

US Patent No. 6,177,078 discloses the anti-IL-3Receptor alpha chain (IL-3R α , CD123) monoclonal antibody 7G3, and the ability of 7G3 to bind to the N-terminal domain, specifically amino acid residues 19-49, of IL-3R α . US Patent No. 6,733,743 discloses a method of impairing a hematologic cancer progenitor cell that expresses CD123 but does not significantly express CD131, by contacting the cell with a composition of an antibody and a cytotoxic agent (selected from a chemotherapeutic agent, a toxin or an alpha-emitting radioisotope) whereby the composition binds

selectively to CD123 in an amount effective to cause cell death. However, it has remained unclear whether targeting CD123 can functionally impair AML-LSCs.

A reference sequence of full-length human CD123 protein, including signal peptide, is available from the NCBI database under the accession number NP_002174.1 and under the Uniprot accession number P26951 and is herein disclosed under SEQ ID NO: 194 (as available on December 14, 2014). A reference sequence of full-length *Macaca fascicularis* CD123 protein, including signal peptide, is available from GenBank database under the accession number EHH61867.1 and under the Uniprot accession number G8F3K3 and is herein disclosed under SEQ ID NO: 195 (as available on December 14, 2014).

A sequence of a mature human CD123 Strep-II tagged Fc-fusion protein, cloned by the inventors from genomic DNA, is disclosed under SEQ ID NO: 196. Said mature human CD123 Fc-fusion protein comprises amino acids 19 to 305 of the full-length human CD123 protein and thus comprises the extracellular domain of human CD123.

A sequence of a mature *Macaca fascicularis* CD123 Strep-II tagged Fc-fusion protein, cloned by the inventors from cDNA, is disclosed under SEQ ID NO: 197. Said mature *Macaca fascicularis* CD123 Fc-fusion protein comprises amino acids 19 to 305 of the full-length *Macaca fascicularis* CD123 protein and thus comprises the extracellular domain of *Macaca fascicularis* CD123.

Domain organization of human and *Macaca fascicularis* CD123 is as follows (based on the human CD123 sequence accessible in the NCBI database under accession NP_002174.1 (SEQ ID NO: 194) and based on the *Macaca fascicularis* CD123 sequence accessible in the Uniprot database under accession number G8F3K3, SEQ ID NO: 195):

Human CD123 domains	Positions on SEQ ID NO: 194 (human)	Positions on SEQ ID NO: 195 (<i>Macaca fascicularis</i>)
Extracellular	19 - 305	19 - 305
Transmembrane domain	306 - 325	306 - 325
Cytoplasmic	326 - 378	326 - 378

Accordingly, the extracellular domain of human CD123 consists of amino acids at positions 19 – 305 of SEQ ID NO: 194.

CD123 (the interleukin-3 receptor alpha chain IL-3R α) is a tumor antigen over-expressed in a variety of hematological neoplasms. The majority of AML blasts express surface CD123 and this expression does not vary by subtype of AML. Higher expression of CD123 on AML at diagnosis has been reported to be associated with poorer prognosis. CD123 expression has been reported in other hematological malignancies including myelodysplasia, systemic mastocytosis, blastic plasmacytoid dendritic cell neoplasm (BPDCN), ALL and hairy cell leukemia.

CD123 is expressed on AML leukemic stem cells and growing evidences suggest that AML arises from these LSCs, which have been shown to be quiescent and relatively resistant to DNA damaging chemotherapy. It is hypothesized that the persistence of LSCs underpins relapse after initial remission and thus the eradication of LSCs can be considered a requirement for cure, and an important therapeutic goal.

“Leukemic stem cells (LSCs)” are cancer cells that possess characteristics associated with normal stem cells, that is, the property of self renewal and the capability to develop multiple lineages. Such cells are proposed to persist in hematological cancers such as AML as distinct populations. The LSC present in AML patients are so called “AML-LCSs”.

“Acute myelogenous leukemia (AML)” is a clonal disorder clinically presenting as increased proliferation of heterogeneous and undifferentiated myeloid blasts. The leukemic hierarchy is maintained by a small population of LSCs (AML-LCSs), which have the distinct ability for self-renewal, and are able to differentiate into leukemic progenitors. These progenitors generate the large numbers of leukemic blasts readily detectable in patients at diagnosis and relapse, leading ultimately to mortality. AML-LSC have been commonly reported as quiescent cells, in contrast to rapidly dividing clonogenic progenitors. This property of AML-LSCs renders conventional chemotherapeutics that target proliferating cells less effective, potentially explaining the current experience in which a high proportion of AML patients enter complete remission, but almost invariably relapse, with <30% of adults surviving for more than 4 years. In addition, minimal residual disease occurrence and poor survival has been attributed to high LSC frequency at diagnosis in AML patients. Consequently, it is imperative for the long-term management of AML (and similarly other above mentioned hematological cancer conditions) that new treatments are developed to specifically eliminate LSCs.

Over-expression of CD123 has been reported on AML blasts and on CD34⁺/CD38 AML- LSCs relative to normal hematopoietic cells.

CD123 thus provides an important therapeutic target for cancer therapy, in particular for cancer therapy in patients having poor prognosis.

The inventors have succeeded in generating, screening and selecting specific mouse and rat anti-CD123 antibodies displaying high affinity for both human and *Macaca fascicularis* CD123 protein, and which do not significantly cross-react with human CSF2RA and IL5RA proteins, and with *Macaca fascicularis* CD3 proteins.

The inventors have determined the sequence of variable heavy and light chains of such monoclonal antibodies, the so-called "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", and "9F6-G3" anti-CD123 antibodies.

The so-called "3E3-D3" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence QVQLQESGPGLVQPSQTLSTCTVS**GFSLT****TYD**VHWVRQPPGKGLEWMGRI**QNGGIT** DYN SALKSR LIISRDTSKSQVFLKMNSVQTEDTAMYFC**AKTGSYFYAFDH**WGQGT LVT VSS (SEQ ID NO: 226, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 227, a CDR2-H of sequence SEQ ID NO: 228, and a CDR3-H of sequence SEQ ID NO: 229, or

a heavy chain variable domain consisting of sequence QVQLQESGPGLVQPSQTLSTCTVS**GFSLT****TYD**VHWVRQPPGKGLEWMGRI**QNAGIT** DYN SALKSR LIISRDTSKSQVFLKMNSVQTEDTAMYFC**AKTGSYFYAFDH**WGQGT LVT VSS (SEQ ID NO: 277, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 227, a CDR2-H of sequence SEQ ID NO: 353, and a CDR3-H of sequence SEQ ID NO: 229, or

a heavy chain variable domain consisting of sequence QVQLQESGPGLVQPSQTLSTCTVS**GFSLT****TYD**VHWVRQPPGKGLEWMGRI**QDGGIT** DYN SALKSR LIISRDTSKSQVFLKMNSVQTEDTAMYFC**AKTGSYFYAFDH**WGQGT LVT VSS (SEQ ID NO: 278, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 227, a CDR2-H of sequence SEQ ID NO: 279, and a CDR3-H of sequence SEQ ID NO: 229, and- a light chain variable domain consisting of sequence QFVLTQPNVSTNLGSTVKLSCKRNT**TGNIGSNY**VNWYQQHEGRSPTTMIY**RDD**KRPD GVPDRFSGSIDRSSNSALLTINNVTEDTADYFC**QSYSSGINII**FGGGTKLTVL (SEQ ID

NO: 230, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 231, a CDR2-L consisting of sequence 'RDD', and a CDR3-L of sequence SEQ ID NO: 232.

5 The so-called "1E1-G5" anti-CD123 antibody comprises:

-a heavy chain variable domain consisting of sequence QVQLQESGPTLVKPGDSVKMSCKAF**GYTFTDHI**HWVKQSHGKSLEWIGY**INPYSGGT** NYNEKFKSKATLTVDKSSSTAYMEFSRLTSEDAICYC**ALNYGSYYAMDA**WGQGTSV TVSS (SEQ ID NO: 198, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 199, a CDR2-H of sequence SEQ ID NO: 200, and a CDR3-H of sequence SEQ ID NO: 201 and

10 a light chain variable domain consisting of sequence DIQMTQSPASLSASLGQTVTIECRP**SEDIYSN**LAWFQQKPGSSPQLLIY**DANNLADGVP** SRFSGSGSGTQYSLKINSLQSEDVASYFC**QQYNKYPYT**FGTGTKLELK (SEQ ID NO: 202, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 203, a CDR2-L consisting of sequence 'DAN', and a CDR3-L of sequence SEQ ID NO: 204.

The so-called "2B8-F3" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVESGGGLVQPGRSLKLSAAS**GFTFSDYN**MAWVRQAPKKGLEWVAT**ILYDGG** **RTYYRGSVKGRFTISR**DNAKSTLYLRMDSLRSEDTATYYC**ATHSRGTDYFDY**WGQGV MVTVSS (SEQ ID NO: 205, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 206, a CDR2-H of sequence SEQ ID NO: 207, and a CDR3-H of sequence SEQ ID NO: 208 and

25 - a light chain variable domain consisting of sequence EIVLTQSPSMTASPGEQVTITCRASS**SSINYM**HWWYQQKPGASPRPWIY**ETSKLASGVP** DRFSGSASGTSYSLTINNMEAEDAATYYC**QQWNYP**SWTFGGGTKLELK (SEQ ID NO: 209, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 210, a CDR2-L consisting of sequence 'ETS', and a CDR3-L of sequence SEQ ID NO: 211.

30

The so-called "2F8-D6" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence QVQLKESGPGLVQPSQTLSTCTVS**GFSLTSYG**VSWVRQPPGKGLEWIAT**ISSAGSTY**

YDLVLKSRLSITRDTSKSQVFLKVHSLQTEDTAIYLC**ARDAPVFNYGSYNAMDS**WGQG
TSVTVSS (SEQ ID NO: 212, with CDRs shown in bold characters) comprising CDR1-H
of sequence SEQ ID NO: 213, a CDR2-H of sequence SEQ ID NO: 214, and a CDR3-H
of sequence SEQ ID NO: 215 and

- 5 - a light chain variable domain consisting of sequence
DIQMTQSPSFLSATVGDRVITINCKAS**QNINKY**LNWYQQKLGEAPKRLIY**NTN**SLQTGIP
SRFSGSGSGTDYTLTISSLQPEDVATYFC**LQHKSGLT**FGSGTKLEIK (SEQ ID NO:
216, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID
NO: 217, a CDR2-L consisting of sequence 'NTN', and a CDR3-L of sequence SEQ ID
10 NO: 218.

The so-called "3B10-E6" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence
QVQLQQSGPELRRPGSSVRLSCKAS**GYRIKDFL**IHWIKNRPEHGLEWIGW**IDPEDGET**
15 KYAQKFQTKATLTADTSSNTAYMQLSSLTSEDATYFC**ARWGDVYYGLMRGHVMDA**
WGQGTSTVTSS (SEQ ID NO: 219, with CDRs shown in bold characters) comprising
CDR1-H of sequence SEQ ID NO: 220, a CDR2-H of sequence SEQ ID NO: 221, and a
CDR3-H of sequence SEQ ID NO: 222 and

- a light chain variable domain consisting of sequence
20 DVLMTQTPVSLPVSLGGQVSISCRSS**QSLVHSDGDTYL**HWYLQKPGQSPQLLIY**RVS**N
RFSGVPDRFSGSGSGTDFTLKISRVEPEDLGLYYC**LQTTHFPPWT**FGGGTKLEMK
(SEQ ID NO: 223, with CDRs shown in bold characters) comprising CDR1-L of
sequence SEQ ID NO: 224, a CDR2-L consisting of sequence 'RVS', and a CDR3-L of
sequence SEQ ID NO: 225.

25

The so-called "5A5-B4" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence
QIQIVQSGSDVTSTCNGCGTCYFS**GFSLSSTTGIC**VSWIRQPSGKGQEWLAD**FCWDDG**
30 **KGYNPSLKNRLSISKDTSNNQVFLKITSVDTADTATYYC****ARRRVYYGIYFDY**WGQGVM
VTVSS (SEQ ID NO: 233, with CDRs shown in bold characters) comprising CDR1-H of
sequence SEQ ID NO: 234, a CDR2-H of sequence SEQ ID NO: 235, and a CDR3-H of
sequence SEQ ID NO: 236 and

- a light chain variable domain consisting of sequence
DIVMTQSPALAVSPGERVSISCRAS**NSVSTR**MHWYQQKPGQPKLLIY**GAS**NLESGVP

ARFSGSGSGTDFTLTIDPVEADDIATYFC**QQSWNDPLT**FGSGGTKLEIK (SEQ ID NO: 237, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 238, a CDR2-L consisting of sequence 'GAS', and a CDR3-L of sequence SEQ ID NO: 239.

5

The so-called "6B10-E4" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence EVQLVESGGGLVQPGRSLKLSKAAS**GFTFSHYN**MAWVRQAPKKGLEWVAT**ITYDDHS**
IYYRDSVKGRFTISRDTAKSTLYLQMDSLRSEDTATYYC**CARLVNYAFAY**WGQGTLVTV
 SS (SEQ ID NO: 240, with CDRs shown in bold characters) comprising CDR1-H of
 sequence SEQ ID NO: 241, a CDR2-H of sequence SEQ ID NO: 242, and a CDR3-H of
 sequence SEQ ID NO: 243 and

10

- a light chain variable domain consisting of sequence NIVMTQSPKSMISVGDRVMTNCKAS**QTVGN**IAWYQQKPGLSPELLID**YAS**NRYTGV
 PNRFTGGGYGTDFILTINSVQAEDAAFYCY**QRMYSPT**FGGGTKLELK (SEQ ID NO:
 244, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO:
 245, a CDR2-L consisting of sequence 'YAS', and a CDR3-L of sequence SEQ ID NO:
 246.

15

20

The so-called "6C10-C4" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence EVKLQESGPSLVQPSETLSLTCTVS**GFSLT**SYSVHWVRQHSGLSLEWMGR**MWNDGD**
ISYNSAFTSRLSISRDTSKGQVFLKMNSLQTEDTGTYCY**CARGHRT**PFDYWGQGVMT
 VSS (SEQ ID NO: 247, with CDRs shown in bold characters) comprising CDR1-H of
 sequence SEQ ID NO: 248, a CDR2-H of sequence SEQ ID NO: 249, and a CDR3-H of
 sequence SEQ ID NO: 250 and

25

- a light chain variable domain consisting of sequence DIVMTQSPSSLAVSAGETVTINCKSS**QSFLSSG**DERNYVAWYQHKPGQSPKLLIY**WAS**
 TRHSGVPDRFIGSGSGTDFTLTISVQAEDLAIYYC**QQYYDTPLT**FGSGGTKLEIK (SEQ
 ID NO: 251, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ
 ID NO: 252, a CDR2-L consisting of sequence 'WAS', and a CDR3-L of sequence SEQ
 ID NO: 253.

30

The so-called "6D6-B8" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence QVQLQESGPTLVKPGDSVKMSCKAS**AYTFTDNI**HWVKQSHGKSLDWIGY**INPYSGGT** NYNGWFRSKATLTVDKSSSTAYMEFSRLTSDDSAIIYYC**ALNYGSYYAMDAWGQGTS** VTVSS (SEQ ID NO: 254, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 255, a CDR2-H of sequence SEQ ID NO: 200, and a CDR3-H of sequence SEQ ID NO: 201 and

- a light chain variable domain consisting of sequence DIQMTQSPASLSASLGETVTIDCRPS**EDIFNN**LAWYQQKPGNSPQLLIY**DAN**SLADGVP SRFSGSGSGTQYSLMIIRLQSEDVASYFCH**HQYNIYPYT**FGAGTKLELK (SEQ ID NO: 256, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 257, a CDR2-L consisting of sequence 'DAN', and a CDR3-L of sequence SEQ ID NO: 258.

The so-called "8B11-B7" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence QVQLQESGPTLVNPGDSVKMSCKAS**GYTFTDHI**HWVKQSHGKSLEWIGY**INPYSGGA** NYNGKFKSKATLTIDKSSSTAYMEFSRLTSGDSAIIYYC**ALNYGSYYAMDAWGQGSVT** VSS (SEQ ID NO: 259, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 199, a CDR2-H of sequence SEQ ID NO: 260, and a CDR3-H of sequence SEQ ID NO: 201 and

- a light chain variable domain consisting of sequence DIQMTQSPASLSASLGETVTIECRTS**KDIYSN**LAWFQQEPGNPQLLIY**DAS**NLADGVP SRFSGSGSGTQYSLQINNLSQSEDVASYFCH**HQYNNYPYT**FGTGTKLELK (SEQ ID NO: 261, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 262, a CDR2-L consisting of sequence 'DAS', and a CDR3-L of sequence SEQ ID NO: 263.

The so-called "9B8-G6" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence EVKLQESGPSLVQSSQTLSTCTVS**GFSLT****SYHI**HWVRQPPGKGLEWMGV**MWSDGD** **ISYSSALKSRLSISRDT**SQSQVFLKMNSLQTEDTATYYC**ARGDYSSYIYLWFAYWGQG** TLTVSS (SEQ ID NO: 264, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 265, a CDR2-H of sequence SEQ ID NO: 266, and a CDR3-H of sequence SEQ ID NO: 267 and

- a light chain variable domain consisting of sequence DIVMTQSPSSLAVSAGETVTINCKSS**QSFLSSGDERNY**VAWYQHKPGQSPKLLIY**WAS** TRHSGVPDRFIGSGSGTDFTLTISVQAEDLAIYYC**QQYYDTPLT**FGSGTKLEIK (SEQ ID NO: 251, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 252, a CDR2-L consisting of sequence 'WAS', and a CDR3-L of sequence SEQ ID NO: 253.

The so-called "9D7-C8" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence EVKLQESGPSLVQSSQTLSTCTVSS**GFSLT****SYHI**HWVRQTPGKGLEWMGV**MWSDGD** **TSYNSALKSRLSISRDT****SQSQVFLKMNSLQ****TEDTATYYC****ARGYYSSYLYLWFAYWGQ** GTLTVVSS (SEQ ID NO: 268, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 265, a CDR2-H of sequence SEQ ID NO: 266, and a CDR3-H of sequence SEQ ID NO: 269 and
- a light chain variable domain consisting of sequence DIVMTQSPSSLAVSEGETVTINCKSS**QSFLSSGDGKNY**VAWYQYKPGQSPKLLIY**WAS** TRQSGVPDRFIGSGSGTDFTLTISTVQAEDLAIYYC**QQYYDTPLT**FGSGTKLEIK (SEQ ID NO: 270, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 271, a CDR2-L consisting of sequence 'WAS', and a CDR3-L of sequence SEQ ID NO: 253.

The so-called "9F6-G3" anti-CD123 antibody comprises:

- a heavy chain variable domain consisting of sequence QVQLQESGPTLVKPGDSVKMSCKAS**GYTFTDYI**HWVKQSHGKSLEWIGY**INPYSDGT** NYNEKFKSKATLTVDKSTSTAYMEFSRLTSEDSAIYFC**ALNYGSYYAMDA**WGQGTSTV TVSS (SEQ ID NO: 272, with CDRs shown in bold characters) comprising CDR1-H of sequence SEQ ID NO: 273, a CDR2-H of sequence SEQ ID NO: 274, and a CDR3-H of sequence SEQ ID NO: 201 and
- a light chain variable domain consisting of sequence DIQMTQSPASLSASLGETVTIECRPSE**EDIHSN**VAWYQQKPGNSPQLLIY**DAS**NLADGVP SRFSGSGSGTQYSLKINSLQSEDVASYFC**HQYNIYPYT**FGSGTKLELK (SEQ ID NO: 275, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 276, a CDR2-L consisting of sequence 'DAS', and a CDR3-L of sequence SEQ ID NO: 258.

In one aspect of the invention, the anti-CD123 antibody binds to human CD123. In another embodiment, the anti-CD123 antibody further binds to *Macaca fascicularis* CD123. In particular, the anti-CD123 antibody of the invention binds to the extracellular domain of human CD123, or of both, human and *Macaca fascicularis* CD123. More specifically, the anti-CD123 antibody binds to the distal moiety of CD123, for example, to the amino acids at position 19 to 49 of human CD123 of the amino acid sequence SEQ ID NO: 194. The anti-CD123 antibody binds to CD123, indifferently whether expressed in isolated form, or present in a soluble extracellular domain or full-length membrane-anchored CD123 as present in CD123 expressing cells such as AML cells or CD123 transfected cells. The anti-CD123 antibody according to the invention is specific to cells that express human and *Macaca fascicularis* CD123 proteins on their surface, for example CD123 expressing cancer cells.

The anti-CD123 antibody according to the invention has a ratio of affinity for *Macaca fascicularis* CD123 on affinity for human CD123 ($KD(\textit{Macaca fascicularis})/KD(\text{human})$) which is ≤ 10 , in particular ≤ 6 , ≤ 5 , ≤ 4 , ≤ 3 , ≤ 2 , ≤ 1 or ≤ 0.5 . Thus, the polypeptide according to the invention may be used in toxicological studies performed in monkeys the toxicity profile observed in monkeys relevant to anticipate potential adverse effects in humans.

In particular, the anti-CD123 antibody of the invention does not bind to, or does not significantly cross-react with CSF2RA and IL5RA proteins.

In particular, the antibody does not bind to, or does not significantly cross-react with the extracellular domain of the aforementioned human and *Macaca fascicularis* CSF2RA and IL5RA proteins.

Furthermore, the anti-CD123 antibody according to the invention has an affinity (KD) for human CD123 or *Macaca fascicularis* CD123, or both, of $\leq 50\text{nM}$, $\leq 40\text{nM}$, $\leq 30\text{nM}$ for instance $\leq 20\text{nM}$, $\leq 15\text{nM}$, $\leq 10\text{nM}$, $\leq 8\text{nM}$, $\leq 6\text{nM}$, $\leq 4\text{nM}$, $\leq 2\text{nM}$, $\leq 1\text{nM}$ or $\leq 0.5\text{ nM}$, for instance an affinity of 0.1 nM to 20 nM, for example 0.1 nM to 10 nM, in particular of 0.1 nM to 2 nM, or of 0.1 nM to 1 nM.

In one example, affinity for human CD3 or for *Macaca fascicularis* CD3 is determined as the KD value with surface plasmon resonance using recombinant CD123

protein from human and *Macaca fascicularis* as capture antigen, for example with human and *Macaca fascicularis* CD123-Fc fusion protein.

As antigen may be used for example the human or *Macaca fascicularis* CD123 extracellular domain including the signal sequence corresponding to the amino acid sequence from position M1 to R305 of the wild type protein (SEQ ID NO : 194 (human), SEQ ID NO : 195 (*Macaca fascicularis*)). The resulting amino acid sequence for human or *Macaca fascicularis* CD123 mature protein is listed as SEQ ID NO: 196 and SEQ ID NO: 197, respectively. Biacore measurements are known to the skilled in the art. In the present example the Biacore measurement may be performed as described in the section "anti CD3 antibodies" above.

The anti-CD123 antibody of the invention may also have an apparent affinity constant (apparent KD), as, for example, determined by FACS analysis on CD123 expressing cells such as CD123 transfected HEK293 cells using non purified anti-CD123 antibody in hybridoma supernatant, which is $\leq 20\text{nM}$, for instance $\leq 15\text{nM}$, $\leq 10\text{nM}$, $\leq 6\text{nM}$, $\leq 5\text{nM}$, $\leq 4\text{nM}$, $\leq 3\text{nM}$, $\leq 2\text{nM}$ or $\leq 1\text{nM}$. Typically, the apparent KD is within the range 0.1 to 20 nM, in particular 0.1 to 10 nM, for example 0.1 to 5nM.

Alignments of the sequences of the VH and VL regions of the so-called "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies.. The comparison of the CDR-H and CDR-L sequences tends to indicate that, structurally, "1E1-G5", "6D6-B8", "8B11-B7" and "9F6-G3" anti-CD123 antibodies, on one hand, and "6C10-C4", 9B8-G6", "9D7-C8" anti-CD123 antibodies, on the other hand, are closely related, said antibodies probably binding to the same epitope. The alignment of the so-called "1E1-G5", "6D6-B8", "8B11-B7" and "9F6-G3" anti-CD123 antibodies and "6C10-C4", 9B8-G6", "9D7-C8" anti-CD123 antibodies are shown in figures 3 and 4, respectively. The comparison of the CDR-H and CDR-L sequences further identifies CDR positions that are strictly conserved between the two groups of antibodies and which are thus assumed to be important for specificity, whereas other positions could support substitution.

Accordingly, the antibody according to the invention comprises:

- a) a heavy chain variable domain comprising a CDR1-H consisting of sequence $X_1\text{YTFTDX}_2\text{I}$ (SEQ ID NO: 336) wherein X_1 is G or A and X_2 is H, Y or N, or any combination thereof; and

- a CDR2-H consisting of sequence INPYSX₁GX₂ (SEQ ID NO: 337) wherein X₁ is G or D and X₂ is T or A, or any combination thereof; and
- a CDR3-H consisting of sequence ALNYGSYYAMDA (SEQ ID NO 201), and
- a light chain variable domain comprising a CDR1-L consisting of sequence X₁DIX₂X₃N (SEQ ID NO: 338) wherein X₁ is E or K, X₂ is F, H or Y and X₃ is N or S, or any combination thereof; and
- a CDR2-L consisting of sequence 'DAN' or 'DAS'; and
- a CDR3-L consisting of sequence X₁QYNX₂YPYT (SEQ ID NO: 339) wherein X₁ is H or Q and X₂ is I, K or N, or any combination thereof; or
- b) a heavy chain variable domain comprising a CDR1-H consisting of sequence GFSLTSYX₁ (SEQ ID NO: 340) wherein X₁ is H or S; and
- a CDR2-H consisting of sequence MWX₁DGDT (SEQ ID NO: 341) wherein X₁ is S or N; and
- a CDR3-H consisting of sequence ARGX₁X₂X₃X₄X₅X₆X₇X₈X₉FX₁₀Y (SEQ ID NO: 342) wherein X₁ is D, Y or H, X₂ is Y or R, X₃ is S or T, X₄ is S or P, X₅ is Y or no amino acid, X₆ is L, I or no amino acid, X₇ is Y or no amino acid, X₈ is L or no amino acid, X₉ is W or no amino acid, X₁₀ is A or D, or any combination thereof, and
- a light chain variable domain comprising a CDR1-L consisting of sequence QSFLSSGD₁X₂NY (SEQ ID NO: 343) wherein X₁ is E or G and X₂ is R or K, or any combination thereof; and
- a CDR2-L consisting of sequence 'WAS'; and
- a CDR3-L consisting of sequence QQYYDTPLT (SEQ ID NO: 253).
- According to an embodiment, the anti-CD123 antibody according to the invention comprises the CDR sequences of the heavy and/or light chains of one of 13 so-called "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", and "9F6-G3" anti-CD123 antibodies listed above.
- Therefore, the invention relates to an anti-CD123 antibody, which comprises:
- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 227 or a sequence differing from SEQ ID NO: 227 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 228 or SEQ ID NO: 353 or SEQ ID NO: 279 or a sequence differing from SEQ ID NO: 228 or SEQ ID NO:

- 353 or SEQ ID NO: 279 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 229 or a sequence differing from SEQ ID NO: 229 by one amino acid substitution; and
- 5 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 231 or a sequence differing from SEQ ID NO: 231 by one amino acid substitution; CDR2-L of sequence 'RDD' or a sequence differing from 'RDD' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 232 or a sequence differing from SEQ ID NO: 232 by one amino acid substitution; or
- 10 b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 199 or a sequence differing from SEQ ID NO: 199 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 200 or a sequence differing from SEQ ID NO: 200 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 201 or a sequence differing from SEQ ID NO: 201 by one amino acid substitution; and
- 15 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 203 or a sequence differing from SEQ ID NO: 203 by one amino acid substitution; CDR2-L of sequence 'DAN' or a sequence differing from 'DAN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 204 or a sequence differing from SEQ ID NO: 204 by one amino acid substitution; or
- 20 c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 206 or a sequence differing from SEQ ID NO: 206 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 207 or a sequence differing from SEQ ID NO: 207 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 208 or a sequence differing from SEQ ID NO: 208 by one amino acid substitution; and
- 25 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 210 or a sequence differing from SEQ ID NO: 210 by one amino acid substitution; CDR2-L of sequence 'ETS' or a sequence differing from 'ETS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 211 or a sequence differing from SEQ ID NO: 211 by one amino acid substitution; or
- 30 d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 213 or a sequence differing from SEQ ID NO: 213 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 214 or a sequence differing from SEQ ID NO: 214 by one or more amino acid substitutions; CDR3-H of

sequence SEQ ID NO: 215 or a sequence differing from SEQ ID NO: 215 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 217 or a sequence differing from SEQ ID NO: 217 by one amino acid substitution; CDR2-L of sequence 'NTN' or a sequence differing from 'NTN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 218 or a sequence differing from SEQ ID NO: 218 by one amino acid substitution; or

e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 220 or a sequence differing from SEQ ID NO: 220 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 221 or a sequence differing from SEQ ID NO: 221 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 222 or a sequence differing from SEQ ID NO: 222 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 224 or a sequence differing from SEQ ID NO: 224 by one amino acid substitution; CDR2-L of sequence 'RVS' or a sequence differing from 'RVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 225 or a sequence differing from SEQ ID NO: 225 by one amino acid substitution; or

f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 234 or a sequence differing from SEQ ID NO: 234 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 235 or a sequence differing from SEQ ID NO: 235 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 236 or a sequence differing from SEQ ID NO: 236 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 238 or a sequence differing from SEQ ID NO: 238 by one amino acid substitution; CDR2-L of sequence 'GAS' or a sequence differing from 'GAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 239 or a sequence differing from SEQ ID NO: 239 by one amino acid substitution; or

g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 241 or a sequence differing from SEQ ID NO: 241 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 242 or a sequence differing from SEQ ID NO: 242 by one or more amino acid substitutions; CDR3-H of

sequence SEQ ID NO: 243 or a sequence differing from SEQ ID NO: 243 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 245 or a sequence differing from SEQ ID NO: 245 by one amino acid substitution; CDR2-L of sequence 'YAS' or a sequence differing from 'YAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 246 or a sequence differing from SEQ ID NO: 246 by one amino acid substitution; or

h) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 248 or a sequence differing from SEQ ID NO: 248 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 249 or a sequence differing from SEQ ID NO: 249 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 250 or a sequence differing from SEQ ID NO: 250 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 252 or a sequence differing from SEQ ID NO: 252 by one amino acid substitution; CDR2-L of sequence 'WAS' or a sequence differing from 'WAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 253 or a sequence differing from SEQ ID NO: 253 by one amino acid substitution; or

i) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 255 or a sequence differing from SEQ ID NO: 255 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 200 or a sequence differing from SEQ ID NO: 200 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 201 or a sequence differing from SEQ ID NO: 201 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 257 or a sequence differing from SEQ ID NO: 257 by one amino acid substitution; CDR2-L of sequence 'DAN' or a sequence differing from 'DAN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 258 or a sequence differing from SEQ ID NO: 258 by one amino acid substitution; or

j) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 199 or a sequence differing from SEQ ID NO: 199 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 260 or a sequence differing from SEQ ID NO: 260 by one or more amino acid substitutions; CDR3-H of

sequence SEQ ID NO: 201 or a sequence differing from SEQ ID NO: 201 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 262 or a sequence differing from SEQ ID NO: 262 by one amino acid substitution; CDR2-L of sequence 'DAS' or a sequence differing from 'DAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 263 or a sequence differing from SEQ ID NO: 263 by one amino acid substitution; or

k) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 265 or a sequence differing from SEQ ID NO: 265 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 266 or a sequence differing from SEQ ID NO: 266 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 267 or a sequence differing from SEQ ID NO: 267 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 252 or a sequence differing from SEQ ID NO: 252 by one amino acid substitution; CDR2-L of sequence 'WAS' or a sequence differing from 'WAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 253 or a sequence differing from SEQ ID NO: 253 by one amino acid substitution; or

l) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 265 or a sequence differing from SEQ ID NO: 265 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 266 or a sequence differing from SEQ ID NO: 266 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 269 or a sequence differing from SEQ ID NO: 269 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 271 or a sequence differing from SEQ ID NO: 271 by one amino acid substitution; CDR2-L of sequence 'WAS' or a sequence differing from 'WAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 253 or a sequence differing from SEQ ID NO: 253 by one amino acid substitution; or

m) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 273 or a sequence differing from SEQ ID NO: 273 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 274 or a sequence differing from SEQ ID NO: 274 by one or more amino acid substitutions; CDR3-H of

sequence SEQ ID NO: 201 or a sequence differing from SEQ ID NO: 201 by one amino acid substitution; and

5 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 276 or a sequence differing from SEQ ID NO: 276 by one amino acid substitution; CDR2-L of sequence 'DAS' or a sequence differing from 'DAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 258 or a sequence differing from SEQ ID NO: 258 by one amino acid substitution.

10 One or more individual amino acids may be altered by substitution, in particular by conservative substitution, in one or more of the above CDR sequences. Such an alteration may be intended for example to remove a glycosylation site or a deamidation site, in connection with humanization of the antibody.

15 Based on the alignments of the sequences of the VH and VL regions of "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies different amino acid substitutions were identified. Therefore, in one embodiment, an amino acid may be substituted:

- in CDR1-H at one or more of positions 1 and 7, for instance at position 7 of CDR1-H of sequence GYTFTDHI (SEQ ID NO:199) or GYTFTDYI (SEQ ID NO:273) for instance at position 1 and 5 of CDR1-H of sequence AYTFTDNI (SEQ ID NO: 255); and/or
- 20 - in CDR2-H, at one or more of positions 6 and 8, for instance at positions 6 and/or 8 of CDR2-H of sequence INPYSGGT (SEQ ID NO: 200) or INPYSDGT (SEQ ID NO: 274) or INPYSGGA (SEQ ID NO : 260); and/or
- in CDR1-L, at one or more of positions 1, 4 and 5, for example at position 1 or 1, 4 and 5 of CDR1-L of sequence KDIYSN (SEQ ID NO : 262) or EDIFNN (SEQ ID
- 25 NO: 257); and/or
- in CDR2-L, at positions 3 of sequence 'DAN' or 'DAS'; and/or
- in CDR3-L, at one or more of positions 1 and 5, for instance at position 1 and/or 5 of CDR3-L of sequence HQYNIYPYT (SEQ ID NO: 258) or QQYNKYPYT (SEQ ID NO: 204) or HQYNNYPYT (SEQ ID NO : 263).

30 In a further embodiment, an amino acid is substituted:

- in CDR1-H at position 8, for instance at position 8 of CDR1-H of sequence GFSLTSYH (SEQ ID NO:265) or GFSLTSYS (SEQ ID NO: 248); and/or
- in CDR2-H at positions 3, for instance at positions 3 of CDR2-H of sequence MWSDGD (SEQ ID NO: 265) or MWNDGD (SEQ ID NO: 248); and/or

- in CDR3-H at positions 4 and 9, for instance at positions 4 and 9 of CDR3-H of sequence ARGDYSSYIYLWFAY (SEQ ID NO : 267) or ARGYYSSYLYLWFAY(SEQ ID NO : 269); and/or
- in CDR1-L, at one or more of positions 9 and 10, for example at position 9 and 10 of CDR1-L of sequence QSFLSSGDERNY (SEQ ID NO: 252) or QSFLSSGDGKNY (SEQ ID NO: 271).

According to an embodiment, the antibody comprises

- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 227, CDR2-H of sequence SEQ ID NO: 228, SEQ ID NO:353, SEQ ID NO:279, CDR3-H of sequence SEQ ID NO: 229 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 231, CDR2-L of sequence 'RDD' and CDR3-L of sequence SEQ ID NO: 232;
or
- n) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 199, CDR2-H of sequence SEQ ID NO: 200, CDR3-H of sequence SEQ ID NO: 201 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 203, CDR2-L of sequence 'DAN' and CDR3-L of sequence SEQ ID NO: 204;
or
- o) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 206, CDR2-H of sequence SEQ ID NO: 207, CDR3-H of sequence SEQ ID NO: 208 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 210, CDR2-L of sequence 'ETS' and CDR3-L of sequence SEQ ID NO: 211; or
- p) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 213, CDR2-H of sequence SEQ ID NO: 214, CDR3-H of sequence SEQ ID NO: 215 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 217, CDR2-L of sequence 'NTN' and CDR3-L of sequence SEQ ID NO: 218;
or
- q) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 220, CDR2-H of sequence SEQ ID NO: 221, CDR3-H of sequence SEQ ID NO: 222 , and

- a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 224, CDR2-L of sequence 'RVS' and CDR3-L of sequence SEQ ID NO: 225;
or
- 5 r) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 234, CDR2-H of sequence SEQ ID NO: 235, CDR3-H of sequence SEQ ID NO: 236 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 238, CDR2-L of sequence 'GAS' and CDR3-L of sequence SEQ ID NO: 239;
or
- 10 s) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 241, CDR2-H of sequence SEQ ID NO: 242, CDR3-H of sequence SEQ ID NO: 243 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 245, CDR2-L of sequence 'YAS' and CDR3-L of sequence SEQ ID NO: 246;
or
- 15 t) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 248, CDR2-H of sequence SEQ ID NO: 249, CDR3-H of sequence SEQ ID NO: 250 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 252, CDR2-L of sequence 'WAS' and CDR3-L of sequence SEQ ID NO: 253;
or
- 20 u) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 255, CDR2-H of sequence SEQ ID NO: 200, CDR3-H of sequence SEQ ID NO: 201 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 257, CDR2-L of sequence 'DAN' and CDR3-L of sequence SEQ ID NO: 258;
or
- 25 v) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 199, CDR2-H of sequence SEQ ID NO: 260, CDR3-H of sequence SEQ ID NO: 201 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 262, CDR2-L of sequence 'DAS' and CDR3-L of sequence SEQ ID NO: 263;
or
- 30

- w) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 265, CDR2-H of sequence SEQ ID NO: 266, CDR3-H of sequence SEQ ID NO: 267 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 252, CDR2-L of sequence 'WAS' and CDR3-L of sequence SEQ ID NO: 253;
or
- x) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 265, CDR2-H of sequence SEQ ID NO: 266, CDR3-H of sequence SEQ ID NO: 269 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 271, CDR2-L of sequence 'WAS' and CDR3-L of sequence SEQ ID NO: 253;
or
- y) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 273, CDR2-H of sequence SEQ ID NO: 274, CDR3-H of sequence SEQ ID NO: 201 , and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 276, CDR2-L of sequence 'DAS' and CDR3-L of sequence SEQ ID NO: 258.

The anti-CD123 antibody according to the invention is in particular a conventional antibody, in particular a conventional monoclonal antibody, or an antibody fragment, a bispecific or multispecific antibody.

The anti-CD123 antibody according to the invention in particular comprises or consists of an IgG, or a fragment thereof.

The invention also provides an anti-CD123 antibody as defined above further comprising at least the heavy chain variable domain and/or the light chain variable domain of one of the so-called anti-CD123 antibodies listed above.

Thus, the invention relates in particular to an anti-CD123 antibody, which comprises:

- 5 a) a heavy chain variable domain of sequence SEQ ID NO: 226 or SEQ ID NO: 277 or SEQ ID NO: 278 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 230, or a sequence at least 85% identical thereto; or
- b) a heavy chain variable domain of sequence SEQ ID NO: 198 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 202, or a sequence at least 85% identical thereto; or
- 10 c) a heavy chain variable domain of sequence SEQ ID NO: 205 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 209, or a sequence at least 85% identical thereto; or
- d) a heavy chain variable domain of sequence SEQ ID NO: 212 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 216, or a sequence at least 85% identical thereto; or
- 15 e) a heavy chain variable domain of sequence SEQ ID NO: 219 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 223, or a sequence at least 85% identical thereto; or
- f) a heavy chain variable domain of sequence SEQ ID NO: 233 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 237, or a sequence at least 85% identical thereto; or
- 20 g) a heavy chain variable domain of sequence SEQ ID NO: 240 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 244, or a sequence at least 85% identical thereto; or
- 25 h) a heavy chain variable domain of sequence SEQ ID NO: 247 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 251, or a sequence at least 85% identical thereto; or
- i) a heavy chain variable domain of sequence SEQ ID NO: 254 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 256, or a sequence at least 85% identical thereto; or
- 30 j) a heavy chain variable domain of sequence SEQ ID NO: 259 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 261, or a sequence at least 85% identical thereto; or

- k) a heavy chain variable domain of sequence SEQ ID NO: 264 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 251, or a sequence at least 85% identical thereto; or
- l) a heavy chain variable domain of sequence SEQ ID NO: 268 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 270, or a sequence at least 85% identical thereto; or
- m) a heavy chain variable domain of sequence SEQ ID NO: 272 or a sequence at least 85% identical thereto, and/or a light chain variable domain of sequence SEQ ID NO: 275, or a sequence at least 85% identical thereto.

For instance, the sequence of the variable domain of heavy or light chain may differ from the reference sequence SEQ ID NO: 226, 277, 278, 230, 198, 202, 205, 209, 212, 216, 219, 223, 233, 237, 240, 244, 247, 251, 254, 256, 259, 261, 264, 251, 268, 270, 272 or 275, as appropriate, by one or more amino acid substitution(s), in particular by one or more conservative amino acid substitution(s) and/or substitution(s) with canonical residues. In particular, the sequence of the variable domain of heavy or light chain may differ from the reference sequence SEQ ID NO: 226, 277, 278, 230, 198, 202, 205, 209, 212, 216, 219, 223, 233, 237, 240, 244, 247, 251, 254, 256, 259, 261, 264, 251, 268, 270, 272 or 275 by conservative amino acid substitution(s), only.

The sequence alterations as compared with sequence SEQ ID NO: 226, 277, 278, 230, 198, 202, 205, 209, 212, 216, 219, 223, 233, 237, 240, 244, 247, 251, 254, 256, 259, 261, 264, 251, 268, 270, 272 or 275 will in particular be present essentially in one or more of the framework regions, FR1-L, FR2-L, FR3-L, FR4-L and/or FR1-H, FR2-H, FR3-H, FR4-H.

In one embodiment, the anti-CD123 antibody of the invention and a fragment thereof is, respectively, a rat antibody and a fragment of a rat antibody.

The anti-CD123 antibody of the invention may also be a chimeric antibody, and in particular a rat/human antibody, e.g. an antibody comprising rat variable domains of heavy and light chains and a CH domain and a CL domain from a human antibody. The polypeptide may be a fragment of such an antibody. The anti-CD123 antibody may also be a humanized antibody or a fragment of a humanized antibody obtained by CDR-grafting or by the 4D method (US20110027266).

Accordingly, in one embodiment, the anti-CD123 antibody of the invention is a humanized antibody comprising :

- a) a heavy chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 280, SEQ ID NO: 281, SEQ ID NO: 282, SEQ ID NO: 283, SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 288, SEQ ID NO: 289, SEQ ID NO: 290, SEQ ID NO: 291, SEQ ID NO: 301 and SEQ ID NO: 302; and
- b) a light chain variable domain comprising of an amino acid sequence selected from the group consisting of SEQ ID NO: 292, SEQ ID NO: 293, SEQ ID NO: 294, SEQ ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299, SEQ ID NO: 300, SEQ ID NO: 303, SEQ ID NO: 304 and SEQ ID NO: 305.

In one embodiment, the anti-CD123 antibody according to the invention comprises the three CDR sequences or the variable domain of the heavy chain, or the six CDR sequences or variable domains of the heavy and light chains of one of so-called anti-CD123 antibodies listed above.

The invention further refers to a fragment of the humanized anti-CD123 antibody as defined above. In one embodiment, the humanized anti-CD123 antibody described above is a chimeric antibody.

The anti-CD123 antibody according to the invention may also be a single domain antibody or a fragment thereof. In particular, a single domain antibody fragment may consist of a variable heavy chain (VHH) which comprises the CDR1-H, CDR2-H and CDR3-H of one of the antibodies described above. The CD123-antibody may also be a heavy chain antibody, i.e. an antibody devoid of light chain, which may or may not contain a CH1 domain.

The single domain antibody or a fragment thereof may also comprise the framework regions of a camelid single domain antibody, and optionally the constant domain of a camelid single domain antibody.

The anti-CD123 antibody according to the invention may also be an antibody fragment, in particular a humanized antibody fragment, selected from the group consisting of Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, and diabodies.

The CD123-antibody may also be a bispecific or multispecific antibody formed from at least one antibody fragment or at least one variable domains of the anti-CD123

antibody of the invention. Multispecific antibodies are polyvalent protein complexes as described for instance in EP 2 050 764 A1 or US 2005/0003403 A1.

The bispecific or multispecific CD123-antibodies according to the invention can have specificity for (a) extracellular domain of human or human and *Macaca fascicularis* CD123 targeted by one of the above described anti-CD123 antibodies and (b) at least one other antigen.

In a particular embodiment, the other antigen is CD3 and accordingly the resulting bispecific antibody is a CD3/CD123 bispecific antibody. Conventional bi-specific antibodies can be produced by techniques that are known to the skilled in the art.

Antibodies and fragments thereof according to the invention can be used in an isolated (e.g., purified) form or contained in a vector, such as a membrane or lipid vesicle (e.g. a liposome).

In one further embodiment, the anti-CD123 antibody of the invention is used for the preparation of antibody-like binding protein of the invention further defined in the section "antibody-like binding protein".

Any combination of the above embodiments makes part of the invention.

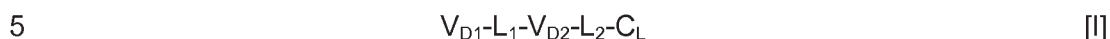
Antibody-like binding proteins

The inventors have generated several antibody-like binding proteins the so called "7G3x20G6", "7G3x4E7", "7G3x4B4", "7G3x18F5", "hz20G6x7G3", "7G3xhz4B4", "hz4B4x3E3", "hz20G6x7G3-TL4" and "hz20G6xhz7G3" antibody-like binding proteins, wherein the term "hz" denotes humanized antibodies. These antibody-like binding proteins have a CODV design, in particular a CODV-Fab or CODV-Ig design.

The "CODV format" in context of the present invention refers to the cross-over dual variable (CODV) configuration of bispecific antibodies or multispecific antibodies. The CODV format allows an interchangeability of variable domains with retention of folding and ultimate binding affinity.

The CODV format has been previously described in the international patent application WO2012/135345. Accordingly, in one embodiment, the antibody-like binding protein of the invention is in the CODV format as previously described in the international patent application WO2012/135345, which is incorporated herein by reference.

In one embodiment, the invention refers to an antibody-like binding protein in the CODV-Fab format. Accordingly, in one embodiment, the invention refers to an antibody-like binding protein comprising two polypeptide chains that form two antigen-binding sites, wherein a first polypeptide has a structure represented by the formula [I]:



and a second polypeptide has a structure represented by the formula [II]:



wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

10 V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

15 L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the first and the second polypeptide form a cross-over light chain-heavy chain pair, and

wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains

20 if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.

Adding a Fc domain to the antibody-like binding protein in the CODV-Fab further stabilizes the antibody-like binding protein. More precisely, adding a Fc domain to the polypeptide of formula (II) of antibody-like binding protein in the CODV-Fab increases the half-life of the antibody-like binding protein and thus improves the pharmacokinetic profile of the antibody-like binding protein. Adding one Fc region to the CODV-Fab results in dimerization of polypeptides containing the Fc domain and the resulting antibody-like binding protein is an antibody-like binding protein in the CODV-Ig format.

30 The invention therefore further refers to an antibody like binding protein in the CODV-Ig format.

Therefore, the invention further refers to an antibody-like binding protein comprising four polypeptide chains that form four antigen-binding sites, wherein two polypeptide chains have a structure represented by the formula [I]:



and two polypeptide chains have a structure represented by the formula [III]:



wherein:

- 5 V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;
 V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;
 V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;
 V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;
 C_L is a light chain constant domain of an immunoglobulin;
 10 C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;
 F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;
 L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the polypeptides of formula I and the polypeptides of formula III form a
 15 cross-over light chain-heavy chain pair, and

wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.
 20

In said CODV-Ig format, the two polypeptide chains having a structure represented by the formula [III] dimerize through their F_c domains.

In a further embodiment, a first F_c domain is added to the polypeptide of formula
 25 [II] of antibody-like binding protein CODV-Fab, and a second F_c domain (called F_{c2}) is added to the polypeptide of formula [I] of the antibody-like binding protein CODV-Fab. Furthermore, in the same embodiment a linker L_5 is present between C_L and the F_{c2} domain of the polypeptide chains of formula [I] resulting in the polypeptide chains of formula [IV].

30 Accordingly, the invention further refers to an antibody-like binding protein comprising two polypeptide chains that form two antigen-binding sites, wherein one polypeptide chain has a structure represented by the formula [IV]:



and one polypeptide chain has a structure represented by the formula [III]:



wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

5 V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

10 F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

F_{c2} is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

L_1 , L_2 , L_3 , L_4 and L_5 are amino acid linkers;

15 and wherein the polypeptide of formula [IV] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair, and

20 wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.

This CODV format, in which the polypeptide chains represented by the formulae [III] and [IV] dimerize through their respective F_{c2} and F_c regions, is herein called CODV-Fab-TL.

25 In another embodiment of the CODV-Fab, a first F_c domain is added to the polypeptide chain represented by formula [II] (resulting in formula [III]), and the antibody-like binding protein comprise a third polypeptide chain comprising, or consisting of a second F_c domain (called F_{c3}).

30 The invention further refers to an antibody-like binding protein which comprises three polypeptide chains that form two antigen-binding sites, wherein

a first polypeptide has a structure represented by the formula [I]:



a second polypeptide has a structure represented by the formula [III]:



a third polypeptide F_{c3} (also called Fc stump") which is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

wherein

- 5 V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;
- V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;
- V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;
- V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;
- C_L is a light chain constant domain of an immunoglobulin;
- 10 C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;
- F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;
- L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair, and

- 15 wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains;
- 20

and wherein the polypeptide of formula [III] heterodimerizes with the third polypeptide through its F_c domain.

- Accordingly, in said embodiment, the so-called "Fc stump" (F_{c3}) heterodimerizes with the Fc region of the polypeptide according to formula III. This CODV format is
- 25 herein called CODV-Fab-OL. This construct avoids that the CODV-Fab form aggregates.

- In one embodiment of the CODV-Fab-OL, both F_c and F_{c3} are immunoglobulin variants in which the CH_3 domain has been modified: each of F_c and F_{c3} has been
- 30 genetically engineered at the CH_3 - CH_3 interface to promote heteromultimer formation according to the so-called "Knob-into-Hole" technology which has been described in patents US5731168 and US8216805, notably, and which are herein incorporated by reference.

Accordingly, in an embodiment the CH3 domain of one of F_c and F_{c3} contains the mutations Y349C, T366S, L368A, and Y407V, while the CH3 domain of the other of F_c and F_{c3} contains the mutations S354C and T366W (amino acid position being indicated by reference to an IgG1 sequence).

- 5 Examples of suitable F_c and F_{c3} pairs include the pair SEQ ID NO: 396 (F_c) and SEQ ID NO: 397 (F_{c3}), and the pair SEQ ID NO: 394 (F_c) and SEQ ID NO: 398 (F_{c3}).

10 In one embodiment of the invention, the first immunoglobulin or the second immunoglobulin is one anti-CD3 antibody as defined in the section «anti-CD3 antibodies» above.

 In another embodiment of the invention, the first immunoglobulin or the second immunoglobulin is one anti-CD123 antibody as defined in the section «anti-CD123 antibodies» above.

15 According to one embodiment of the invention, V_{D1} and V_{D2} of polypeptide of formula I or formula [IV] are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} of polypeptide II or III are both variable domains of heavy chains or of light chains. This interchangeability is also referred to as "swapability" and thus determines the cross-over dual variable (CODV) configuration of
20 the antibody-like binding proteins of the invention.

 According to the above definition, V_{D1} and V_{D4} are variable domains of heavy or light chain of a first immunoglobulin and V_{D2} and V_{D3} are variable domains of heavy or light chain of a second immunoglobulin, V_{D1} and V_{D4} are therefore to be considered as cognate domains as well as V_{D2} and V_{D3} .

25 Accordingly, the term "cross-over" refers to the swapped alignment of V_{D1} or V_{D2} of polypeptide of formula [I] or formula [IV] with respect to its cognate variable domain V_{D4} or V_{D3} of polypeptide of formula [II] or formula [III].

30 In one particular embodiment, V_{D1} and V_{D2} are light chain variable domains and V_{D3} and V_{D4} are heavy chain variable domains.

 The antibody-like binding proteins of the invention may be prepared using domains or sequences obtained or derived from any human or non-human antibody, including, for example, human, rat, or humanized antibodies.

In one embodiment, the immunoglobulin is an IgG immunoglobulin.

Accordingly, in one embodiment, C_L is a light chain constant domain of an IgG immunoglobulin. In a further embodiment, C_{H1} is a C_{H1} heavy chain constant domain of an IgG immunoglobulin.

5 In one embodiment, the antibody-like binding protein of the invention may be prepared using domains or sequences of the anti-CD3 antibody and anti-CD123 antibody herein described.

The term "linker" as used herein refers to one or more amino acid residues inserted between immunoglobulin domains to provide sufficient mobility for the domains
 10 of the light and heavy chains to fold into cross over dual variable region immunoglobulins. In some embodiments, a linker consists of 0 amino acid meaning that the linker is absent. A linker is inserted at the transition between variable domains or between variable and constant domains, respectively, at the sequence level. The transition between domains can be identified because the approximate size of the
 15 immunoglobulin domains is well understood. The precise location of a domain transition can be determined by locating peptide stretches that do not form secondary structural elements such as beta-sheets or alpha-helices as demonstrated by experimental data or as can be assumed by techniques of modeling or secondary structure prediction. The linkers described in context of the invention are the linkers L_1 , L_2 , L_3 , L_4 and L_5 . L_1 is
 20 located between the N-terminal V_{D1} domain and the V_{D2} domain; L_2 is located between the V_{D2} and the C-terminal C_L domain. The linkers L_3 and L_4 are located on polypeptide as defined according to formula II or III of the antibody-like-proteins. More precisely, L_3 is located between the N-terminal V_{D3} and the V_{D4} domains and L_4 is located between the V_{D4} and the C-terminal C_{H1} -Fc domains. L_5 is located between C_L and the N-terminal
 25 F_{c2} . The linkers L_1 , L_2 , L_3 , L_4 and L_5 are independent, but in some embodiments, they have the same sequence and/or length.

In some antibody-like binding proteins of the invention, the length of L_3 is at least twice the length of L_1 . In other antibody-like binding proteins of the invention, the length of L_4 is at least twice the length of L_2 . In some antibody-like binding proteins of the
 30 invention, the length of L_1 is at least twice the length of L_3 . In other antibody-like binding proteins of the invention, the length of L_2 is at least twice the length of L_4 .

In one embodiment, the linker L_1 , L_2 , L_3 and L_4 comprise 0 to 20 amino acids. In one embodiment, L_5 comprises 0 to 10 amino acids.

In some antibody-like binding proteins of the invention, L_1 is 3 to 12 amino acid residues in length, L_2 is 3 to 14 amino acid residues in length, L_3 is 1 to 8 amino acid residues in length, and L_4 is 1 to 3 amino acid residues in length. In other antibody-like binding proteins, L_1 is 5 to 10 amino acid residues in length, L_2 is 5 to 8 amino acid residues in length, L_3 is 1 to 5 amino acid residues in length, and L_4 is 1 to 2 amino acid residues in length. In a preferred antibody-like binding protein, L_1 is 7 amino acid residues in length, L_2 is 5 amino acid residues in length, L_3 is 1 amino acid residues in length, and L_4 is 2 amino acid residues in length.

In some antibody-like binding proteins of the invention, L_1 is 1 to 3 amino acid residues in length, L_2 is 1 to 4 amino acid residues in length, L_3 is 2 to 15 amino acid residues in length, and L_4 is 2 to 15 amino acid residues in length. In other antibody-like binding proteins, L_1 is 1 to 2 amino acid residues in length, L_2 is 1 to 2 amino acid residues in length, L_3 is 4 to 12 amino acid residues in length, and L_4 is 2 to 12 amino acid residues in length. In a preferred antibody-like binding protein, L_1 is 1 amino acid residue in length, L_2 is 2 amino acid residues in length, L_3 is 7 amino acid residues in length, and L_4 is 5 amino acid residues in length.

In some antibody-like binding proteins of the invention, L_1 , L_3 , or L_4 may be equal to zero. However, in antibody-like binding proteins wherein L_3 , or L_4 is equal to zero, the corresponding transition linker between the variable region and constant region or between the dual variable domains on the other chain cannot be zero. In some embodiments, L_1 is equal to zero and L_3 is 2 or more amino acid residues, L_3 is equal to zero and L_1 is equal to 1 or more amino acid residues, or L_4 is equal to 0 and L_2 is 3 or more amino acid residues.

In some antibody-like binding proteins of the invention, at least one of the linkers selected from the group consisting of L_2 , L_3 , and L_4 contains at least one cysteine residue.

Examples of suitable linkers include a single glycine, threonine or serine residue; a dipeptide such as a diglycine peptide, histidine-threonine peptide or glycine-serine dipeptide; a tripeptide with three glycines, the tripeptide Thr-His-Thr, the tripeptide Gly-Gly-Ser; a peptide with four glycine residues; a peptide with five glycine residues; a peptide with six glycine residues; a peptide with seven glycine residues; a peptide with eight glycine residues. Other combinations of amino acid residues may be used such as the peptide Gly-Gly-Gly-Ser (SEQ ID NO: 354), the peptide Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 344), the peptide Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 355), the peptide Gly-

Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 356), the peptide Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 357), the peptide Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 358), and the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 345). Other suitable linkers include a single Ser, and Val residue; the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Lys-Thr-His-Thr (SEQ ID NO: 359); Lys-Thr-His-Thr-Ser (SEQ ID NO: 360); Asp-Lys-Thr-His-Thr-Ser (SEQ ID NO: 361); Asp-Lys-Thr-His-Thr-Ser-Pro (SEQ ID NO: 362); Ser-Asp-Lys-Thr-His-Thr-Ser-Pro (SEQ ID NO: 363); Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro (SEQ ID NO: 364); Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser (SEQ ID NO: 365); Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser (SEQ ID NO: 366); Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro (SEQ ID NO: 367); Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro (SEQ ID NO: 368); Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly (SEQ ID NO: 369); Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly (SEQ ID NO: 370); Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly (SEQ ID NO: 371); Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly (SEQ ID NO: 372); Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly (SEQ ID NO: 373); Gly-Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly (SEQ ID NO: 374); Thr-Val-Ala-Ala-Pro (SEQ ID NO: 346), Gln-Pro-Lys-Ala-Ala (SEQ ID NO: 347), Gln-Arg-Ile-Glu-Gly (SEQ ID NO: 348); Ala-Ser-Thr-Lys-Gly-Pro-Ser (SEQ ID NO: 349), Arg-Thr-Val-Ala-Ala-Pro-Ser (SEQ ID NO: 350), Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 307), Thr-Lys-Gly-Pro-Ser (SEQ ID NO: 309), His-Ile-Asp-Ser-Pro-Asn-Lys (SEQ ID NO: 351), and Gly-Gly-Ser-Gly-Ser-Ser-Gly-Ser-Gly-Gly (SEQ ID NO: 389). The examples listed above are not intended to limit the scope of the invention in any way, and linkers comprising randomly selected amino acids selected from the group consisting of valine, leucine, isoleucine, serine, threonine, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, glycine, and proline have been shown to be suitable in the antibody-like binding proteins of the invention.

The identity and sequence of amino acid residues in the linker may vary depending on the type of secondary structural element necessary to achieve in the linker. For example, glycine, serine, and alanine are best for linkers having maximum flexibility. Some combination of glycine, proline, threonine, and serine are useful if a more rigid and extended linker is necessary. Any amino acid residue may be considered

as a linker in combination with other amino acid residues to construct larger peptide linkers as necessary depending on the desired properties.

In one embodiment, the linker L₁ is of sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 307), the linker L₂ is of sequence Thr-Lys-Gly-Pro-Ser (SEQ ID NO: 309),
 5 the linker L₃ is of sequence 'S' and the linker L₄ is of sequence 'RT'.

In a further embodiment, the sequences of linkers L₁, L₂, L₃, and L₄ are selected from the group consisting of threonine; a dipeptide such as a histidine-threonine peptide; the tripeptide Thr-His-Thr, Lys-Thr-His-Thr (SEQ ID NO: 359); Lys-Thr-His-Thr-Ser (SEQ ID NO: 360); Asp-Lys-Thr-His-Thr-Ser (SEQ ID NO: 361); Asp-Lys-Thr-His-Thr-
 10 Ser-Pro (SEQ ID NO: 362); Ser-Asp-Lys-Thr-His-Thr-Ser-Pro (SEQ ID NO: 363); Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro (SEQ ID NO: 364); Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser (SEQ ID NO: 365); Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser (SEQ ID NO: 366); Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro (SEQ ID NO: 367); Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro (SEQ ID NO: 368);
 15 Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly (SEQ ID NO: 369); Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly (SEQ ID NO: 370); Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly (SEQ ID NO: 371); Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly (SEQ ID NO: 372); Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly (SEQ ID NO: 373) and Gly-Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly (SEQ ID NO: 374). In one embodiment the sequence of linker L₅ is selected from the group consisting of a single serine residue, a dipeptide such as a glycine-serine dipeptide; a tripeptide Gly-Gly-Ser, the peptide Gly-Gly-Gly-Ser (SEQ ID NO: 354), the peptide Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 344), the peptide Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 355), the peptide Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 356), the peptide Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 357), the peptide Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 358), the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 345), and the peptide Gly-Gly-Ser-Gly-Ser-Ser-Gly-Ser-Gly-Gly (SEQ ID NO: 389).
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The term "Fc domain" as used herein encompasses native Fc and Fc variants and sequences as defined above. As with Fc variants and native Fc molecules, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

The term "native Fc" as used herein refers to a molecule comprising the sequence of a non-antigen-binding fragment resulting from digestion of an antibody or produced by other means, whether in monomeric or multimeric form, and can contain the hinge region. The original immunoglobulin source of the native Fc is, in particular, of human origin and can be any of the immunoglobulins, although IgG1 and IgG2 are preferred. Native Fc molecules are made up of monomeric polypeptides that can be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, and IgE) or subclass (e.g., IgG1, IgG2, IgG3, IgA1, and IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG. The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

The term "Fc variant" as used herein refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn (neonatal Fc receptor). Exemplary Fc variants, and their interaction with the salvage receptor, are known in the art. Thus, the term "Fc variant" can comprise a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises regions that can be removed because they provide structural features or biological activity that are not required for the antibody-like binding proteins of the invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues, or in which one or more Fc sites or residues has been modified, that affect or are involved in: (1) disulfide bond formation, (2) incompatibility with a selected host cell, (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC).

In some embodiments, when the antibody like binding protein contains two F_c domains, i.e. in the CODV-Ig (F_c and F_{c2}), CODV-Fab-TL (two F_c domains), and CODV-Fab-OL (F_c and F_{c3}), the two F_c domains are of the same immunoglobulin isotype or isotype subclass. Accordingly, in some embodiments both F_c and F_{c2} of CODV-Ig, or both F_c domains of CODV-Fab-TL, or both F_c and F_{c3} of CODV-Fab-OL are of the IgG1 subclass, or of the IgG2 subclass, or of the IgG3 subclass, or of the IgG4 subclass.

All antibody like binding proteins as described herein have no effector function. This means that when the antibody like binding protein contains one or more F_c

domain(s) (i.e. F_c in formula [III], F_{c2} in formula [IV] and/or F_{c3}) of the IgG1 subclass, said on or more F_c domain(s) of IgG1 backbone contain(s) a double mutation L234A and L235A (so-called "LALA mutation") which abolishes F_c effector function. The F_c double mutant L234A and L235A does not bind $Fc\gamma R$ or C1q, and both ADCC and CDC
 5 functions of the F_c domain of IgG1 subclass are abolished (Hezareh, M. et al., J Virol. 2001 Dec; 75(24): 12161–12168).

In one example, the F_c region comprises the amino acid sequence SEQ ID NO: 330, SEQ ID NO: 394, or SEQ ID NO: 396. In one embodiment, the F_{c2} region
 10 comprises two amino acid exchanges within the CH3 domain H435R and Y436F, as described in Jendeborg, L. et al. (1997, J. Immunological Meth. 201: 25-34). Accordingly, in one embodiment the F_{c2} region comprises the amino acid sequence SEQ ID NO: 327. In another embodiment, the F_{c2} region comprises the amino acid sequence SEQ ID NO: 392.

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The so-called CODV-Fab "7G3x20G6" antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence
 DVVMTQTPVSLSVSLGGQVSISCRSSQSLVHNNGNTYLSWYLQKPGQSPQSLIYKVS
 RFSGFSDRFSGSGSGTDFTLKISRVPDDLGVYYCGQGTQYPFTFGSGTKLEIK**GQPK**
 20 **AAP**DFVMTQSPSSLTVTAGEKVTMSCKSSQSLNNGNQNLYLTWYLQKPGQPPKLLIY
 WASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEIK
TKGPSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPREAKVQWKVDNALQSGNS
 QESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
 (SEQ ID NO: 306, linkers are indicated in bold and underlined) comprising V_{D1} of
 25 sequence SEQ ID NO: 9, L_1 of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID
 NO: 308, L_2 of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and
 - one polypeptide according to formula II of the amino acid sequence
 EVQLQQSGPELVKPGASVKMSCKASGYFTFDYYMKWVKQSHGKSLEWIGDIIPSNGA
 TFYNQKFKGKATLTVDRSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGT
 30 LVTVS**A**EVQLVETGGSLVQPGKSLKLTCAATSGFTFTKAWMHWRQSPKQLEWVAQ
 IKDKSNSYATYYAESVKGRFTISRDDSKSTIYLMNSLKEEDTAIYYCRGVYALSPFDY
 WGQGVMVT**SSRT**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
 ALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ
 ID NO: 311, linkers are indicated in bold and underlined) comprising V_{D3} of sequence

SEQ ID NO: 312, L₃ of the amino acid sequence 'S', V_{D4} of sequence SEQ ID NO: 5, L₄ of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab "7G3x4E7" antibody-like binding protein comprises:

- 5 - one polypeptide according to formula I of the amino acid sequence
DVVMTQTPVSLSVSLGGQVSISCRSSQSLEHNNGNTYLSWYLQKPGQSPQPLIYKVS
NRFSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYCGQGTQYPFTFGPGTKLELK**GQPK**
AAPDFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLLIY
WASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEIK
10 **TKGPS**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 314, linkers are indicated in bold and underlined) comprising V_{D1} of
sequence SEQ ID NO: 21, L₁ of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID
NO: 308, L₂ of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and
15 - one polypeptide according to formula II of the amino acid sequence
EVQLQQSGPELVKPGASVKMSCKASGYTFTDYMKWVKQSHGKSLEWIGDIIPSNGA
TFYNQKFKGKATLTVDRSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGT
LVTVSA**SE**EVQVETGGSLVQPGKSLKLTCASTGFTFSNAWMHWVRQSPEKQLEWVA
QIKDKSNNYATYYAESLKGRFTISRDDPKRSIYLQMNSLREEDTAIYYCRYVHYGIGYA
20 MDAWGQGTSTVTVSS**RT**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
(SEQ ID NO: 315, linkers are indicated in bold and underlined) comprising V_{D3} of
sequence SEQ ID NO: 312, L₃ of the amino acid sequence 'S', V_{D4} of sequence SEQ ID
NO: 18, L₄ of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

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The so-called CODV-Fab "7G3x4B4" antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence
DVVMTQTPVSLSVSLGGQVSISCRSSQSLVHDNGNTYLSWSLQRPQGSPQVLIYKVS
NRFSGTSDRFTGSGSGTDFTLKISRVEPDDLGVYYCGQGTQYPFTFGSGTKLEIK**GQP**
30 **KAAP**DFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLL
IYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEI
KTKGPSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 316, linkers are indicated in bold and underlined) comprising V_{D1} of

sequence SEQ ID NO: 16, L₁ of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID NO: 308, L₂ of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and

- one polypeptide according to formula II of the amino acid sequence EVQLQQSGPELVKPGASVKMSCKASGYTFTDYMKWVKQSHGKSLEWIGDIIPSNGA

5 TFYNQKFKGKATLTVDRSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGT
LVTVSASEVQLVETGGRLVQPGRSLKLTCATSGFTFSNAWMHWVRQSPEKQLEWVA
QIKARSNNYATYYAESVKGRFTISRDDSKSTIYLQMNSLKEEDTAIYYCRGTYASKPF
DYWGQGVMTVSSRTASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV

10 (SEQ ID NO: 317, linkers are indicated in bold and underlined) comprising V_{D3} of
sequence SEQ ID NO: 312, L₃ of the amino acid sequence 'S', V_{D4} of sequence SEQ ID
NO: 12, L₄ of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab "7G3x18F5" antibody-like binding protein comprises:

15 - one polypeptide according to formula I of the amino acid sequence
DVLMTQTPVSLSVSLGGQVSISCRSSQSLVHTNGNTYLSWYLQKPGQSPQLLIYKVSN
RLSGISDRFSGSGSGTDFTLKISRVEPDDLGVYYCGQGTHYPFTFGAGTKLELKGQPK

AAPDFVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYLQKPGQPPKLLIY
WASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGKLEIK

20 TKGPSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

(SEQ ID NO: 318, linkers are indicated in bold and underlined) comprising V_{D1} of
sequence SEQ ID NO: 26, L₁ of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID
NO: 308, L₂ of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and

25 - one polypeptide according to formula II of the amino acid sequence
EVQLQQSGPELVKPGASVKMSCKASGYTFTDYMKWVKQSHGKSLEWIGDIIPSNGA
TFYNQKFKGKATLTVDRSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGT

LVTVSASEVQVVETGGSLVQPGKSLKLTCATSGFTFTNAWMHWVRRSPEKQLEWVA
QIKDKSNNYATYYAESVKGRFTISRDDSKSSIYLQMNSLKEEDTAIYYCRYVHYRFAYAL

30 DAWGRGTSVSVSSRTASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV

(SEQ ID NO: 319, linkers are indicated in bold and underlined) comprising V_{D3} of
sequence SEQ ID NO: 312, L₃ of the amino acid sequence 'S', V_{D4} of sequence SEQ ID
NO: 23, L₄ of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab “hz20G6x7G3” antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence
DFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLLIYWA
STRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEIK**GQ**
5 **PKAAP**DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNGNTYLSWYLQKPGQSPQSLI
YKVSNRFGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTKVEI
KTGPSRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 320, linkers are indicated in bold and underlined) comprising V_{D1} of
10 sequence SEQ ID NO: 308, L_1 of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID
NO: 143, L_2 of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and
- one polypeptide according to formula II of the amino acid sequence
QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKQLEWVAQIKDKSN
SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGQ
15 GTLTVTVSS**S**EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQSHGKSLEWI
GDIIPSNQATFYNQKFKGKATLTVDSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWF
AYWGQGTLVTVS**ART**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
(SEQ ID NO: 321, linkers are indicated in bold and underlined) comprising V_{D3} of
20 sequence SEQ ID NO: 138, L_3 of the amino acid sequence ‘S’, V_{D4} of sequence SEQ ID
NO: 312, L_4 of the amino acid sequence ‘RT’ and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab “7G3xhz4B4” antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence
25 DVVMTQTTPVSLSVSVGGRVSISSCRSSQSLVHDNGNTYLSWSLQKPGKSPKVLIIYKVSN
RFSGVSSRFTGSGSGTDFTLKISSVQPDDLGVYYCGQGTQYPFTFGSGTKLEIK**GQPK**
AAPDFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLLIY
WASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEIK
TKGPSRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
30 QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 322, linkers are indicated in bold and underlined) comprising V_{D1} of
sequence SEQ ID NO: 158, L_1 of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID
NO: 308, L_2 of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and

- one polypeptide according to formula II of the amino acid sequence
EVQLQQSGPELVKPGASVKMSCKASGYTFTDYMKWVKQSHGKSLEWIGDIIPSNGA
TFYNQKFKGKATLTVDSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGT
LVTVSASQVQLVETGGGLVKPGQSLKLTCAATSGFTFSNAWMHWVRQSPEKGLEWVA
5 QIKARSNNYATYYAESVKGRFTISRDDSKSTIYLQMNSLTPEDTAIYYCRGTYTYPASKPFD
YWGQGVMTVSSRTASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
(SEQ ID NO: 323, linkers are indicated in bold and underlined) comprising V_{D3} of
sequence SEQ ID NO: 312, L_3 of the amino acid sequence 'S', V_{D4} of sequence SEQ ID
10 NO: 171, L_4 of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab "hz4B4x3E3" antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence
QFVLTQPNVSTNLGSTVKLSCKRNTGNIGSNYVNWYQQHEGRSPTTMIYRDDKRPD
15 GVPDRFSGSIDRSSNSALLTINNVQTEDEADYFCQSYSSGINIIFGGGTKLTVLGQPKA
APDVVMTQTPVSLSVSVGGGRVSISCRSSQSLVHDNGNTYLSWSLQKPGKSPKVLIIYKV
SNRFSGVSSRFTGSGSGTDFTLKISSVQPDDLGVYYCGQGTQYPFTFGSGTKLEIKTK
GPSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE
SVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ
20 ID NO: 324, linkers are indicated in bold and underlined) comprising V_{D1} of sequence
SEQ ID NO: 230, L_1 of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID NO: 158, L_2
of sequence SEQ ID NO: 309 and C_L of sequence SEQ ID NO: 310, and
- one polypeptide according to formula II of the amino acid sequence
QVQLVETGGGLVKPGQSLKLTCAATSGFTFSNAWMHWVRQSPEKGLEWVAQIKARSN
25 NYATYYAESVKGRFTISRDDSKSTIYLQMNSLTPEDTAIYYCRGTYTYPASKPFDYWGQG
VMVTVSSSQVQLQESGPGLVQPSQTLSTCTVSGFSLTTYDVHWVRQPPGKGLEWM
GRIQNGGITDYNALKSRLLISRDTSKSQVFLKMNSVQTEDTAMVFCAGTGSYFYAFDH
WGQGTLLTVVSSRTASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ
30 ID NO: 325, linkers are indicated in bold and underlined) comprising V_{D3} of sequence
SEQ ID NO: 171, L_3 of the amino acid sequence 'S', V_{D4} of sequence SEQ ID NO: 226,
 L_4 of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

The so-called CODV-Fab “hz20G6xhz7G3” antibody-like binding protein comprises:

- one polypeptide according to formula [I] consisting essentially of the amino acid sequence:

5 DIVMTQSPDSLAVSLGERATINCESSQSLNLSGNQKNYLTWYQQKPGQPPKPLIYWAS
TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYSYPYTFGQGTKLEIK**GGS**
GSSGSGGDIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQ
SLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGGTQYPFTFGSGTK
VEIK**GSGSGSGSGG**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
10 NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSF
NRGEC (SEQ ID NO: 388, linkers are indicated in bold and underlined) comprising V_{D1}
of sequence SEQ ID NO: 385, L₁ of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ
ID NO: 141, L₂ of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310, and
- one polypeptide according to formula [II] consisting essentially of the amino acid
15 sequence
QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHVWRQAPGKQLEWVAQIKDKSN
SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGG
GTLVTVSSEVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKGLEWM
GDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMYCARSHLLRASWF
20 AYWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ
ID NO: 390) comprising V_{D3} of sequence SEQ ID NO: 138, L₃ is 0 amino acid, V_{D4} of
sequence SEQ ID NO: 383 (in italic and underlined), L₄ is 0 amino acid and C_{H1} of
sequence SEQ ID NO: 313.

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In one example, the polypeptides according to formula II of the so-called CODV-Fab “7G3x20G6”, “7G3x4E7”, “7G3x4B4”, “7G3x18F5”, “hz20G6x7G3”, “7G3xhz4B4”, “hz4B4x3E3” and “hz20G6xhz7G3” antibody-like binding proteins further comprise the sequence EPKSCDKTHHHHHHH (SEQ ID NO: 352) corresponding to a hinge
30 sequence and a Histag used, for example, for purification.

The so-called CODV-Fab “hz20G6x7G3-TL4” (also called CODV-Fab-TL4 “hz20G6x7G3”) antibody-like binding protein comprises:

- one polypeptide according to formula [IV] consisting essentially of the amino acid sequence

DFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLLIYWA
STRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPYTFGGGTKLEIK**GQ**

5 **PKAAP**DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNGNTYLSWYLQKPGQSPQSLI
YKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTKVEI
KTGPSRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**ESK**
YGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWY

10 **VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI**
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQKSLSLSLG (SEQ
ID NO: 326, linkers are indicated in bold and underlined) comprising V_{D1} of sequence
SEQ ID NO: 308, L_1 of sequence SEQ ID NO: 307, V_{D2} of sequence SEQ ID NO: 143, L_2
15 of sequence SEQ ID NO: 309, C_L of sequence SEQ ID NO: 310 and F_{c2} (underlined) of
sequence SEQ ID NO: 327, and

- one polypeptide according to formula [III] of the amino acid sequence
QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHVVRQAPGKQLEWVAQIKDKSN
SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGG

20 GTLTVTVSS**S**EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQSHGKSLEWI
GDIIPSNNGATFYNQKFKGKATLTVDRSSSTAYMHLNSLTSEDSAVYYCTRSHLLRASWF
AYWGQGTLVTVS**ART**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTITYTCNVDHKPSNTKVDKRVES
KYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNW
25 YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ
ID NO: 328, linkers are indicated in bold and underlined) comprising V_{D3} of sequence
SEQ ID NO: 138, L_3 of the amino acid sequence 'S', V_{D4} of sequence SEQ ID NO: 312,
30 L_4 of the amino acid sequence 'RT', and C_{H1} of sequence SEQ ID NO: 329, and F_c of
sequence SEQ ID NO: 330.

In the above CODV-Fab-TL4 "hz20G6x7G3" antibody-like binding protein, the F_c
of sequence SEQ ID NO: 330 and F_{c2} of sequence SEQ ID NO: 327 are from an IgG4

backbone. Said antibody-like binding protein is in a CODV-Fab-TL format and contains or consists of one polypeptide of formula III and one polypeptide of formula IV.

The so-called CODV-Fab-TL1 “hz20G6xhz7G3” antibody-like binding protein comprises:

- one polypeptide according to formula IV of the amino acid sequence

DIVMTQSPDSLAVSLGERATINCESSQSLLNSGNQKNYLTWYQQKPGQPPKPLIYWAS
TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYSYPYTFGQGTKLEIK**GGSGSSGSGG**DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQ
SLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGGQTQYPFTFGSGTK
VEIK**GGSGSSGSGG**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSF
NRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPG

(SEQ ID NO: 391, linkers are indicated in bold and underlined) comprising V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389, C_L of sequence SEQ ID NO: 310, L_5 which contains 0 amino acid, and F_{c2} (underlined) of sequence SEQ ID NO: 392; and

- one polypeptide according to formula III of the amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKQLEWVAQIKDKSN
SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGG
GTLVTVSSEVQLVQSGAEVKKPGESLKISCKGSGYSFTDYIMKWARQMPGKGLEWM
GDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMYYCARSHLLRASWF
AYWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSKVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKEPKSC
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPG

(SEQ ID NO : 393) comprising V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383 (in italic), L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c (underlined) of sequence SEQ ID NO: 394.

In the CODV-Fab-TL1 “hz20G6x7G3” antibody-like binding protein, the Fc of sequence SEQ ID NO: 394 and F_{c2} of sequence SEQ ID NO: 392 are from an IgG1 backbone. Said antibody-like binding protein is in a CODV-Fab-TL format. It contains or consists of one polypeptide of formula IV and one polypeptide of formula III.

5

The so-called CODV-Fab-OL1 “hz20G6xhz7G3” antibody-like binding protein comprises:

- one polypeptide according to formula I of the amino acid sequence

DIVMTQSPDSLAVSLGERATINCESSQSLLNSGNQKNYLTWYQQKPGQPPKPLIYWAS
 10 TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYSYPYTFGGGTKLEIK**GGS**
GSSGSGGDIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQ
 SLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGGTQYPFTFGSGTK
 VEIK**GSGSGSGSGG**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
 NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
 15 NRGEC

(SEQ ID NO: 388, linkers are indicated in bold and underlined) comprising V_{D1} of sequence SEQ ID NO: 385, L₁ of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L₂ of sequence SEQ ID NO: 389, and C_L of sequence SEQ ID NO: 310; and

- one polypeptide according to formula III of the amino acid sequence

20 QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKQLEWVAQIKDKSN
 SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGG
 GTLTVTSSEVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKGLEWM
GDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMYCARSHLLRASWF
AYWGQGTMTVTSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
 25 ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN**RFT**QKSLSLSPG

30 (SEQ ID NO : 395) comprising V_{D3} of sequence SEQ ID NO: 138, L₃ is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383 (in italic and underlined), L₄ is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c (underlined) of sequence SEQ ID NO: 396;

and wherein the so-called CODV-Fab-OL1 “hz20G6xhz7G3” antibody-like binding protein further comprises a Fc stump (F_{c3}) of the amino acid sequence:

GSDKTHTCPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

- 5 (SEQ ID NO: 397) and which heterodimerises with the Fc region of the polypeptide according to formula III.

Said antibody-like binding protein is in a CODV-Fab-OL format, i.e. it contains or consists of one polypeptide of formula I, one polypeptide of formula III, and one Fc stump. Its F_c and F_{c3} sequences have been engineered according to the “Knob-into-
 10 Hole” technology and further contain the double mutation L234A and L235A.

The F_c sequence of sequence SEQ ID NO: 396 has been designed to contain RF residues at positions 200-221 (in bold above), instead of HY residues which would have otherwise been present at these positions of the Fc region. The HY > RF mutation (i.e. H435R and Y436F in CH3 domain as described by Jendeborg, L. et al. 1997, J.
 15 Immunological Meth., 201: 25-34) is advantageous for purification purposes as it abolishes binding to protein A. In the case of CODV-Fab-OL1 “hz20G6xhz7G3”, the F_c stump of sequence SEQ ID NO: 397 comprises HY residues at positions 217-218 (in bold above).

- 20 The so-called CODV-Fab-OL1a “hz20G6xhz7G3” antibody-like binding protein comprise:

- one polypeptide according to formula I of the amino acid sequence

DIVMTQSPDSLAVSLGERATINCESSQSLLNSGNQKNYLTWYQQKPGQPPKPLIYWAS
 TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYSYPYTFGQGTKLEIK**GGS**
 25 **GSSGSGG**DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQ
 SLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTK
 VEIK**GSGSGSGG**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
 NALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
 NRGEC

- 30 (SEQ ID NO: 388) which comprises V_{D1} of sequence SEQ ID NO: 385, L₁ of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L₂ of sequence SEQ ID NO: 389, and C_L of sequence SEQ ID NO: 310;

- one polypeptide according to formula III of the amino acid sequence:

QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHVVRQAPGKQLEWVAQIKDKSN
 SYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGG
 GTLVTVSSEVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKGLEWM
GDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMYCARSHLLRASWF
 5 AYWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
 ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
 DKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT
 10 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

(SEQ ID NO: 399) comprising V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383 (in italic and underlined), L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c (underlined) of sequence SEQ ID NO: 400;

- and wherein the so-called CODV-Fab-OL1a “hz20G6xhz7G3” antibody-like

15 binding protein further comprises a F_c stump (F_{c3}) of the amino acid sequence:

GSDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY
 KTTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHEALHN**RF**TQKSLSLSPG

20 (SEQ ID NO: 398) which heterodimerises with the F_c region of the polypeptide according to formula III.

The F_c of sequence SEQ ID NO: 400 comprises HY residues at positions 200-221 (in bold above) while the F_c stump of sequence SEQ ID NO: 398 comprises RF residues at positions 217-218 (in bold above).

25 Said antibody-like binding protein is in a CODV-Fab-OL format, i.e. it contains or consists of one polypeptide of formula I, one polypeptide of formula III and one F_c stump. Its F_c and F_{c3} sequences have been engineered according to the “Knob-into-Hole” technology and contain the double mutation L234A and L235A.

30 In one embodiment, the first immunoglobulin or the second immunoglobulin is one anti-CD123 antibody selected from the so-called “3E3-D3”, “1E1-G5”, “2B8-F3”, “2F8-D6”, “3B10-E6”, “5A5-B4”, “6B10-E4”, “6C10-C4”, “6D6-B8”, “8B11-B7”, “9B8-G6”, “9D7-C8”, and “9F6-G3” anti-CD123 antibodies, or a humanized form thereof, or the anti-

CD123 antibody "7G3" described herein below, for example the anti-CD123 antibodies "3E3-D3" or "7G3", or a humanized form thereof.

In one embodiment, the first immunoglobulin or the second immunoglobulin is one
 5 anti-CD3 antibody selected from the so-called "20G6-F3", "4B4-D7", "4E7-C9", "18F5-H10", "12D2-E5", "11D7-C3", "11H3-E5", "13H2-C2", "13C1-F6", "18H11-F10", "1E6-C9", "10F4-C10", "10E6-G6", "18G9-H11", "11F3-B9", "12G3-E8", "5B1-G2", "16F8-A7", "11F9-F8", "3G5-E10", "9D7-F3", "8C2-F7", "20E5-F10", "20B5-F10", "6C9-C9", "3E8-G1", "3H6-D2", and "8H2" anti-CD3 antibodies, or a humanized form thereof, for
 10 example the so-called "20G6-F3", "4B4-D7", "4E7-C9", "18F5-H10", and "hz20G6" anti-CD3 antibodies, for instance the so-called so-called "20G6-F3", "4B4-D7" anti-CD3 antibodies.

Accordingly, V_{D1} and V_{D4} , or V_{D2} and V_{D3} are the variable domains of a heavy or
 15 light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody comprises:

- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6, CDR2-H of sequence SEQ ID NO: 7, CDR3-H of sequence SEQ ID NO: 8 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or SEQ ID NO: 142, CDR2-L of sequence 'KVS' and CDR3-L of
 20 sequence SEQ ID NO: 11; or
- b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 14, CDR3-H of sequence SEQ ID NO: 15 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or SEQ ID NO: 184, CDR2-L of sequence 'KVS' and CDR3-L of
 25 sequence SEQ ID NO: 11; or
- c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 20 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 22, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO:
 30 11; or
- d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 24, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 25 and a light chain variable domain comprising CDR1-L of sequence SEQ ID

NO: 27, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28, and

wherein V_{D1} is the variable domain of heavy chain as defined above if V_{D4} is the variable domain of light chain or V_{D1} is the variable domain of the light chain as defined above if V_{D4} is the variable domain of the heavy chain, or V_{D2} is the variable domain of heavy chain as defined above if V_{D3} is the variable domain of light chain or V_{D2} is the variable domain of the light chain as defined above if V_{D3} is the variable domain of the heavy chain.

In a further embodiment, V_{D1} and V_{D4} or V_{D2} and V_{D3} are the variable domains of a heavy or light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody is a humanized antibody and comprises:

- a) a heavy chain variable domain of sequence SEQ ID NO: 138, or a sequence at least 85% identical thereto and/or a light chain variable domain of sequence SEQ ID NO: 143, or a sequence at least 85% identical thereto; or
- b) a heavy chain variable domain of sequence SEQ ID NO: 171, or a sequence at least 85% identical thereto and/or a light chain variable domain of sequence SEQ ID NO: 158, or a sequence at least 85% identical thereto; or
- c) a heavy chain variable domain of sequence SEQ ID NO: 176, or a sequence at least 85% identical thereto and/or a light chain variable domain of sequence SEQ ID NO: 164, or a sequence at least 85% identical thereto; or

wherein V_{D1} is the variable domain of heavy chain as defined above if V_{D4} is the variable domain of light chain or V_{D1} is the variable domain of the light chain as defined above if V_{D4} is the variable domain of the heavy chain, or V_{D2} is the variable domain of heavy chain as defined above if V_{D3} is the variable domain of light chain or V_{D2} is the variable domain of the light chain as defined above if V_{D3} is the variable domain of the heavy chain.

In said sequence at least 85% identical to SEQ ID NO: 138, SEQ ID NO: 143, SEQ ID NO: 171, SEQ ID NO: 158, SEQ ID NO: 176, or SEQ ID NO: 164, the sequences of the 6 CDRs are unchanged compared to the 6 CDRs present in the reference sequence SEQ ID NO: 138, SEQ ID NO: 143, SEQ ID NO: 171, SEQ ID NO: 158, SEQ ID NO: 176, or SEQ ID NO: 164.

In an embodiment, the antibody-like binding protein of the invention binds to human CD3. In another embodiment, the antibody-like binding protein of the invention further binds to *Macaca fascicularis* CD3. In particular, the antibody-like binding protein of the invention binds to the extracellular domain of human CD3, or of both human and *Macaca fascicularis* CD3. More specifically, the antibody binds to CD3 ϵ . More specifically, the antibody-like binding protein binds to the human or human and *Macaca fascicularis* extracellular domain of CD3 ϵ . The antibody-like binding protein binds to CD3 ϵ when present in the form of a complex, such as a CD3 ϵ/δ complex, or when present as single protein, indifferently whether expressed in isolated form, or present in a soluble extracellular domain or full-length membrane-anchored CD3 ϵ as present in for example in T-cells. The antibody-like binding protein according to the invention is specific for the surface human CD3 protein, or of both human and *Macaca fascicularis* CD3 proteins, in particular to CD3 ϵ .

The antibody-like binding according to the invention has a ratio of affinity for *Macaca fascicularis* CD3 on affinity for human CD3 ($KD(\textit{Macaca fascicularis})/KD(\text{human})$) which is ≤ 10 , in particular ≤ 6 , ≤ 5 , ≤ 4 , ≤ 3 , ≤ 2 , ≤ 1 or ≤ 0.5 . Thus, the antibody-like binding protein according to the invention may be used in toxicological studies performed in monkeys the toxicity profile observed in monkeys relevant to anticipate potential adverse effects in humans.

Furthermore, the antibody-like binding protein according to the invention has an affinity (KD) for human CD3 or *Macaca fascicularis* CD3, or both, which is $\leq 50\text{nM}$, $\leq 40\text{nM}$, or $\leq 30\text{nM}$, for instance $\leq 20\text{nM}$, for example an affinity of 0.1 nM to 30 nM, in particular of 0.4 nM to 20 nM, or of 0.4 nM to 15 nM.

In one embodiment, the antibody-like binding protein of the invention has a T-cell activation that is lower than less than 20%, less than 18%, less than 16%, less than 14%, less than 12%, less than 10% in the absence of target cells.

In one embodiment, the antibody-like binding protein of the invention has a T-cell activation that is higher higher than 55%, higher than 60%, higher than 62%, higher than 64%, higher than 66%, higher than 68%, higher than 70% in the presence of target cells.

“Low T-cell activation” in the context of the antibody-like binding proteins of the invention refers to a T-cell activation less than 20%, less than 18%, less than 16%, less than 14%, less than 12%, less than 10%.

“Target cells” herein refer to cells that express the second antigen, in one example
5 target cells herein refer to CD123 expressing cells such as THP-1 cells.

“High T-cell activation” herein refers to a T-cell activation higher than 50%, higher than 55%, higher than 60%, higher than 62%, higher than 64%, higher than 66%, higher than 68%, higher than 70%.

10 In a further embodiment, the invention relates to an antibody-like binding protein having biological and immunological specificity to at least one further target antigen.

Therefore, in one aspect of the invention, the antibody-like binding protein of the invention binds further to at least one other target antigen. Accordingly, in one embodiment, the antibody-like binding protein of the invention is bispecific and capable
15 of binding two different antigen targets or epitopes.

Accordingly, in one embodiment the first immunoglobulin is an immunoglobulin directed against at least one further target if the second immunoglobulin is one anti-CD123 antibody as defined in the section «anti-CD123 antibodies» above, or the second immunoglobulin is an immunoglobulin directed against at least one further target
20 if the first immunoglobulin is one anti-CD123 antibody as defined in the section «anti-CD123 antibodies» above.

In one further embodiment the first immunoglobulin is an immunoglobulin directed against at least one further target if the second immunoglobulin is one anti-CD3 antibody as defined in the section «anti-CD3 antibodies» above, or the second
25 immunoglobulin is an immunoglobulin directed against at least one further target if the first immunoglobulin is one anti-CD3 antibody as defined in the section «anti-CD3 antibodies» above.

The antibody-like binding protein of the invention has a T-cell engaging effect. This
30 T-cell engaging effect induces cytotoxicity in the target cell. In one embodiment, the target cell is a CD123 expressing cell, such as a CD123 expressing cancer cell, for example THP-1 or TF-1.

Accordingly, in one embodiment the antibody-like binding protein according to the invention is able to engage primary T-cells and to lyse target cells *in vitro* wherein the (EC_{50}) is $\leq 40\text{pM}$, $\leq 35\text{pM}$, for instance $\leq 30\text{pM}$.

5 “Cytotoxicity” herein refers to the quality of a compound, such as the antibody-like binding protein or an anti-CD123 antibody of the invention, to be toxic to cells. Cytotoxicity may be induced by different mechanisms of action and can thus be divided into cell-mediated cytotoxicity, apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

10 “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies.

15 “Complement-dependent cytotoxicity” or “CDC”, in the context of the invention, refers to lysis of a target cell in the presence of complement system proteins.

 “Cell-mediated cytotoxicity” refers to cytolysis of a target cell by effector lymphocytes, such as cytotoxic T lymphocytes or natural killer cells and can thus be distinguished into T-cell-mediated cytotoxicity and NK-cell cytotoxicity.

20 In one embodiment, cytotoxicity herein refers to Cell-mediated cytotoxicity for example T-cell-mediated cytotoxicity.

 Furthermore, in one embodiment the cell-mediated cytotoxicity refers to cell-mediated cytotoxicity by T-cells.

 Accordingly, the antibody-like binding protein of the invention induces cell-mediated cytotoxicity in the target cell mediated by T-cells.

25 Methods to measure cytotoxicity are known to the skilled in the art and include using 51-Chromium (Cr) release assay, live/dead cell staining of target cells including propidium iodide, 7-AAD, and other stains that are known to the skilled in the art, detection of lytic molecules released by T cells including granzyme and perforin by flow cytometry or ELISA, detection of lactate dehydrogenase (LDH) released into the media
30 from damaged cells as a biomarker for cellular cytotoxicity and cytolysis, detection of cell surface mobilization of CD107a, Annexin V (calcium-dependent phospholipid-binding proteins) staining of apoptotic target cells and for example detection of activated Caspase-3 (CASP3).. Furthermore, the skilled in the art can distinguish between the

different mechanisms of cytotoxicity based on the test selected and based on the experimental set up.

In one example, cell-mediated cytotoxicity may be for example measured using CFSE to label target cells and 7-AAD to label dead cells as described, for instance, in
5 example 3.2.

In a further embodiment, the antibody-like binding protein is capable of binding to CD3 and at least one further antigen target, for example CD123.

In one embodiment, the antibody-like binding protein is capable of inhibiting the
10 function of this further antigen target, for example CD123.

In one aspect of the invention, the antibody-like binding protein binds to human CD123. In another embodiment, the antibody-like binding protein further binds to *Macaca fascicularis* CD123. In particular, the antibody-like binding protein of the
15 invention binds to the extracellular domain of human CD123, or of both, human and *Macaca fascicularis* CD123. More specifically, the antibody-like binding protein binds to the distal moiety of CD123, for example, to the amino acids starting from position 19 to 49 of human CD123 of the amino acid sequence SEQ ID NO: 104. The antibody-like binding protein binds to CD123, indifferently whether expressed in isolated form, or
20 present in a soluble extracellular domain or full-length membrane-anchored CD123 as present in CD123 expressing cells such as AML cells or CD123 transfected cells. The antibody-like binding protein according to the invention is specific to cells that express human or human and *Macaca fascicularis* CD123 proteins on their surface, for example CD123 expressing cancer cells.

25 Accordingly, the antibody-like binding protein according to the invention has an affinity (KD) for human CD123 or *Macaca fascicularis* CD123, or both, which is ≤ 20 nM, ≤ 15 nM, or ≤ 10 nM, for instance ≤ 5 nM, for example an affinity of 0.01 nM to 5 nM, in particular of 0.1 nM to 5 nM.

30 Accordingly, in one embodiment, the first immunoglobulin is one anti-CD3 antibody selected from the so-called "20G6-F3", "4B4-D7", "4E7-C9", "18F5-H10", "12D2-E5", "11D7-C3", "11H3-E5", "13H2-C2", "13C1-F6", "18H11-F10", "1E6-C9", "10F4-C10", "10E6-G6", "18G9-H11", "11F3-B9", "12G3-E8", "5B1-G2", "16F8-A7", "11F9-F8", "3G5-E10", "9D7-F3", "8C2-F7", "20E5-F10", "20B5-F10", "6C9-C9", "3E8-G1", "3H6-D2", and

“8H2” anti-CD3 antibodies, or a humanized form thereof, for example the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “hz4B4” and “hz20G6” anti-CD3 antibodies, and the second immunoglobulin is one anti-CD123 antibody selected from the so-called “3E3-D3”, “1E1-G5”, “2B8-F3”, “2F8-D6”, “3B10-E6”, “5A5-B4”, “6B10-E4”,
 5 “6C10-C4”, “6D6-B8”, “8B11-B7”, “9B8-G6”, “9D7-C8”, and “9F6-G3” anti-CD123 antibodies.

Accordingly, in a further embodiment, the second immunoglobulin is one anti-CD3 antibody selected from the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “12D2-E5”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “18H11-F10”, “1E6-C9”,
 10 “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “3G5-E10”, “9D7-F3”, “8C2-F7”, “20E5-F10”, “20B5-F10”, “6C9-C9”, “3E8-G1”, “3H6-D2”, and “8H2” anti-CD3 antibodies, or a humanized form thereof, for example the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “hz4B4” and “hz20G6” anti-CD3 antibodies, and the first immunoglobulin is one anti-CD123 antibody
 15 selected from the so-called “3E3-D3”, “1E1-G5”, “2B8-F3”, “2F8-D6”, “3B10-E6”, “5A5-B4”, “6B10-E4”, “6C10-C4”, “6D6-B8”, “8B11-B7”, “9B8-G6”, “9D7-C8”, and “9F6-G3” anti-CD123 antibodies.

Accordingly, in one embodiment, the V_{D1} and V_{D4} or V_{D2} and V_{D3} comprise a heavy
 20 chain variable domain and a light chain variable domain, each of them either defined by three CDR sequences or by heavy and light chain variable domain sequences of one the 13 so-called “3E3-D3”, “1E1-G5”, “2B8-F3”, “2F8-D6”, “3B10-E6”, “5A5-B4”, “6B10-E4”, “6C10-C4”, “6D6-B8”, “8B11-B7”, “9B8-G6”, “9D7-C8”, and “9F6-G3” anti-CD123 antibodies as defined above,

25 wherein V_{D1} and V_{D4} , both comprise the three CDR sequences of the heavy and light chain variable domain sequences of one anti-CD123 antibody as defined above, if V_{D2} and V_{D3} , both comprise three CDR sequences of heavy and light chain variable domain sequences of one of the anti-CD3 antibodies as defined above, or

30 wherein V_{D2} and V_{D3} , both comprise the three CDRs of the heavy and light chain variable domain sequences of one anti-CD123 antibody as defined above, if V_{D1} and V_{D4} comprise CDR sequences of heavy and light chain variable domain sequences of one of the anti-CD3 antibodies as defined above.

Accordingly, in one embodiment V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6, CDR2-H of sequence SEQ ID NO: 7, CDR3-H of sequence SEQ ID NO: 8 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or SEQ ID NO: 142, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11 and V_{D2} and V_{D3} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

Accordingly, in a further embodiment V_{D2} and V_{D3} are the variable domain of heavy or light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6, CDR2-H of sequence SEQ ID NO: 7, CDR3-H of sequence SEQ ID NO: 8 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or SEQ ID NO: 142, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11 and V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

Accordingly, in a further embodiment V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 14, CDR3-H of sequence SEQ ID NO: 15 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or SEQ ID NO: 184, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11 and V_{D2} and V_{D3}

are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

- 5 wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

Accordingly, in a further embodiment V_{D1} and V_{D4} are the variable domain of heavy
10 or light chain of an anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 14, CDR3-H of sequence SEQ ID NO: 15 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or SEQ ID NO: 184, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11 and V_{D2} and V_{D3}
15 are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

 wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are
20 both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

In a further embodiment V_{D1} and V_{D4} are the variable domain of heavy or light
chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a
25 heavy chain variable a heavy chain variable domain of sequence SEQ ID NO: 138 and/or a light chain variable domain of sequence SEQ ID NO: 143 and V_{D2} and V_{D3} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123
30 antibodies as described above under the section "anti-CD123 antibodies",

 wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

In a further embodiment V_{D2} and V_{D3} are the variable domain of heavy or light chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain variable a heavy chain variable domain of sequence SEQ ID NO: 138 and/or a light chain variable domain of sequence SEQ ID NO: 143 and V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

In a further embodiment V_{D1} and V_{D4} are the variable domain of heavy or light chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain a heavy chain variable domain of sequence SEQ ID NO: 171 and/or a light chain variable domain of sequence SEQ ID NO: 158 and V_{D2} and V_{D3} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

In a further embodiment V_{D2} and V_{D3} are the variable domain of heavy or light chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain a heavy chain variable domain of sequence SEQ ID NO: 171 and/or a light chain variable domain of sequence SEQ ID NO: 158 and V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

5 In a further embodiment V_{D1} and V_{D4} are the variable domain of heavy or light chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain a heavy chain variable domain of sequence SEQ ID NO: 176 and/or a light chain variable domain of sequence SEQ ID NO: 164 and V_{D2} and V_{D3} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group
10 consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.
15

In a further embodiment V_{D2} and V_{D3} are the variable domain of heavy or light chain of a humanized anti-CD3 antibody, wherein said anti-CD3 antibody comprises a heavy chain a heavy chain variable domain of sequence SEQ ID NO: 176 and/or a light chain variable domain of sequence SEQ ID NO: 164 and V_{D1} and V_{D4} are the variable domain of heavy or light chain of an anti-CD123 antibody selected from the group
20 consisting of "3E3-D3", "1E1-G5", "2B8-F3", "2F8-D6", "3B10-E6", "5A5-B4", "6B10-E4", "6C10-C4", "6D6-B8", "8B11-B7", "9B8-G6", "9D7-C8", "9F6-G3" anti-CD123 antibodies as described above under the section "anti-CD123 antibodies",

25 wherein V_{D1} and V_{D2} are both variable domains of light chains if V_{D3} and V_{D4} are both variable domains of heavy chains or V_{D1} and V_{D2} are both variable domains of heavy chains if V_{D3} and V_{D4} are both variable domains of light chains.

According to a further aspect of the invention, the first or second immunoglobulin
30 is the anti-CD123 antibody 7G3. Therefore, in one embodiment V_{D1} and V_{D4} or V_{D2} and V_{D3} comprise a heavy chain variable domain and a light chain variable domain as defined by CDR sequences of heavy and light chain variable domain sequences of the antibody 7G3 as defined herein below. In one embodiment V_{D1} and V_{D4} , or V_{D2} and V_{D3} comprise a heavy chain variable domain and a light chain variable domain of the

antibody 7G3 as described in the patent application WO2013/173820, which is incorporated herein by reference.

Accordingly, the so-called "7G3" anti-CD123 antibody as herein used comprises:

5 -a heavy chain variable domain consisting of sequence
 EVQLQQSGPELVKPGASVKMSCKAS**GYTFTDY**MKWVKQSHGKSLEWIGDI**IIP****SN****G**A
 TFYNQKFKGKATLTVDSSSTAYMHLNSLTSEDSAVYYCT**RS****HLL****R**AS**W**FAYWGQGT
 LVTVSA (SEQ ID NO: 312, with CDRs shown in bold characters) comprising CDR1-H of
 sequence SEQ ID NO: 375, a CDR2-H of sequence SEQ ID NO: 376, and a CDR3-H of
 10 sequence SEQ ID NO: 377 and

 -a light chain variable domain consisting of sequence
 DFVMTQSPSSLTVTAGEKVTMSCKSS**QSLL****NS****GN****Q**KNYLTWYLQKPGQPPKLLIY**W**A
STRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYC**Q****N****D****Y****S****P****Y****T**FGGGTKLEIK
 (SEQ ID NO: 308, with CDRs shown in bold characters) comprising CDR1-L of
 15 sequence SEQ ID NO: 378, a CDR2-L of sequence 'WAS', and a CDR3-L of sequence
 SEQ ID NO: 379.

In a further aspect of the invention, the antibody 7G3 may also be a humanized antibody or a fragment of a humanized antibody. Accordingly, in one embodiment, the antibody 7G3 of the invention is a humanized antibody comprising

20 - a heavy chain variable domain consisting of sequence
 EVQLVQSGAEVKKPGESLKISCKGS**GY****S****F****T****D****Y****M****K****W****A****R****Q****M****P****G****K****G****L****E****W****M****G****D****I****I****P****S****N****G****A**
 TFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMY**C****A****R****S****H****L****L****R****A****S****W****F****A****Y****W****G****Q****G****T**
 MVTVSS (SEQ ID NO: 380, with CDRs shown in bold characters) comprising CDR1-H
 of sequence SEQ ID NO: 381, a CDR2-H of sequence SEQ ID NO: 377, and a CDR3-H
 25 of sequence SEQ ID NO: 382, or

 a heavy chain variable domain consisting of sequence
 EVQLVQSGAEVKKPGESLKISCKGS**GY****S****F****T****D****Y****M****K****W****A****R****Q****M****P****G****K****G****L****E****W****M****G****D****I****I****P****S****S****G****A**
 TFYNQKFKGQVTISADKSISTTYLQWSSLKASDTAMY**C****A****R****S****H****L****L****R****A****S****W****F****A****Y****W****G****Q****G****T**
 MVTVSS (SEQ ID NO: 383, with CDRs shown in bold characters) comprising CDR1-H
 30 of sequence SEQ ID NO: 381, a CDR2-H of sequence SEQ ID NO: 384, and a CDR3-H
 of sequence SEQ ID NO: 382, or

 - a light chain variable domain consisting of sequence
 DIVMTQSPDSLAVSLGERATINCESS**QSLL****NS****GN****Q**KNYLTWYQQKPGQPPKPLIY**W**A**S**
 TRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYC**Q****N****D****Y****S****P****Y****T**FGQG**T****K****L****E****I****K**

(SEQ ID NO: 385, with CDRs shown in bold characters) comprising CDR1-L of sequence SEQ ID NO: 378, a CDR2-L of sequence 'WAS', and a CDR3-L of sequence SEQ ID NO: 379.

5 In one embodiment, the antibody-like binding protein that binds specifically to human CD3ε and human CD123 comprises

- 10 a) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 9 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 5 or a sequence at least 85% identical thereto, or
- 15 b) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 21 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 18 or a sequence at least 85% identical thereto, or
- 20 c) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 16 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO 12 or a sequence at least 85% identical thereto, or
- 25 d) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 26 or a sequence at least 85% identical
- 30

thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 23 or a sequence at least 85% identical thereto, or

e) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 308 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 143 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 138 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, or

f) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 158 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto and a heavy chain variable domain of a first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 171 or a sequence at least 85% identical thereto,

g) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 230 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 158 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 171 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 226 or a sequence at least 85% identical thereto,

h) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 385 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO: 141 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO: 138 or a sequence at least 85% identical thereto and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO: 383 or a sequence at least 85% identical thereto.

10 In said above sequence at least 85% identical to a reference sequence (e.g. a sequence at least 85% identical to SEQ ID NO: 383 or SEQ ID NO: 385), the sequences of the 6 CDRs are unchanged compared to the 6 CDRs present in the reference sequence.

15 In one embodiment the antibody binding protein according to any of the definitions a) to g) further comprises the linker L_1 of sequence SEQ ID NO: 307, L_2 of sequence SEQ ID NO: 309, L_3 of the amino acid sequence 'S', L_4 of the amino acid sequence 'RT' and C_{H1} of sequence SEQ ID NO: 313.

In one embodiment, the antibody binding protein according to any of the definitions a) to g) further comprises F_{c2} of sequence SEQ ID NO: 327.

20 In one embodiment, the antibody binding protein according to definition h) further comprises the linker L_1 of sequence SEQ ID NO: 389, L_2 of sequence SEQ ID NO: 389, L_3 and L_4 consisting of 0 amino acid, and C_{H1} of sequence SEQ ID NO: 313.

In one embodiment, the antibody binding protein according to definition h) further comprises F_{c2} of sequence SEQ ID NO: 392.

25 In one embodiment, L_5 of the antibody binding protein according to any of the definitions a) to h) contains 0 amino acids.

In a further embodiment the antibody binding protein according to any of the definitions a) to g) further comprises the linker L_1 of sequence SEQ ID NO: 307, L_2 of sequence SEQ ID NO: 309, L_3 of the amino acid sequence 'S', L_4 of the amino acid sequence 'RT', C_{H1} of sequence SEQ ID NO: 329 and F_c of sequence SEQ ID NO: 330.

30 In a further embodiment, the antibody binding protein according to definition h) further comprises the linker L_1 of sequence SEQ ID NO: 389, L_2 of sequence SEQ ID

NO: 389, L₃ and L₄ consisting of 0 amino acid, C_{H1} of sequence SEQ ID NO: 313 and F_c of sequence SEQ ID NO: 394.

In a further embodiment, the antibody binding protein according to definition h) further comprises a Fc stump of sequence SEQ ID NO: 397 or SEQ ID NO: 398, or a
5 sequence at least 85% identical to SEQ ID NO: 397 or SEQ ID NO: 398.

In an embodiment, the antibody binding protein that binds specifically to human CD3ε and human CD123 comprises, or essentially consists of:

- 10 a) one polypeptide of formula [I] consisting of sequence SEQ ID NO: 388 (V_{D1} of sequence SEQ ID NO: 385, L₁ of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L₂ of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310),
or a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of hz7G3 light chain variable domain (V_{D1} of sequence SEQ ID NO: 385), and the 3 CDRs of
15 sequences. SEQ ID NO: 142, 'KVS' and SEQ ID NO: 11 of hz20G6 light chain variable domain (V_{D2} of sequence SEQ ID NO: 141) are unaltered; and
- b) one polypeptide of formula [II] consisting of sequence SEQ ID NO: 390 (V_{D3} of sequence SEQ ID NO: 138, L₃ is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L₄ is 0 amino acid and C_{H1} of sequence SEQ ID NO: 313), or
20 a sequence at least 85% identical to SEQ ID NO: 390 in which the 3 CDRs of sequences SEQ ID NO: 381, SEQ ID NO: 384, and SEQ ID NO: 382 of the hz7G3 heavy chain variable domain (V_{D4} of sequence SEQ ID NO: 383), and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of
25 hz20G6 heavy chain variable domain (V_{D3} of sequence SEQ ID NO: 138) are unaltered;

and wherein the polypeptide of formula [I] and the polypeptide of formula [II] form a cross-over light chain-heavy chain pair.

30 In an embodiment, the antibody binding protein that binds specifically to human CD3ε and human CD123 comprises, or essentially consists of:

- a) one polypeptide according to formula [IV] of the amino acid sequence SEQ ID NO: 391 (V_{D1} of sequence SEQ ID NO: 385, L₁ of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L₂ of sequence SEQ ID NO: 389, C_L of

sequence SEQ ID NO: 310, L_5 which contains 0 amino acid, and F_{c2} of sequence SEQ ID NO: 392), or

a sequence at least 85% identical to SEQ ID NO: 391 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of hz7G3 light chain variable domain (V_{D1} of sequence SEQ ID NO: 385), and the 3 CDRs of sequences. SEQ ID NO:142, 'KVS' and SEQ ID NO:11 of hz20G6 light chain variable domain (V_{D2} of sequence SEQ ID NO: 141) are unaltered; and

b) one polypeptide according to formula [III] of the amino acid sequence SEQ ID NO : 393 (V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c of sequence SEQ ID NO: 394), or

a sequence at least 85% identical to SEQ ID NO : 393 in which the the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of the hz7G3 heavy chain variable domain (V_{D4} of sequence SEQ ID NO: 383), and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of hz20G6 heavy chain variable domain (V_{D3} of sequence SEQ ID NO: 138) are unaltered;

and wherein the polypeptide of formula [IV] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair.

In said antibody like binding protein, the polypeptide chains represented by the formulae [III] and [IV] dimerize through their respective F_{c2} and F_c regions.

In an embodiment, the antibody binding protein that binds specifically to human CD3 ϵ and human CD123 comprises, or essentially consists of:

a) one polypeptide according to formula [I] consisting of the amino acid sequence SEQ ID NO: 388 (V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310) or

a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of hz7G3 light chain variable domain (V_{D1} of sequence SEQ ID NO: 385), and the 3 CDRs of sequences. SEQ ID NO:142, 'KVS' and SEQ ID NO:11 of hz20G6 light chain variable domain (V_{D2} of sequence SEQ ID NO: 141) are unaltered; and

- b) one polypeptide according to formula [III] of the amino acid sequence SEQ ID NO : 395 (V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c of sequence SEQ ID NO: 396), or
- 5 a sequence at least 85% identical to SEQ ID NO : 395 in which the the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of the hz7G3 heavy chain variable domain (V_{D4} of sequence SEQ ID NO: 383), and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of hz20G6 heavy chain variable domain (V_{D3} of sequence SEQ ID NO: 138) are unaltered; and
- 10 c) one F_c stump (polypeptide F_{c3}) of the amino acid sequence SEQ ID NO : 397, or a sequence at least 85% identical thereto, wherein said F_{c3} stump or the sequence at least 85% identical thereto heterodimerises with the F_c region of the polypeptide according to formula [III];
- and wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a
- 15 cross-over light chain-heavy chain pair.

In an embodiment, the antibody binding protein that binds specifically to human CD3 ϵ and human CD123 comprises, or essentially consists of:

- a) one polypeptide according to formula [I] consisting of the amino acid sequence
- 20 SEQ ID NO: 388 (V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310) or
- a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of hz7G3 light chain
- 25 variable domain (V_{D1} of sequence SEQ ID NO: 385), and the 3 CDRs of sequences. SEQ ID NO:142, 'KVS' and SEQ ID NO:11 of hz20G6 light chain variable domain (V_{D2} of sequence SEQ ID NO: 141) are unaltered;
- b) a polypeptide according to formula III of the amino acid sequence SEQ ID NO: 399 (V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of
- 30 sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c of sequence SEQ ID NO: 400), or
- a sequence at least 85% identical thereto in which the the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of the hz7G3 heavy chain variable domain (V_{D4} of sequence SEQ ID NO: 383), and

the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of hz20G6 heavy chain variable domain (V_{D3} of sequence SEQ ID NO: 138) are unaltered; and

- 5 c) one Fc stump (polypeptide F_{c3}) of the amino acid sequence SEQ ID NO : 398, or a sequence at least 85% identical thereto, wherein said F_{c3} stump or the sequence at least 85% identical thereto heterodimerises with the Fc region of the polypeptide according to formula [III];

and wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair.

10

Immunoconjugates

In one embodiment, the anti-CD123 antibody of the invention is conjugated or linked to a growth inhibitory agent, cytotoxic agent, or a prodrug-activating enzyme. In particular, anti-CD123 antibodies of the invention are indeed useful for targeting said growth inhibitory agent, cytotoxic agent, or a prodrug to the cancerous cells expressing or over-expressing CD123 on their surface.

15

Nucleic acids, vectors and recombinant host cells

A further object of the invention relates to a nucleic acid sequence comprising or consisting of a sequence encoding an anti-CD3 antibody, anti-CD123 antibody or an antibody-like binding protein as defined above.

20

Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector.

25

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

30

So, a further object of the invention relates to a vector comprising a nucleic acid of the invention.

Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said polypeptide upon

administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40 (Mizukami T. et al. 1987), LTR promoter and enhancer of Moloney mouse leukemia virus (Kuwana Y et al. 1987), promoter (Mason JO et al. 1985) and enhancer (Gillies SD et al. 1983) of immunoglobulin H chain and the like.

Any expression vector for animal cell can be used, so long as a gene encoding the human antibody C region can be inserted and expressed. Examples of suitable vectors include pAGE107 (Miyaji H et al. 1990), pAGE103 (Mizukami T et al. 1987), pHSG274 (Brady G et al. 1984), pKCR (O'Hare K et al. 1981), pSG1 beta d2-4-(Miyaji H et al. 1990) and the like. Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like.

Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

A further object of the present invention relates to a cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the invention.

The term "transformation" means the introduction of a "foreign" (i.e. extrinsic) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

The nucleic acids of the invention may be used to produce a recombinant antibody of the invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell.

Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other

examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include *E. coli*, *Kluyveromyces* or *Saccharomyces* yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as
 5 primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G et al; 1980), rat
 10 YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as "YB2/0 cell"), and the like. The YB2/0 cell is preferred, since ADCC activity of chimeric or humanized antibodies is enhanced when expressed in this cell.

In particular, for expression of humanized antibody or antibody-like binding protein, the expression vector may be either of a type in which a gene encoding an antibody heavy chain and a gene encoding an antibody light chain exists on separate
 15 vectors or of a type in which both genes exist on the same vector (tandem type). In respect of easiness of construction of a humanized antibody and antibody-like binding protein expression vector, easiness of introduction into animal cells, and balance between the expression levels of antibody H and L chains in animal cells, humanized
 20 antibody expression vector of the tandem type is preferred (Shitara K et al. J Immunol Methods. 1994 Jan. 3;167(1-2):271-8). Examples of tandem type humanized antibody expression vector include pKANTEX93 (WO 97/10354), pEE18 and the like.

The present invention also relates to a method of producing a recombinant host cell expressing an anti-CD3 antibody, anti-CD123 antibody or an antibody-like binding
 25 protein according to the invention, said method comprising the steps consisting of : (i) introducing *in vitro* or *ex vivo* a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing *in vitro* or *ex vivo* the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said antibody.

30 Such recombinant host cells can be used for the production of anti-CD3 antibody, at least one anti-CD123 antibody or at least one antibody-like binding protein of the invention.

Methods of producing antibodies antibody-like binding protein of the invention

One embodiment of the invention provides a method for making an antibody-like binding protein comprising two polypeptide chains that form two antigen-binding sites, wherein a first polypeptide has a structure represented by the formula [I]:

$$V_{D1}-L_1-V_{D2}-L_2-C_L \quad [I]$$

and a second polypeptide has a structure represented by the formula [II]:

$$V_{D3}-L_3-V_{D4}-L_4-C_{H1} \quad [II]$$

wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

10 V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

15 L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the first and the second polypeptide form a cross-over light chain-heavy chain pair, and

20 wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.

25 In a further embodiment, the invention provides a method for making an antibody-like binding protein comprising four polypeptide chains that form four antigen-binding sites, wherein two polypeptide chains have a structure represented by the formula [I]:

$$V_{D1}-L_1-V_{D2}-L_2-C_L \quad [I]$$

and two polypeptide chains have a structure represented by the formula (III):

$$V_{D3}-L_3-V_{D4}-L_4-C_{H1}-F_c \quad [III]$$

30 wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

5 L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the polypeptides of formula I and the polypeptides of formula III form a cross-over light chain-heavy chain pair, and

10 wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.

15 In a further embodiment, the invention provides a method for making an antibody-like binding protein comprising four polypeptide chains that form four antigen-binding sites, wherein two polypeptide chains have a structure represented by the formula [IV]:



and two polypeptide chains have a structure represented by the formula [III]:



wherein:

20 V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

25 C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

F_{c2} is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

30 L_1 , L_2 , L_3 , L_4 and L_5 are amino acid linkers;

and wherein the polypeptides of formula I and the polypeptides of formula III form a cross-over light chain-heavy chain pair, and

wherein V_{D1} and V_{D2} are both either variable domains of light chains, or variable domains of heavy chains, and V_{D3} and V_{D4} are both variable domains of heavy

chains if V_{D1} and V_{D2} are variable domains of light chains, or V_{D3} and V_{D4} are both variable domains of light chains if V_{D1} and V_{D2} are variable domains of heavy chains.

5 In one embodiment of the invention, the first immunoglobulin or the second immunoglobulin is one anti-CD3 antibody as defined in the section «anti-CD3 antibodies» above.

In another embodiment of the invention, the first immunoglobulin or the second immunoglobulin is one anti-CD123 antibody as defined in the section «anti-CD123
10 antibodies» above.

An Anti-CD3 antibody, anti-CD123 antibody and/or antibody-like binding proteins of the invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in
15 combination.

Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said antibodies or immunoglobulin chains, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, in particular using a commercially available peptide synthesis
20 apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, antibodies, immunoglobulin chains and antibody-like binding proteins of the invention can be synthesized by recombinant DNA techniques as is well-known in the art. For example, these fragments can be obtained as DNA expression products after incorporation of DNA sequences
25 encoding the desired (poly)peptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired polypeptide, from which they can be later isolated using well-known techniques.

In particular, the invention further relates to a method of producing anti-CD3 antibodies, anti-CD123 antibodies and/or antibody-like binding proteins of the invention,
30 which method comprises the steps consisting of: (i) culturing a transformed host cell according to the invention; (ii) expressing said antibody or polypeptide; and (iii) recovering the expressed antibody or polypeptide.

Anti-CD3 antibodies, anti-CD123 antibodies and/or antibody-like binding proteins of the invention are suitably separated from the culture medium by conventional

immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

5 In a particular embodiment, a humanized chimeric anti-CD3 antibodies and/or anti-CD123 of the present invention can be produced by obtaining nucleic sequences encoding humanized VL and VH domains as previously described, constructing a human chimeric antibody expression vector by inserting them into an expression vector for animal cell having genes encoding human antibody CH and human antibody CL, and expressing the coding sequence by introducing the expression vector into an animal
10 cell. In analogy thereto, a humanized antibody-like binding protein can be obtained by using for the variable domain of heavy or light chain of a first humanized immunoglobulin (V_{D1}), for the variable domain of heavy or light chain of a second humanized immunoglobulin (V_{D2}), for the variable domain of heavy or light chain of said second immunoglobulin (V_{D3}), and for the variable domain of heavy or light chain of said
15 first immunoglobulin the variable domains of the heavy and light chains of two humanized antibodies.

As the CH domain of a human chimeric antibody or the CH domain of antibody-like binding protein of the invention, it may be any region which belongs to human immunoglobulin heavy chains, but those of IgG class are suitable and any one of
20 subclasses belonging to IgG class, such as IgG1, IgG2, IgG3 and IgG4, can also be used. Also, as the CL of a human chimeric antibody or the CL of an antibody-like binding protein of the invention, it may be any region which belongs to human immunoglobulin light chains, and those of kappa class or lambda class can be used.

Methods for producing humanized or chimeric antibodies involve conventional
25 recombinant DNA and gene transfection techniques are well known in the art (See Morrison SL. et al. (1984) and patent documents US5,202,238; and US5,204, 244).

Methods for producing humanized antibodies based on conventional recombinant DNA and gene transfection techniques are well known in the art (See, e. g., Riechmann L. et al. 1988; Neuberger MS. et al. 1985). Antibodies can be humanized using a variety
30 of techniques known in the art including, for example, the technique disclosed in the application WO2009/032661, CDR-grafting (EP 239,400; PCT publication WO91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan EA (1991); Studnicka GM et al. (1994); Roguska MA. et al. (1994)), and chain shuffling (U.S. Pat. No.5,565,332). The general recombinant DNA

technology for preparation of such antibodies is also known (see European Patent Application EP 125023 and International Patent Application WO 96/02576).

5 The Fab of the present invention can be obtained by treating an antibody which specifically reacts with CD3 or CD123 with a protease, such as papaine. Also, the Fab can be produced by inserting DNA sequences encoding both chains of the Fab of the antibody into a vector for prokaryotic expression, or for eukaryotic expression, and introducing the vector into prokaryotic or eukaryotic cells (as appropriate) to express the Fab.

10 The F(ab')₂ of the present invention can be obtained treating an antibody which specifically reacts with CD3 or CD123 with a protease, pepsin. Also, the F(ab')₂ can be produced by binding Fab' described below via a thioether bond or a disulfide bond.

15 The Fab' of the present invention can be obtained treating F(ab')₂ which specifically reacts with CD3 or CD123 with a reducing agent, such as dithiothreitol. Also, the Fab' can be produced by inserting DNA sequences encoding Fab' chains of the antibody into a vector for prokaryotic expression, or a vector for eukaryotic expression, and introducing the vector into prokaryotic or eukaryotic cells (as appropriate) to perform its expression.

20 The scFv of the present invention can be produced by taking sequences of the CDRs or VH and VL domains as previously described, constructing a DNA encoding an scFv fragment, inserting the DNA into a prokaryotic or eukaryotic expression vector, and then introducing the expression vector into prokaryotic or eukaryotic cells (as appropriate) to express the scFv. To generate a humanized scFv fragment, a well known technology called CDR grafting may be used, which involves selecting the complementary determining regions (CDRs) according to the invention, and grafting them onto a human scFv fragment framework of known three dimensional structure (see, e. g., W098/45322; WO 87/02671; US5,859,205; US5,585,089; US4,816,567; EP0173494).

Modification of the antibodies of the invention

30 Amino acid sequence modification(s) of the antibodies or antibody-like binding proteins as described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody nor or antibody-like binding protein. For instance, it is known that when a humanized antibody is produced by simply grafting only CDRs in VH and VL of an antibody derived from a

non-human animal in FRs of the VH and VL of a human antibody, the antigen binding activity may be reduced in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the VH and VL of the non-human antibody, not only in CDRs but also in FRs, may be directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the VH and VL of the human antibody would reduce the binding activity. In order to solve the problem, in human antibodies grafted with non-human CDRs, attempts have to be made to identify, among amino acid sequences of the FR of the VH and VL of human antibodies, an amino acid residue which is directly associated with binding of the antibody, or which interacts with an amino acid residue of a CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding to the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal. An antibody-like binding protein of the invention may comprise the variable regions of a humanized antibody and therefore herein mentioned considerations apply equally to antibody-like binding proteins of the invention.

Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still result in a functional antibody, antibody-like binding protein or polypeptide with desirable characteristics.

In making the changes in the amino sequences of polypeptide, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8) ; phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate -3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

A further object of the present invention also encompasses function-conservative variants of the polypeptides of the anti-CD3 antibodies, anti-CD123 antibodies and antibody-like binding proteins of the present invention.

For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a protein define its biological functional activity, certain amino acid substitutions can be made in a protein sequence, and of course in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the antibodies sequences of the invention, or corresponding DNA sequences which encode said polypeptides, without appreciable loss of their biological activity.

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein. It is also possible to use well-established technologies, such as alanine-scanning approaches, to identify, in an antibody or polypeptide of the invention, all the amino acids that can be substituted without significant loss of binding to the antigen. Such residues can be qualified as neutral, since they are not involved in antigen binding or in maintaining the structure of the antibody. One or more of these neutral positions can be substituted by alanine or by another amino acid can without changing the main characteristics of the antibody or polypeptide of the invention.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

It may be also desirable to modify the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein of the present invention with respect to effector function, e.g. so as to enhance or reduce antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody, herein also called Fc-variants in context with the antibody-like binding proteins of the present invention. Alternatively or additionally, cysteine residue(s) may be introduced in

the Fc region, thereby allowing inter-chain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved or reduced internalization capability and/or increased complement-mediated cell killing and/or antibody-dependent cellular cytotoxicity (ADCC) (Caron PC. et al. 1992; and Shopes B. 1992).

5 Another type of amino acid modification of the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein of the invention may be useful for altering the original glycosylation pattern of the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein, i.e. by deleting one or more carbohydrate moieties found in the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein, and/or
10 adding one or more glycosylation sites that are not present in the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein. The presence of either of the tripeptide sequences asparagine-X-serine, and asparagine-X-threonine, where X is any amino acid except proline, creates a potential glycosylation site. Addition or deletion of glycosylation sites to the anti-CD3 antibody, anti-CD123 antibody and antibody-like
15 binding protein is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites).

 Another type of modification involves the removal of sequences identified, either in silico or experimentally, as potentially resulting in degradation products or heterogeneity
20 of anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein preparations. As examples, deamidation of asparagine and glutamine residues can occur depending on factors such as pH and surface exposure. Asparagine residues are particularly susceptible to deamidation, primarily when present in the sequence Asn-Gly, and to a lesser extent in other dipeptide sequences such as Asn-Ala. When such a
25 deamidation site, in particular Asn-Gly, is present in an anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein or polypeptide of the invention, it may therefore be desirable to remove the site, typically by conservative substitution to remove one of the implicated residues. Such substitutions in a sequence to remove one or more of the implicated residues are also intended to be encompassed by the present
30 invention.

 Another type of covalent modification involves chemically or enzymatically coupling glycosides to the anti-CD3 antibody, anti-CD123 antibody and antibody-like binding protein. These procedures are advantageous in that they do not require production of anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein in

a host cell that has glycosylation capabilities for N-or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. For example, such methods are described in WO87/05330.

Removal of any carbohydrate moieties present on the anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the antibody intact. Chemical deglycosylation is described by Sojahn H. et al. (1987) and by Edge, AS. et al. (1981). Enzymatic cleavage of carbohydrate moieties on antibodies can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura, NR. et al. (1987).

Another type of covalent modification of the anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein comprises linking the antibody to one of a variety of non proteinaceous polymers, eg. , polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in US Patent Nos. 4,640, 835; 4,496, 689; 4,301, 144; 4,670, 417; 4,791, 192 or 4,179,337.

Pharmaceutical compositions

The anti-CD3 antibody, anti-CD123 antibody and/or antibody-like binding protein of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

Thus, another object of the invention relates to a pharmaceutical composition comprising an anti-CD3 antibody, an anti-CD123 antibody or antibody-like binding protein of the invention and a pharmaceutically acceptable carrier.

The invention also relates to an anti-CD3 antibody, an anti-CD123 antibody or antibody-like binding protein according to the invention, for use as a medicament. The

invention also relates to a pharmaceutical composition of the invention for use as a medicament.

The terms "pharmaceutical composition" or "therapeutic composition" as used herein refer to a compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

Such therapeutic or pharmaceutical compositions may comprise a therapeutically effective amount of an anti-CD3 antibody, an anti-CD123 antibody or antibody-like binding protein or drug conjugates thereof, in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

As used herein, "pharmaceutically-acceptable carriers" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, and the like that are physiologically compatible. Examples of suitable carriers, diluents and/or excipients include one or more of water, amino acids, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, as well as combination thereof. In many cases, it will be preferable to include isotonic agents, such as sugars, polyalcohols, or sodium chloride in the composition and formulation may also contain an antioxidant such as tryptamine and a stabilizing agent such as Tween 20.

The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and gender of the patient, etc.

The pharmaceutical compositions of the invention can be formulated for a topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

In particular, the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on

the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the
5 relevant pathology, or alternatively of the desired duration of treatment.

To prepare pharmaceutical compositions, an effective amount of the antibody or immunoconjugate of the invention may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

The pharmaceutical forms suitable for injectable use include sterile aqueous
10 solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
15 microorganisms, such as bacteria and fungi.

Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid
20 polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

A anti-CD3 antibody, anti-CD123 antibody or antibody-like binding of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of
25 the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, glycine,
30 histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the

5 maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

10 Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and 15 freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

20 The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

25 Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

30 For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage

will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

5 In one embodiment, an anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein of the invention is formulated within a therapeutic mixture to comprise about 0.01 to 100 milligrams, per dose or so.

10 In addition to the anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time-release capsules; and any other form currently used.

In certain embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of polypeptides such as anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

15 Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) are generally designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made.

20 Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm . Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations.

25 Once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations can be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

Therapeutic methods and uses

The inventors have shown for several bi-specific compounds of the invention, such as “hz20G6x7G3”, “7G3xhz4B4”, “hz4B4x3E3” and “hz20G6x7G3–TL4” T-cell mediated cytotoxicity on a CD123 positive tumor cell line model. Furthermore, the inventors demonstrated the capacity of for several bi-specific compounds of the invention to activate T-cells in presence of target cells leading to cytotoxicity of the tumor cells. The inventors further demonstrated the low activation of T-cells in the absence of T-cell activation in absence of target cells.

It is well known that therapeutic anti-CD123 monoclonal antibodies can lead to the depletion of cells bearing the antigen specifically recognized by the antibody. This depletion can be mediated through at least three mechanisms: antibody mediated cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and direct anti-tumour inhibition of tumour growth through signals given via the antigen targeted by the antibody. In one embodiment, the anti-CD123 antibodies of the invention induce cytotoxicity in a CD123 expressing cell by antibody mediated cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC).

“Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system to antibodies which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al. (1997) may be performed.

“Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted antibodies bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed.

As described above in the section “anti-CD3 antibodies” the anti-CD3 antibodies of the invention have a low T-cell activation they thus have a therapeutic potential in a subject for use as an immunosuppressive agent.

Furthermore, an antibody-like binding protein as defined above aims at enhancing the patient’s immune response to tumors by targeting T-cells to tumor cells. In one embodiment, the antibody-like binding protein as defined above targets CD3ε subunit of

the T-cell Receptor (TCR) at the surface of the T-cell and the other arm targets a CD123 expressing cancer cell, wherein the co-engagement of T-cell and tumor cell by the bispecific construct leads to the formation of a cytolytic synapse which induces T-cell activation and results in tumor cell killing. The tumor cell killing may be mediated through at least two mechanisms: Perforin/Granzyme Killing and FasL/Fas Killing, for example Perforin/Granzyme Killing.

Therefore, in one embodiment the invention provides a method of treating or preventing a disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of an anti-CD3 antibody, an anti-CD123 antibody, antibody-like binding protein or a pharmaceutical composition of the invention as defined above in the section "Pharmaceutical composition".

The invention further refers to the use of anti-CD3 antibody, an anti-CD123 antibody, antibody-like binding protein or a pharmaceutical composition of the invention for the preparation of a medicament for treating or preventing a disease or disorder in a subject. In one embodiment, the invention refers to the use of an of anti-CD3 antibody, anti-CD123 antibody, antibody-like binding protein or a pharmaceutical composition for treating or preventing a disease or disorder in a subject.

The term "subject" or "individual" are used interchangeably and may be, for example, a human or a non-human mammal. For example, the subject is a bat; a ferret; a rabbit; a feline (cat); a canine (dog); a primate (monkey), an equine (horse); a human, including man, woman and child. In one embodiment a "subject" refers to a human.

In the context of the invention, the term "treating" or "treatment", refers to a therapeutic use (i.e. on a subject having a given disease) and means reversing, alleviating, inhibiting the progress of one or more symptoms of such disorder or condition. Therefore, treatment does not only refer to a treatment that leads to a complete cure of the disease, but also to treatments that slow down the progression of the disease and/or prolong the survival of the subject.

By "preventing" is meant a prophylactic use (i.e. on a subject susceptible of developing a given disease).

In one embodiment, a "disease" or "disorder" is any condition that would benefit from treatment with the anti-CD123 antibody or antibody-like binding protein of the invention. In one embodiment, this includes chronic and acute disorders or diseases including those pathological conditions which predisposes the subject to the disorder in

question. The term “in need of treatment” refers to a subject having already the disorder as well as those in which the disorder is to be prevented.

In one embodiment, a “disease” or “disorder” is any condition that would benefit from treatment with the anti-CD3 antibody of the invention. Therefore, in one embodiment, this includes diseases or disorders characterized by pathological immune responses.

A “pathological immune response” in the context of the invention is an inflammatory immune response.

In one embodiment, the disease, characterized by a pathological immune response, is an autoimmune diseases, a transplantation-related diseases, or an inflammation-associated diseases.

The auto-immune disease is, for example, Crohn's disease, ulcerative colitis and type 1 diabetes or a transplantation-related disease such as graft-versus-host disease (GVHD).

Therefore, in one embodiment, the subject has been diagnosed to suffer from a disease or disorder characterized by a pathological immune response.

In one embodiment, the subject has been diagnosed to suffer from an auto-immune disease.

In another embodiment, the disorder refers to cancer.

In a further embodiment, cancer relates to hematological cancer, in particular to hematological cancer associated with CD123 expression.

In one embodiment, expression of CD123 by cancer cells is readily assayed for instance by using an anti-CD123 antibody according to the invention, as described in the following section “Diagnostic uses”.

“Hematological cancers associated with CD123 expression” include leukemias (such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia, hairy cell leukemia and myelodysplasia syndrome) and malignant lymphoproliferative conditions, blastic plasmacytoid dendritic cell neoplasm (BPDCN), systemic mastocytosis, including lymphomas (such as multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-follicular lymphoma).

As described above in the section ‘anti-CD123 antibodies’ LSCs express CD123.

Thus, in a related embodiment cancer refers to hematological cancer associated associated with leukemic stem cells.

The hematologic cancer conditions associated with leukemic stem cells (LSCs) which are to be treated in accordance with the present invention include leukemias (such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplasia syndrome) and malignant lymphoproliferative conditions, including lymphomas (such as multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-follicular lymphoma).

In one aspect of the invention, the hematologic cancer is acute myelogenous leukemia (AML).

10 In one embodiment, the subject has been diagnosed to suffer from AML.

In a further embodiment, the subject has already been treated with chemotherapy until complete remission but relapsed.

The "relapse" is defined as the reoccurrence of AML after complete remission.

15 "Complete remission" or "CR" is defined as follows: normal values for neutrophil ($>1.0 \times 10^9/L$), haemoglobin level of 10g/dL and platelet count ($>100 \times 10^9/L$) and independence from red cell transfusion; blast cells less than 5%, no clusters or collections of blasts, and absence of Auer rods on bone marrow examination; and normal maturation of blood cells (morphology; myelogramme) and absence of extramedullary leukemia.

20 In one embodiment, the anti-CD3 antibody, anti-CD123 antibody or antibody-like binding protein of the invention is used alone or in combination with any suitable growth-inhibitory agent.

By a "therapeutically effective amount" of the polypeptide of the invention is meant a sufficient amount of the polypeptide to treat said cancer disease, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the polypeptides and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; 25 activity of the specific polypeptide employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific polypeptide employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is 30

well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

5 In one embodiment, efficacy of the treatment with an anti-CD3 antibody, an anti-CD123 antibody or antibody-like binding protein of the invention or immunoconjugate according to the invention is readily assayed *in vivo*, for instance in a mouse model of cancer and by measuring, for example, changes in tumor volume between treated and control groups.

Diagnostic uses

10 CD123 has been reported to be over-expressed on the surface of a variety of hematological neoplasms.

Therefore, CD123 constitutes a cancer marker and, therefore, has the potential to be used to indicate the effectiveness of an anti-cancer therapy or detecting recurrence of the disease.

15 In an embodiment, the anti-CD123 antibody of the invention is used as component of an assay in the context of a therapy targeting CD123 expressing tumours, in order to determine susceptibility of the patient to the therapeutic agent, monitor the effectiveness of the anti-cancer therapy or detect recurrence of the disease after treatment. In particular, the same anti-CD123 antibody of the invention is used both as component of
20 the therapeutic agent and as component of the diagnostic assay.

Thus, a further object of the invention relates to an anti-CD123 antibody according to the invention for use for *in vivo* detecting CD123 expression in a subject, or for use for *ex vivo* detecting CD123 expression in biological sample of a subject. In one embodiment, said detection is intended, in particular for:

- 25 a) diagnosing the presence of a cancer in a subject, or
b) determining susceptibility of a patient having cancer to a therapeutic agent targeting CD123, in particular an anti-CD123 antibody or antibody-like binding protein according to the invention, or
c) monitoring effectiveness of anti-CD123 cancer therapy or detecting cancer
30 relapse after anti-CD123 cancer therapy, in particular for therapy with an anti-CD123 antibody or antibody-like binding protein according to the invention; by detecting expression of the surface protein CD123 on tumor cells.

In an embodiment, the antibody is intended for an *in vitro* or *ex vivo* use. For example, CD123 is detected *in vitro* or *ex vivo* in a biological sample obtained from a subject, using an anti-CD123 antibody of the invention. The use according to the invention may also be an *in vivo* use. For example, an anti-CD123 antibody or antibody-like binding protein according to the invention is administered to the subject and antibody-cells complexes are detected and/or quantified, whereby the detection of said complexes is indicative of a cancer.

The invention further relates to an *in vitro* or *ex vivo* method of detecting the presence of a cancer in a subject, comprising the steps consisting of:

- (a) contacting a biological sample of a subject with an anti-CD123 antibody according to the invention, in particular in conditions sufficient for the antibody to form complexes with said biological sample;
- (b) measuring the level of antibody bound to said biological sample,
- (c) detecting the presence of a cancer by comparing the measured level of bound antibody with a control, an increased level of bound antibody compared to control being indicative of a cancer.

The invention also relates to an *in vitro* or *ex vivo* method of determining susceptibility of a patient having cancer to a therapeutic agent targeting CD123, in particular to an anti-CD123 antibody or antibody-like binding protein according to the invention, which method comprises the steps consisting of:

- (a) contacting a biological sample sample of a patient having cancer with an anti-CD123 antibody according to the invention, in particular in conditions sufficient for the antibody to form complexes with said biological sample;
- (b) measuring the level of antibody bound to said biological sample sample,
- (c) comparing the measured level of bound antibody to said biological sample sample with the level of antibody bound to a control;

wherein an increased level of bound antibody to said biological sample sample compared to control is indicative of a patient susceptible to a therapeutic agent targeting at least CD123.

In the above methods, said control can be a normal, non cancerous, biological sample of the same type, or a reference value determined a representative of the antibody binding level in normal biological sample of the same type.

5 In an embodiment, the anti-CD123 antibody of the invention are useful for diagnosing hematological cancers associated with CD123 expression including leukemias (such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia, hairy cell leukemia and myelodysplasia syndrome) and malignant lymphoproliferative conditions, blastic plasmacytoid dendritic
10 cell neoplasm (BPDCN), systemic mastocytosis, including lymphomas (such as multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-follicular lymphoma).

The invention further relates to an *in vitro* or *ex vivo* method of monitoring
15 effectiveness of anti-CD123 cancer therapy, comprising the steps consisting of:

- (a) contacting a biological sample of a subject undergoing anti-CD123 cancer therapy, with an anti-CD123 antibody or antibody-like binding protein according to the invention, in particular in conditions sufficient for the antibody to form complexes with said biological sample;
- 20 (b) measuring the level of antibody bound to said biological sample,
- (c) comparing the measured level of bound antibody with the level of antibody bound to a control;

wherein a decreased level of bound antibody to said biological sample compared to control is indicative of effectiveness of said anti-CD123 cancer therapy.

25 In said method, an increased level of bound antibody to said biological sample compared to control is indicative of ineffectiveness of said anti-CD123 cancer therapy.

Said control is in particular a biological sample of the same type as the biological sample submitted to analysis, but which was obtained from the subject previously in time, during the course of the anti-CD123 cancer therapy.

30 The invention further relates to an *in vitro* or *ex vivo* method of detecting cancer relapse after anti-CD123 cancer therapy, comprising the steps consisting of:

- (a) contacting a biological sample of a subject having completed anti-CD123 cancer therapy, with an anti-CD123 antibody or antibody-like

binding protein according to the invention, in particular in conditions sufficient for the antibody to form complexes with said biological sample;

(b) measuring the level of antibody bound to said biological sample,

5 (c) comparing the measured level of bound antibody with the level of antibody bound to a control;

wherein an increased level of bound antibody to said biological sample compared to control is indicative of cancer relapse after anti-CD123 cancer therapy.

10 Said control is in particular a biological sample of the same type as the biological sample submitted to analysis, but which was obtained from the subject previously in time, upon or after completion of the anti-CD123 cancer therapy.

Said anti-CD123 cancer therapy is in particular a therapy using an anti-CD123 antibody or antibody-like binding protein or immunoconjugate according to the invention.

15 Said anti-CD123 cancer therapy targets a CD123 expressing cancer, in particular a hematological cancers associated with CD123 expression including leukemias (such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia, hairy cell leukemia and myelodysplasia syndrome) and malignant lymphoproliferative conditions, blastic plasmacytoid dendritic cell neoplasm (BPDCN), systemic mastocytosis, including lymphomas (such as multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-follicular lymphoma).

20

In an embodiment, anti-CD123 of the invention is labelled with a detectable molecule or substance, such as a fluorescent molecule, a radioactive molecule or any other labels known in the that provide (either directly or indirectly) a signal.

25

As used herein, the term "labeled", with regard to the anti-CD123 antibody according to the invention, is intended to encompass direct labeling of the anti-CD123 antibody by coupling (i.e., physically linking) a detectable substance, such as a radioactive agent or a fluorophore (e.g. fluorescein isothiocyanate (FITC) or phycoerythrin (PE) or Indocyanine (Cy5)) to the polypeptide, as well as indirect labeling of the polypeptide by reactivity with a detectable substance.

30

In a further embodiment, an anti-CD123 antibody of the invention is labelled with a radioactive molecule by any method known to the art. For example radioactive

molecules include but are not limited radioactive atom for scintigraphic studies such as I^{123} , I^{124} , In^{111} , Re^{186} , Re^{188} , Tc^{99} . In one example, polypeptides of the invention are also labelled with a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

A "biological sample" encompasses a variety of sample types obtained from a subject and can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. Therefore, biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples, in particular tumor sample.

The invention also relates to an *in vivo* method of detecting the presence of a cancer in a subject, comprising the steps consisting of:

- a) administering an antibody according to the invention detectably labelled to a patient;
- b) detecting localization of said detectably labelled antibody in the patient by imaging.

In one embodiment, antibodies of the invention are useful for staging of cancer (e.g., in radioimaging). They are used, for example, alone or in combination with other cancer markers.

The terms "detection" or "detected" as used herein includes qualitative and/or quantitative detection (measuring levels) with or without reference to a control.

In the context of the invention, the term "diagnosing", as used herein, means the determination of the nature of a medical condition intended to identify a pathology which affects the subject from a number of collected data.

In said method, the cancer is a CD123 expressing cancer as defined above.

Kits

Finally, the invention also provides kits comprising at least one anti-CD3 antibody, at least one anti-CD123 antibody or at least one antibody-like binding protein of the invention. Kits containing anti-CD123 or anti-CD3 antibodies of the invention find use in detecting the surface protein CD123 or CD3, or in therapeutic or diagnostic assays. Kits

of the invention can contain a polypeptide or anti-CD3 antibody, at least one anti-CD123 antibody or at least one antibody-like binding protein coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantification of the surface protein CD123 or CD3
 5 *in vitro*, e.g. in an ELISA or a Western blot. In one embodiment, said antibody is useful for detection and is provided with a label such as a fluorescent or radiolabel.

In one embodiment, the invention encompasses kits for producing a single-dose administration unit. The kits can each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the
 10 scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

Throughout the instant application, the term “comprising” is to be interpreted as encompassing all specifically mentioned features as well optional, additional,
 15 unspecified ones. As used herein, the use of the term “comprising” also discloses the embodiment wherein no features other than the specifically mentioned features are present (*i.e.* “consisting of”). Furthermore the indefinite article “a” or “an” does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be
 20 used to advantage.

The invention will now be described in more details with reference to the following figures and examples. All literature and patent documents cited herein are hereby incorporated by reference. While the invention has been illustrated and described in
 25 detail in the foregoing description, the examples are to be considered illustrative or exemplary and not restrictive.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 shows the amino acid sequence of full-length human CD3ε protein, including the signal peptide, as available from the Uniprot database under accession
 30 number P07766.

SEQ ID NO: 2 shows the amino acid sequence of full-length *Macaca fascicularis* CD3ε protein, including the signal peptide, as available from the Uniprot database under accession number Q95LI5.

SEQ ID NO: 3 shows the amino acid sequence of mature human CD3ε His-tagged Fc-fusion comprising amino acids 23 to 126 of the full-length wild-type human CD3ε protein.

5 SEQ ID NO: 4 shows the amino acid sequence of mature *Macaca fascicularis* CD3ε Fc-fusion comprising amino acids 23 to 117 of the full-length wild-type *Macaca fascicularis* CD3ε protein (SEQ ID NO:2) containing one Ala to Val exchange at amino acid position 35 in comparison to amino acid position 57 of the wild-type sequence.

SEQ ID NO: 5 shows the amino acid sequence of the heavy chain variable domain of the so-called "20G6-F3" antibody.

10 SEQ ID NO: 6, 7 and 8 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "20G6-F3" antibody.

SEQ ID NO: 9 shows the amino acid sequence of the light chain variable domain of the so-called "20G6-F3" antibody.

15 SEQ ID NO: 10 shows the amino acid sequence of CDR1-L of the so-called "20G6-F3", "11D7-C3", "13H2-C2", "13C1-F6", "1E6-C9", "10F4-C10", "18G9-H11", "12G3-E8", "5B1-G2", "16F8-A7", "11F9-F8", "8C2-F7", "20E5-F10" and "3H6-D2" antibodies.

20 SEQ ID NO: 11 shows the amino acid sequence of CDR3-L of the so-called "20G6-F3", "4B4-D7", "11H3-E5", "13H2-C2", "13C1-F6", "10F4-C10", "4E7-C9", "11F3-B9", "12G3-E8", "5B1-G2", "16F8-A7", "20E5-F10" and "3H6-D2" antibodies.

SEQ ID NO: 12 shows the amino acid sequence of the heavy chain variable domain of the so-called "4B4-D7" antibody.

25 SEQ ID NO: 13 shows the amino acid sequence of CDR1-H of the so-called "4B4-D7", "11D7-C3", "11H3-E5", "13H2-C2", "13C1-F6", "10F4-C10", "18G9-H11", "4E7-C9", "11F3-B9", "16F8-A7", "11F9-F8", "20B5-F10" and "3H6-D2" antibodies.

SEQ ID NO: 14 and 15 show the amino acid sequences of CDR2-H and CDR3-H of the so-called "4B4-D7" antibody.

SEQ ID NO: 16 shows the amino acid sequence of the light chain variable domain of the so-called "4B4-D7" antibody.

30 SEQ ID NO: 17 shows the amino acid sequence of CDR1-L of the so-called "4B4-D7", "11H3-E5" and "11F3-B9" antibodies.

SEQ ID NO: 18 shows the amino acid sequence of the heavy chain variable domain of the so-called "4E7-C9" antibody.

SEQ ID NO: 19 shows the amino acid sequence of CDR2-H of the so-called “4E7-C9”, “18F5-H10”, “20E5-F10” and “3H6-D2” antibodies.

SEQ ID NO: 20 shows the amino acid sequence of CDR3-H of the so-called “4E7-C9” antibody.

5 SEQ ID NO: 21 shows the amino acid sequence of the light chain variable domain of the so-called “4E7-C9” antibody.

SEQ ID NO: 22 shows the amino acid sequence of CDR1-H of the so-called “4E7-C9” antibody.

10 SEQ ID NO: 23 shows the amino acid sequence of the heavy chain variable domain of the so-called “18F5-H10” antibody.

SEQ ID NO: 24 shows the amino acid sequence of CDR1-H of the so-called “18F5-H10” antibody.

SEQ ID NO: 25 shows the amino acid sequence of CDR3-H of the so-called “18F5-H10” antibody.

15 SEQ ID NO: 26 shows the amino acid sequence of the light chain variable domain of the so-called “18F5-H10” antibody.

SEQ ID NO: 27 shows the amino acid sequence of CDR1-L of the so-called “18F5-H10” anti-CD3 antibody.

20 SEQ ID NO: 28 shows the amino acid sequence of CDR3-L of the so-called “18F5-H10”, “11D7-C3”, “1E6-C9” and “10E6-G6” anti-CD3 antibodies.

SEQ ID NO: 29 shows the amino acid sequence of the heavy chain variable domain of the so-called “12D2-E5” anti-CD3 antibody.

SEQ ID NO: 30 and 31 show the amino acid sequences of CDR1-H and CDR2-H of the so-called “12D2-E5” anti-CD3 antibody.

25 SEQ ID NO: 32 shows the amino acid sequence of CDR3-H of the so-called “12D2-E5” and “3G5-E10” anti-CD3 antibodies.

SEQ ID NO: 33 shows the amino acid sequence of the light chain variable domain of the so-called “12D2-E5” anti-CD3 antibody.

30 SEQ ID NO: 34 and 35 show the amino acid sequences of CDR1-H and CDR3-H of the so-called “12D2-E5” anti-CD3 antibody.

SEQ ID NO: 36 shows the amino acid sequence of the heavy chain variable domain of the so-called “11D7-C3” anti-CD3 antibody.

SEQ ID NO: 37 shows the amino acid sequence of CDR2-H of the so-called “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “18G9-H11”, “11F3-B9”, “16F8-A7”, “11F9-F8” and “20B5-F10” anti-CD3 antibodies.

5 SEQ ID NO: 38 shows the amino acid sequence of CDR3-H of the so-called “11D7-C3” anti-CD3 antibody.

SEQ ID NO: 39 shows the amino acid sequence of the light chain variable domain of the so-called “11D7-C3” anti-CD3 antibody.

SEQ ID NO: 40 shows the amino acid sequence of the heavy chain variable domain of the so-called “11H3-E5” anti-CD3 antibody.

10 SEQ ID NO: 41 shows the amino acid sequence of CDR3-H of the so-called called “11H3-E5” anti-CD3 antibody.

SEQ ID NO: 42 shows the amino acid sequence of the light chain variable domain of the so-called “11H3-E5” anti-CD3 antibody.

15 SEQ ID NO: 43 shows the amino acid sequence of the heavy chain variable domain of the so-called “13H2-C2” anti-CD3 antibody.

SEQ ID NO: 44 shows the amino acid sequence of CDR3-H of the so-called “13H2-C2” anti-CD3 antibody.

SEQ ID NO: 45 shows the amino acid sequence of the light chain variable domain of the so-called “13H2-C2” anti-CD3 antibody.

20 SEQ ID NO: 46 shows the amino acid sequence of the heavy chain variable domain of the so-called “13C1-F6” and “11F9-F8” anti-CD3 antibodies.

SEQ ID NO: 47 shows the amino acid sequence of CDR3-H of so-called “13C1-F6”, “10E6-G6” and “11F9-F8” anti-CD3 antibodies.

25 SEQ ID NO: 48 shows the amino acid sequence of the light chain variable domain of the so-called “13H2-C2” anti-CD3 antibody.

SEQ ID NO: 49 shows the amino acid sequence of the heavy chain variable domain of the so-called “18H11-F10” anti-CD3 antibody.

SEQ ID NO: 50, 51 and 52 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called “18H11-F10” anti-CD3 antibody.

30 SEQ ID NO: 53 shows the amino acid sequence of the light chain variable domain of the so-called “18H11-F10” anti-CD3 antibody.

SEQ ID NO: 54 and 55 show the amino acid sequences of CDR1-L and CDR3-L of the so-called “18H11-F10” anti-CD3 antibody.

SEQ ID NO: 56 shows the amino acid sequence of the heavy chain variable domain of the so-called "1E6-C9" anti-CD3 antibody.

SEQ ID NO: 57 and 58 show the amino acid sequences of CDR1-H and CDR3-H of the so-called "1E6-C9" anti-CD3 antibody.

5 SEQ ID NO: 59 shows the amino acid sequence of the light chain variable domain of the so-called "1E6-C9" anti-CD3 antibody.

SEQ ID NO: 60 shows the amino acid sequence of the heavy chain variable domain of the so-called "10F4-C10" anti-CD3 antibody.

10 SEQ ID NO: 61 shows the amino acid sequence of CDR3-H of the so-called "10F4-C10" anti-CD3 antibody.

SEQ ID NO: 62 shows the amino acid sequence of the light chain variable domain of the so-called "10F4-C10" anti-CD3 antibody.

SEQ ID NO: 63 shows the amino acid sequence of the heavy chain variable domain of the so-called "10E6-G6" anti-CD3 antibody.

15 SEQ ID NO: 64 and 65 show the amino acid sequences of CDR1-H and CDR2-H of the so-called "10E6-G6" anti-CD3 antibody.

SEQ ID NO: 66 shows the amino acid sequence of the light chain variable domain of the so-called "10E6-G6" anti-CD3 antibody.

20 SEQ ID NO: 67 shows the amino acid sequence of CDR1-L of the so-called "10E6-G6" anti-CD3 antibody.

SEQ ID NO: 68 shows the amino acid sequence of the heavy chain variable domain of the so-called "18G9-H11" anti-CD3 antibody.

SEQ ID NO: 69 shows the amino acid sequence of CDR3-H of the so-called "18G9-H11" anti-CD3 antibody.

25 SEQ ID NO: 70 shows the amino acid sequence of the light chain variable domain of the so-called "18G9-H11" anti-CD3 antibody.

SEQ ID NO: 71 shows the amino acid sequence of CDR3-L of the so-called "18G9-H11" anti-CD3 antibody.

30 SEQ ID NO: 72 shows the amino acid sequence of the heavy chain variable domain of the so-called "11F3-B9" anti-CD3 antibody.

SEQ ID NO: 73 shows the amino acid sequence of the light chain variable domain of the so-called "11F3-B9" anti-CD3 antibody.

SEQ ID NO: 74 shows the amino acid sequence of the heavy chain variable domain of the so-called "12G3-E8" anti-CD3 antibody.

SEQ ID NO: 75, 76 and 77 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called “12G3-E8” anti-CD3 antibody.

SEQ ID NO: 78 shows the amino acid sequence of the light chain variable domain of the so-called “12G3-E8” anti-CD3 antibody.

5 SEQ ID NO: 79 shows the amino acid sequence of the heavy chain variable domain of the so-called “5B1-G2” anti-CD3 antibody.

SEQ ID NO: 80 and 81 show the amino acid sequences of CDR1-H and CDR3-H of the so-called “5B1-G2” anti-CD3 antibody.

10 SEQ ID NO: 82 shows the amino acid sequence of the light chain variable domain of the so-called “5B1-G2” anti-CD3 antibody.

SEQ ID NO: 83 shows the amino acid sequence of a part of the variable domain of the heavy chain of the so-called “16F8-A7” anti-CD3 antibody.

SEQ ID NO: 84 shows the amino acid sequence of CDR3-H of the so-called “16F8-A7” and “11F3-B9” anti-CD3 antibodies.

15 SEQ ID NO: 85 shows the amino acid sequence of the light chain variable domain of the so-called “16F8-A7” anti-CD3 antibody.

SEQ ID NO: 86 shows the amino acid sequence of full-length human CD3 δ protein, including the signal peptide, as available from the Uniprot database under accession number P04234.

20 SEQ ID NO: 87 shows the amino acid sequence of the light chain variable domain of the so-called “11F9-F8” anti-CD3 antibody.

SEQ ID NO: 88 shows the amino acid sequence of CDR3-L of the so-called “11F9-F8” anti-CD3 antibody.

25 SEQ ID NO: 89 shows the amino acid sequence of the heavy chain variable domain of the so-called “3G5-E10” anti-CD3 antibody.

SEQ ID NO: 90 and 91 show the amino acid sequences of CDR1-H and CDR2-H of the so-called “3G5-E10” anti-CD3 antibody.

SEQ ID NO: 92 shows the amino acid sequence of the light chain variable domain of the so-called “3G5-E10” anti-CD3 antibody.

30 SEQ ID NO: 93 and 94 show the sequences of CDR1-L and CDR3-L of the so-called “3G5-E10” anti-CD3 antibody.

SEQ ID NO: 95 shows the amino acid sequence of the heavy chain variable domain of the so-called “9D7-F3” anti-CD3 antibody.

SEQ ID NO: 96, 97 and 98 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "9D7-F3" anti-CD3 antibody.

SEQ ID NO: 99 shows the amino acid sequence of the light chain variable domain of the so-called "9D7-F3" anti-CD3 antibody.

5 SEQ ID NO: 100 and 101 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "9D7-F3" and "6C9-C9" anti-CD3 antibody.

SEQ ID NO: 102 shows the amino acid sequence of the heavy chain variable domain of the so-called "8C2-F7" anti-CD3 antibody.

10 SEQ ID NO: 103, 104 and 105 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "8C2-F7" anti-CD3 antibody.

SEQ ID NO: 106 shows the amino acid sequence of the light chain variable domain of the so-called "8C2-F7" anti-CD3 antibody.

SEQ ID NO: 107 shows the amino acid sequence of the heavy chain variable domain of the so-called "20E5-F10" anti-CD3 antibody.

15 SEQ ID NO: 108 shows the amino acid sequences of CDR3-H of the so-called "20E5-F10" anti-CD3 antibody.

SEQ ID NO: 109 shows the amino acid sequence of the light chain variable domain of the so-called "20E5-F10" anti-CD3 antibody.

20 SEQ ID NO: 110 shows the amino acid sequence of the heavy chain variable domain of the so-called "20B5-F10" anti-CD3 antibody.

SEQ ID NO: 111 shows the amino acid sequence of CDR3-H of the so-called "20B5-F10" anti-CD3 antibody.

SEQ ID NO: 112 shows the amino acid sequence of the light chain variable domain of the so-called "20B5-F10" anti-CD3 antibody.

25 SEQ ID NO: 113 and 114 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "20B5-F10" anti-CD3 antibody.

SEQ ID NO: 115 shows the amino acid sequence of the heavy chain variable domain of the so-called "6C9-C9" anti-CD3 antibody.

30 SEQ ID NO: 116, 117 and 118 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "6C9-C9" anti-CD3 antibody.

SEQ ID NO: 119 shows the amino acid sequence of the light chain variable domain of the so-called "6C9-C9" anti-CD3 antibody.

SEQ ID NO: 120 shows the amino acid sequence of CDR3-L of the so-called "6C9-C9" anti-CD3 antibody.

SEQ ID NO: 121 shows the amino acid sequence of the heavy chain variable domain of the so-called "3E8-G1" anti-CD3 antibody.

SEQ ID NO: 122, 123 and 124 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "3E8-G1" anti-CD3 antibody

5 SEQ ID NO: 125 shows the amino acid sequence of the light chain variable domain of the so-called "3E8-G1" anti-CD3 antibody.

SEQ ID NO: 126 and 127 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "3E8-G1" anti-CD3 antibody.

10 SEQ ID NO: 128 shows the amino acid sequence of the heavy chain variable domain of the so-called "3H6-D2" anti-CD3 antibody.

SEQ ID NO: 129 show the amino acid sequence of CDR3-H of the so-called "3H6-D2" anti-CD3 antibody.

SEQ ID NO: 130 shows the amino acid sequence of the light chain variable domain of the so-called "3H6-D2" anti-CD3 antibody.

15 SEQ ID NO: 131 shows the amino acid sequence of the heavy chain variable domain of the so-called "8H2" anti-CD3 antibody.

SEQ ID NO: 132 shows the amino acid sequence of the light chain variable domain of the so-called "8H2" anti-CD3 antibody.

20 SEQ ID NO: 133 and 134 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "8H2" anti-CD3 antibody.

SEQ ID NO: 135 shows the VH variant amino acid sequence VH1a of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 136 shows the VH variant amino acid sequence VH1b of humanized "20G6" anti-CD3 antibody.

25 SEQ ID NO: 137 shows the VH variant amino acid sequence VH1c of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 138 shows the VH variant amino acid sequence VH1d of humanized "20G6" anti-CD3 antibody.

30 SEQ ID NO: 139 shows the VL variant amino acid sequence VL1a of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 140 shows the VL variant amino acid sequence VL1b of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 141 shows the VL variant amino acid sequence VL1c of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 142 shows the amino acid sequence of the CDR1-L of the VL1c variant of the humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 143 shows the VL variant amino acid sequence VL1d of humanized "20G6" anti-CD3 antibody.

5 SEQ ID NO: 144 shows the VH variant amino acid sequence VH2a of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 145 shows the VH variant amino acid sequence VH2b of humanized "20G6" anti-CD3 antibody.

10 SEQ ID NO: 146 shows the VH variant amino acid sequence VH2c of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 147 shows the VH variant amino acid sequence VH2d of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 148 shows the VL variant amino acid sequence VL2a of humanized "20G6" anti-CD3 antibody.

15 SEQ ID NO: 149 shows the VL variant amino acid sequence VL2b of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 150 shows the VL variant amino acid sequence VL2c of humanized "20G6" anti-CD3 antibody.

20 SEQ ID NO: 151 shows the VL variant amino acid sequence VL2d of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 152 shows the VH variant amino acid sequence VH3a of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 153 shows the VH variant amino acid sequence VH3b of humanized "20G6" anti-CD3 antibody.

25 SEQ ID NO: 154 shows the VL variant amino acid sequence VL3a of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 155 shows the VL variant amino acid sequence VL3b of humanized "20G6" anti-CD3 antibody.

30 SEQ ID NO: 156 shows the VL variant amino acid sequence VL3c of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 157 shows the VL variant amino acid sequence VL3d of humanized "20G6" anti-CD3 antibody.

SEQ ID NO: 158 shows the VL variant amino acid sequence VL1A of humanized "4B4" anti-CD3 antibody.

SEQ ID NO: 159 shows the VL variant amino acid sequence VL1B of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 160 shows the VL variant amino acid sequence VL2A of humanized “4B4” anti-CD3 antibody.

5 SEQ ID NO: 161 shows the VL variant amino acid sequence VL2B of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 162 shows the VL variant amino acid sequence VL1Cmodif1 of humanized “4B4” anti-CD3 antibody.

10 SEQ ID NO: 163 shows the VL variant amino acid sequence VL1Cmodif2 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 164 shows the VL variant amino acid sequence VL1Cmodif3 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 165 shows the VL variant amino acid sequence VL1Amodif1 of humanized “4B4” anti-CD3 antibody.

15 SEQ ID NO: 166 shows the VL variant amino acid sequence VL1Amodif2 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 167 shows the VL variant amino acid sequence VL1Amodif3 of humanized “4B4” anti-CD3 antibody.

20 SEQ ID NO: 168 shows the VL variant amino acid sequence VL2C of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 169 shows the VL variant amino acid sequence VL2D of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 170 shows the VL variant amino acid sequence VL2F of humanized “4B4” anti-CD3 antibody.

25 SEQ ID NO: 171 shows the VH variant amino acid sequence VH1A of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 172 shows the VH variant amino acid sequence VH1B of humanized “4B4” anti-CD3 antibody.

30 SEQ ID NO: 173 shows the VH variant amino acid sequence VH2A of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 174 shows the VH variant amino acid sequence VH2B of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 175 shows the VH variant amino acid sequence VH6Bmodif1 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 176 shows the VH variant amino acid sequence VH6Bmodif2 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 177 shows the VH variant amino acid sequence VH6Amodif1 of humanized “4B4” anti-CD3 antibody.

5 SEQ ID NO: 178 shows the VH variant amino acid sequence VH6Amodif2 of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 179 shows the VH variant amino acid sequence VH6Amodif3 of humanized “4B4” anti-CD3 antibody.

10 SEQ ID NO: 180 shows the VH variant amino acid sequence VH6C of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 181 shows the VH variant amino acid sequence VH6D of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 182 shows the VL variant amino acid sequence D7-VK3mut of humanized “4B4” anti-CD3 antibody.

15 SEQ ID NO: 183 shows the VH variant amino acid sequence D7-VH1mut of humanized “4B4” anti-CD3 antibody.

SEQ ID NO: 184 shows the amino acid sequence of the CDR1-L of the VL1B, VL2B, VL1Cmodif3 and VL2F variants of the humanized “4B4” anti-CD3 antibody.

20 SEQ ID NO: 185 shows the amino acid sequence of full-length human CD3 γ protein, including the signal peptide, as available from the Uniprot database under accession number P09693.

SEQ ID NO: 186 shows the amino acid sequence of the heavy chain of the Fab of so-called “20G6-F3” anti-CD3 antibody.

25 SEQ ID NO: 187 shows the amino acid sequence of the light chain of the Fab of so-called “20G6-F3” anti-CD3 antibody.

SEQ ID NO: 188 shows the amino acid sequence of the heavy chain of the Fab of so-called “4E7-C9” anti-CD3 antibody.

SEQ ID NO: 189 shows the amino acid sequence of the light chain of the Fab of so-called “4E7-C9” anti-CD3 antibody.

30 SEQ ID NO: 190 shows the amino acid sequence of the heavy chain of the Fab of so-called “4B4-D7” anti-CD3 antibody.

SEQ ID NO: 191 shows the amino acid sequence of the light chain of the Fab of so-called “4B4-D7” anti-CD3 antibody.

SEQ ID NO: 192 shows the amino acid sequence of the heavy chain of the Fab of so-called “18F5-H10” anti-CD3 antibody.

SEQ ID NO: 193 shows the amino acid sequence of the light chain of the Fab of so-called “18F5-H10” anti-CD3 antibody.

5 SEQ ID NO: 194 shows the amino acid sequence of full-length human CD123 protein, including the signal peptide, as available from the NCBI database under NP_002174.1 and from the Uniprot database under P26951.

10 SEQ ID NO: 195 shows the amino acid sequence of full-length *Macaca fascicularis* CD123 protein, including the signal peptide, as available from the GenBank database under EHH61867.1 and Uniprot database under G8F3K3.

SEQ ID NO: 196 shows the amino acid sequence of mature human CD123 Strep-II tagged Fc-fusion comprising amino acids 22 to 305 of the full-length human CD123 protein (SEQ ID NO: 194).

15 SEQ ID NO: 197 shows the amino acid sequence of mature *Macaca fascicularis* CD123 Strep-II tagged Fc-fusion comprising amino acids 22 to 305 of the full-length *Macaca fascicularis* CD123 protein (SEQ ID NO: 195).

SEQ ID NO: 198 shows the amino acid sequence of the heavy chain variable domain of the so-called “1E1-G5” anti-CD123 antibody.

20 SEQ ID NO: 199 shows the amino acid sequences of CDR1-H of the so-called “1E1-G5” and “8B11-B7” anti-CD123 antibody.

SEQ ID NO: 200 shows the amino acid sequences of CDR2-H of the so-called “1E1-G5” and “6D6-B8” anti-CD123 antibody.

SEQ ID NO: 201 show the amino acid sequences of CDR3-H of the so-called “1E1-G5”, “6D6-B8”, “8B11-B7” and “9F6-G3” anti-CD123 antibody.

25 SEQ ID NO: 202 shows the amino acid sequence of the light chain variable domain of the so-called “1E1-G5” anti-CD123 antibody.

SEQ ID NO: 203 and 204 show the amino acid sequences of CDR1-L and CDR3-L of the so-called “1E1-G5” anti-CD123 antibody.

30 SEQ ID NO: 205 shows the amino acid sequence of the heavy chain variable domain of the so-called “2B8-F3” anti-CD123 antibody.

SEQ ID NO: 206, 207 and 208 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called “2B8-F3” anti-CD123 antibody.

SEQ ID NO: 209 shows the amino acid sequence of the light chain variable domain of the so-called “2B8-F3” anti-CD123 antibody.

SEQ ID NO: 210 and 211 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "2B8-F3" anti-CD123 antibody.

SEQ ID NO: 212 shows the amino acid sequence of the heavy chain variable domain of the so-called "2F8-D6" anti-CD123 antibody.

5 SEQ ID NO: 213, 214 and 215 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "2F8-D6" anti-CD123 antibody.

SEQ ID NO: 216 shows the amino acid sequence of the light chain variable domain of the so-called "2F8-D6" anti-CD123 antibody.

10 SEQ ID NO: 217 and 218 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "2F8-D6" anti-CD123 antibody.

SEQ ID NO: 219 shows the amino acid sequence of the heavy chain variable domain of the so-called "3B10-E6" anti-CD123 antibody.

SEQ ID NO: 220, 221 and 222 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "3B10-E6" anti-CD123 antibody.

15 SEQ ID NO: 223 shows the amino acid sequence of the light chain variable domain of the so-called "3B10-E6" anti-CD123 antibody.

SEQ ID NO: 224 and 225 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "3B10-E6" anti-CD123 antibody.

20 SEQ ID NO: 226 shows the amino acid sequence of the heavy chain variable domain of the so-called "3E3-D3" anti-CD123 antibody.

SEQ ID NO: 227, 228 and 229 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "3E3-D3" anti-CD123 antibody.

SEQ ID NO: 230 shows the amino acid sequence of the light chain variable domain of the so-called "3E3-D3" anti-CD123 antibody.

25 SEQ ID NO: 231 and 232 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "3E3-D3" anti-CD123 antibody.

SEQ ID NO: 233 shows the amino acid sequence of the heavy chain variable domain of the so-called "5A5-B4" anti-CD123 antibody.

30 SEQ ID NO: 234, 235 and 236 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "5A5-B4" anti-CD123 antibody.

SEQ ID NO: 237 shows the amino acid sequence of the light chain variable domain of the so-called "5A5-B4" anti-CD123 antibody.

SEQ ID NO: 238 and 239 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "5A5-B4" anti-CD123 antibody.

SEQ ID NO: 240 shows the amino acid sequence of the heavy chain variable domain of the so-called "6B10-E4" anti-CD123 antibody.

SEQ ID NO: 241, 242 and 243 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "6B10-E4" anti-CD123 antibody.

5 SEQ ID NO: 244 shows the amino acid sequence of the light chain variable domain of the so-called "6B10-E4" anti-CD123 antibody.

SEQ ID NO: 245 and 246 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "6B10-E4" anti-CD123 antibody.

10 SEQ ID NO: 247 shows the amino acid sequence of the heavy chain variable domain of the so-called "6C10-C4" anti-CD123 antibody.

SEQ ID NO: 248, 249 and 250 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "6C10-C4" anti-CD123 antibody.

SEQ ID NO: 251 shows the amino acid sequence of the light chain variable domain of the so-called "6C10-C4" and "9B8-G6" anti-CD123 antibody.

15 SEQ ID NO: 252 shows the amino acid sequence of CDR1-L of the so-called "6C10-C4" and "9B8-G6" anti-CD123 antibody.

SEQ ID NO: 253 shows the amino acid sequence of CDR3-L of the so-called "6C10-C4", "9B8-G6" and "9D7-G3" anti-CD123 antibodies.

20 SEQ ID NO: 254 shows the amino acid sequence of the heavy chain variable domain of the so-called "6D6-B8" anti-CD123 antibody.

SEQ ID NO: 255 shows the amino acid sequence of CDR1-H of the so-called "6D6-B8" anti-CD123 antibody.

SEQ ID NO: 256 shows the amino acid sequence of the light chain variable domain of the so-called "6D6-B8" anti-CD123 antibody.

25 SEQ ID NO: 257 and 258 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "6D6-B8" anti-CD123 antibody.

SEQ ID NO: 259 shows the amino acid sequence of the heavy chain variable domain of the so-called "8B11-B7" anti-CD123 antibody.

30 SEQ ID NO: 260 shows the amino acid sequence of CDR2-H of the so-called "8B11-B7" anti-CD123 antibody.

SEQ ID NO: 261 shows the amino acid sequence of the light chain variable domain of the so-called "8B11-B7" anti-CD123 antibody.

SEQ ID NO: 262 and 263 show the amino acid sequences of CDR1-L and CDR3-L of the so-called "8B11-B7" anti-CD123 antibody.

SEQ ID NO: 264 shows the amino acid sequence of the heavy chain variable domain of the so-called "9B8-G6" anti-CD123 antibody.

SEQ ID NO: 265 and 266 show the amino acid sequences of CDR1-H and CDR2-H of the so-called "9B8-G6" and "9D7-C8" anti-CD123 antibodies.

5 SEQ ID NO: 267 shows the amino acid sequences of CDR3-H of the so-called "9B8-G6" anti-CD123 antibody.

SEQ ID NO: 268 shows the amino acid sequence of the heavy chain variable domain of the so-called "9D7-C8" anti-CD123 antibody.

10 SEQ ID NO: 269 shows the amino acid sequences of CDR3-H of the so-called "9D7-C8" anti-CD123 antibody.

SEQ ID NO: 270 shows the amino acid sequence of the light chain variable domain of the so-called "9D7-C8" anti-CD123 antibody.

SEQ ID NO: 271 shows the amino acid sequences of CDR1-L of the so-called "9D7-C8" anti-CD123 antibody.

15 SEQ ID NO: 272 shows the amino acid sequence of the heavy chain variable domain of the so-called "9F6-G3" anti-CD123 antibody.

SEQ ID NO: 273 and 274 show the amino acid sequences of CDR1-H and CDR2-H of the so-called "9F6-G3" anti-CD123 antibody.

20 SEQ ID NO: 275 shows the amino acid sequence of the light chain variable domain of the so-called "9F6-G3" anti-CD123 antibody.

SEQ ID NO: 276 shows the amino acid sequence of CDR1-L of the so-called "9F6-G3" anti-CD123 antibody.

SEQ ID NO: 277 shows the VH variant amino acid sequence VH_G45A of "3E3" anti-CD123 antibody.

25 SEQ ID NO: 278 shows the VH variant amino acid sequence VHmDG of "3E3" anti-CD123 antibody.

SEQ ID NO: 279 shows the amino acid sequence of CDR2-H of the variant VHmDG of "3E3" anti-CD123 antibody and CDR2-H of variant VH1Fm2DG of humanized "3E3" anti-CD123 antibody.

30 SEQ ID NO: 280 shows the VH variant amino acid sequence VH1A of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 281 shows the VH variant amino acid sequence VH1B of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 282 shows the VH variant amino acid sequence VH1C of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 283 shows the VH variant amino acid sequence VH1D of humanized “3E3” anti-CD123 antibody.

5 SEQ ID NO: 284 shows the VH variant amino acid sequence VH1E of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 285 shows the VH variant amino acid sequence VH1F of humanized “3E3” anti-CD123 antibody.

10 SEQ ID NO: 286 shows the VH variant amino acid sequence VH1G of humanized “3E3” anti-CD123 antibody

SEQ ID NO: 287 shows the VH variant amino acid sequence VH1Fm1 of humanized “3E3” anti-CD123 antibody

SEQ ID NO: 288 shows the VH variant amino acid sequence VH1Fm2 of humanized “3E3” anti-CD123 antibody

15 SEQ ID NO: 289 shows the VH variant amino acid sequence VH1Fm2DG of humanized “3E3” anti-CD123 antibody

SEQ ID NO: 290 shows the VH variant amino acid sequence VH1Dm1 of humanized “3E3” anti-CD123 antibody

20 SEQ ID NO: 291 shows the VH variant amino acid sequence VH1Em1 of humanized “3E3” anti-CD123 antibody

SEQ ID NO: 292 shows the VL variant amino acid sequence VL1A of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 293 shows the VL variant amino acid sequence VL1B of humanized “3E3” anti-CD123 antibody.

25 SEQ ID NO: 294 shows the VL variant amino acid sequence VL1C of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 295 shows the VL variant amino acid sequence VL1D of humanized “3E3” anti-CD123 antibody.

30 SEQ ID NO: 296 shows the VL variant amino acid sequence VL1E of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 297 shows the VL variant amino acid sequence VL1F of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 298 shows the VL variant amino acid sequence VL1G of humanized “3E3” anti-CD123 antibody.

SEQ ID NO: 299 shows the VL variant amino acid sequence VL1Fm1 of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 300 shows the VL variant amino acid sequence VL1Fm2 of humanized "3E3" anti-CD123 antibody.

5 SEQ ID NO: 301 shows the VH variant amino acid sequence VH2A of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 302 shows the VH variant amino acid sequence VH3A of humanized "3E3" anti-CD123 antibody.

10 SEQ ID NO: 303 shows the VL variant amino acid sequence VL2A of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 304 shows the VL variant amino acid sequence VL2Am1 of humanized "3E3" anti-CD123 antibody.

SEQ ID NO: 305 shows the VL variant amino acid sequence VL2Am2 of humanized "3E3" anti-CD123 antibody.

15 SEQ ID NO: 306 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab "7G3x20G6" antibody-like binding protein.

SEQ ID NO: 307 shows the amino acid sequence of the linker L1 of the so-called CODV-Fab "7G3x20G6", "7G3x4E7", "7G3x4B4", "7G3x18F5", "hz20G6x7G3", "7G3xhz4B4", "hz4B4x3E3" and CODV-Fab "hz20G6x7G3-TL4" antibody-like binding
20 proteins

SEQ ID NO: 308 shows the amino acid sequence of the variable light chain domain of 7G3 representing the V_{D1} or V_{D2} domain of the so-called CODV-Fab "7G3x20G6", "7G3x4E7", "7G3x4B4", "7G3x18F5", "hz20G6x7G3", "7G3xhz4B4" and CODV-Fab "hz20G6x7G3-TL4" antibody-like binding proteins.

25 SEQ ID NO: 309 shows the amino acid sequence of the linker L2 of the so-called CODV-Fab "7G3x20G6", "7G3x4E7", "7G3x4B4", "7G3x18F5", "hz20G6x7G3", "7G3xhz4B4", "hz4B4x3E3" and CODV-Fab "hz20G6x7G3-TL4" antibody-like binding proteins.

30 SEQ ID NO: 310 shows the amino acid sequence CL of the so-called CODV-Fab "7G3x20G6", "7G3x4E7", "7G3x4B4", "7G3x18F5", "hz20G6x7G3", "7G3xhz4B4" and "hz4B4x3E3" antibody-like binding proteins.

SEQ ID NO: 311 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab "7G3x20G6" antibody-like binding protein.

SEQ ID NO: 312 shows the amino acid sequence of the variable heavy chain domain of 7G3 representing herein the V_{H1} or V_{H2} domain of the so-called CODV-Fab “7G3x20G6“, “7G3x4E7“, “7G3x4B4“, “7G3x18F5“, “hz20G6x7G3“, “7G3xhz4B4“ and CODV- Fab “hz20G6x7G3-TL4“ antibody-like binding proteins.

5 SEQ ID NO: 313 shows the amino acid sequence C_{H1} of the so-called CODV-Fab “7G3x20G6“, “7G3x4E7“, “7G3x4B4“, “7G3x18F5“, “hz20G6x7G3“, “7G3xhz4B4“, “hz4B4x3E3“ antibody-like binding proteins.

SEQ ID NO: 314 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “7G3 x 4E7“ antibody-like binding protein.

10 SEQ ID NO: 315 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “7G3x4E7“ antibody-like binding protein.

SEQ ID NO: 316 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “7G3x4B4“ antibody-like binding protein.

15 SEQ ID NO: 317 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “7G3x4B4“ antibody-like binding protein.

SEQ ID NO: 318 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “7G3x18F5“ antibody-like binding protein.

SEQ ID NO: 319 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “7G3x18F5“ antibody-like binding protein.

20 SEQ ID NO: 320 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “hz20G6x7G3“ antibody-like binding protein.

SEQ ID NO: 321 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “hz20G6x7G3“ antibody-like binding protein.

25 SEQ ID NO: 322 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab 7G3xhz4B4“ antibody-like binding protein.

SEQ ID NO: 323 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “7G3xhz4B4“ antibody-like binding protein.

SEQ ID NO: 324 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “hz4B4x3E3“ antibody-like binding protein.

30 SEQ ID NO: 325 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab “hz4B4x3E3“ antibody-like binding protein.

SEQ ID NO: 326 shows the amino acid sequence of polypeptide according to formula I of the so-called CODV-Fab “hz20G6x7G3 TL4“ antibody-like binding protein.

SEQ ID NO: 327 shows the amino acid sequence F_{c2} of the so-called CODV-Fab “hz20G6x7G3-TL4” antibody-like binding protein.

SEQ ID NO: 328 shows the amino acid sequence of the polypeptide according to formula III of the so-called CODV-Fab “hz20G6x7G3-TL4” antibody-like binding protein.

5 SEQ ID NO: 329 shows the amino acid sequence CH1 of the so-called CODV-Fab “hz20G6x7G3-TL4” antibody-like binding protein.

SEQ ID NO: 330 shows the amino acid sequence F_c of the so-called CODV-Fab “hz20G6x7G3-TL4” antibody-like binding protein.

10 SEQ ID NO: 331 shows a consensus sequence for CDR1-H of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies based on sequence alignment.

15 SEQ ID NO: 332 shows a consensus sequence for CDR2-H of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies based on sequence alignment.

20 SEQ ID NO:333 shows a consensus sequence for CDR3-H of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies based on sequence alignment.

25 SEQ ID NO: 334 shows a consensus sequence for CDR1-L of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies based on sequence alignment.

30 SEQ ID NO:335 shows a consensus sequence for CDR3-L of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies based on sequence alignment.

SEQ ID NO: 336 shows a consensus sequence for CDR1-H of the so-called “1E1-

G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

5 SEQ ID NO:337 shows a consensus sequence for CDR2-H of the so-called "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

SEQ ID NO: 338 shows a consensus sequence for CDR1-L of the so-called "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

10 SEQ ID NO: 339 shows a consensus sequence for CDR3-L of the so-called "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

SEQ ID NO: 340 shows a consensus sequence for CDR1-H of the so-called "6C10-C4", "9B8-G6", "9D7-C8" anti-CD123 antibodies based on sequence alignment.

15 SEQ ID NO: 341 shows a consensus sequence for CDR2-H of the so-called "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

SEQ ID NO:342 shows a consensus sequence for CDR3-H of the so-called "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

20 SEQ ID NO: 343 shows a consensus sequence for CDR1-L of "1E1-G5", "6D6-B8", "8B11-B7", "9F6-G3" anti-CD123 antibodies based on sequence alignment.

SEQ ID NO: 344 shows the amino acid sequence of a linker sequence (Gly-Gly-Gly-Gly-Ser).

25 SEQ ID NO: 345 shows the amino acid sequence of a linker sequence (Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser).

SEQ ID NO: 346 shows the amino acid sequence of a linker sequence (Thr-Val-Ala-Ala-Pro).

SEQ ID NO: 347 shows the amino acid sequence of a linker sequence (Gln-Pro-Lys-Ala-Ala).

30 SEQ ID NO: 348 shows the amino acid sequence of a linker sequence (Gln-Arg-Ile-Glu-Gly).

SEQ ID NO: 349 shows the amino acid sequence of a linker sequence (Ala-Ser-Thr-Lys-Gly-Pro-Ser).

SEQ ID NO: 350 shows the amino acid sequence of a linker sequence (Ala-Ser-

Thr-Lys-Gly-Pro-Ser).

SEQ ID NO: 351 shows the amino acid sequence of a linker sequence (His-Ile-Asp-Ser-Pro-Asn-Lys).

5 SEQ ID NO: 352 shows the amino acid sequence of a linker and His-tag sequence added C-terminally to polypeptide according to formula II of the so-called CODV-Fab “7G3x20G6”, “7G3x4E7”, “7G3x4B4”, “7G3x18F5”, “hz20G6x7G3”, “7G3xhz4B4” and “hz4B4x3E3” antibody-like binding proteins corresponding to a hinge sequence and a His-tag used for example for purification.

10 SEQ ID NO: 353 shows the amino acid sequence of CDR2-H of a variant of the so-called “3E3” anti-CD123 antibody.

SEQ ID NO: 354 shows the amino acid sequence of a linker sequence (Gly-Gly-Gly-Ser).

SEQ ID NO: 355 shows the amino acid sequence of a linker sequence (Ser-Gly-Gly-Gly-Ser).

15 SEQ ID NO: 356 shows the amino acid sequence of a linker sequence (Gly-Ser-Gly-Gly-Gly-Gly-Ser).

SEQ ID NO: 357 shows the amino acid sequence of a linker sequence (Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser).

20 SEQ ID NO: 358 shows the amino acid sequence of a linker sequence (Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser).

SEQ ID NO: 359 shows the amino acid sequence of a linker sequence (Lys-Thr-His-Thr).

SEQ ID NO: 360 shows the amino acid sequence of a linker sequence (Lys-Thr-His-Thr-Ser).

25 SEQ ID NO: 361 shows the amino acid sequence of a linker sequence (Asp-Lys-Thr-His-Thr-Ser).

SEQ ID NO: 362 shows the amino acid sequence of a linker sequence (Asp-Lys-Thr-His-Thr-Ser-Pro).

30 SEQ ID NO: 363 shows the amino acid sequence of a linker sequence (Ser-Asp-Lys-Thr-His-Thr-Ser-Pro).

SEQ ID NO: 364 shows the amino acid sequence of a linker sequence (Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro).

SEQ ID NO: 365 shows the amino acid sequence of a linker sequence (Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser)

SEQ ID NO: 366 shows the amino acid sequence of a linker sequence (Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser).

SEQ ID NO: 367 shows the amino acid sequence of a linker sequence (Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro)

5 SEQ ID NO: 368 shows the amino acid sequence of a linker sequence (Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro)

SEQ ID NO: 369 shows the amino acid sequence of a linker sequence (Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly).

10 SEQ ID NO: 370 shows the amino acid sequence of a linker sequence (Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly).

SEQ ID NO: 371 shows the amino acid sequence of a linker sequence (Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly).

SEQ ID NO: 372 shows the amino acid sequence of a linker sequence (Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly)

15 SEQ ID NO: 373 shows the amino acid sequence of a linker sequence (Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly).

SEQ ID NO: 374 shows the amino acid sequence of a linker sequence (Gly-Gly-Gly-Glu-Pro-Lys-Ser-Asp-Lys-Thr-His-Thr-Ser-Pro-Pro-Ser-Pro-Gly-Gly-Gly)

20 SEQ ID NO: 375, 376 and 377 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the so-called "7G3" antibody.

SEQ ID NO: 378 and 379 show the amino acid sequence of CDR1-L and CDR3-L of the so-called "7G3" antibody.

SEQ ID NO: 380 shows the amino acid sequence of a variant of the heavy chain variable domain of the so-called humanized "7G3" antibody.

25 SEQ ID NO: 381 and 382 show the amino acid sequences of CDR1-H and CDR3-H of the so-called humanized "7G3" antibody.

SEQ ID NO: 383 shows the amino acid sequence of a further variant of the heavy chain variable domain of the so-called humanized "7G3" antibody.

30 SEQ ID NO: 384 shows the amino acid sequences of CDR2-H of one of the so-called humanized "7G3" antibody.

SEQ ID NO: 385 shows the amino acid sequence of the light chain variable domain of the so-called humanized "7G3" antibody.

SEQ ID NO: 386 shows the amino acid sequence SEQ ID NO:1 as shown in WO2015026892.

SEQ ID NO: 387 shows the amino acid sequence SEQ ID NO:3 as shown in WO2015026892.

SEQ ID NO: 388 shows the amino acid sequence of the polypeptide according to formula I of the so-called CODV-Fab "hz20G6xhz7G3", CODV-Fab-OL1
5 "hz20G6xhz7G3" and CODV-Fab-OL1a "hz20G6xhz7G3" antibody-like binding proteins.

SEQ ID NO: 389 shows the amino acid sequence of a linker sequence (Gly-Gly-Ser-Gly-Ser-Ser-Gly-Ser-Gly-Gly).

SEQ ID NO: 390 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab "hz20G6xhz7G3" antibody-like binding protein.

10 SEQ ID NO: 391 shows the amino acid sequence of the polypeptide according to formula IV of the so-called CODV-Fab-TL1 "hz20G6xhz7G3" antibody-like binding protein.

SEQ ID NO: 392 shows the amino acid sequence of the F_{c2} region of the so-called CODV-Fab-TL1 "hz20G6xhz7G3" antibody-like binding protein.

15 SEQ ID NO: 393 shows the amino acid sequence of the polypeptide according to formula III of the so-called CODV-Fab-TL1 "hz20G6xhz7G3" antibody-like binding protein.

SEQ ID NO: 394 shows the amino acid sequence of the F_c region of the so-called CODV-Fab-TL1 "hz20G6xhz7G3". SEQ ID NO: 395 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab-OL1 "hz20G6xhz7G3"
20 antibody-like binding protein.

SEQ ID NO: 396 shows the amino acid sequence of the F_c region of the so-called CODV-Fab-OL1 "hz20G6xhz7G3" antibody-like binding protein.

SEQ ID NO: 397 shows the amino acid sequence of the F_c stump (F_{c3}) of the so-called CODV-Fab-OL1 "hz20G6xhz7G3" antibody-like binding protein.

25 SEQ ID NO: 398 shows the amino acid sequence of the F_c stump (F_{c3}) of the so-called CODV-Fab-OL1a "hz20G6xhz7G3" antibody-like binding protein.

SEQ ID NO: 399 shows the amino acid sequence of the polypeptide according to formula II of the so-called CODV-Fab-OL1a "hz20G6xhz7G3" antibody-like binding protein.

30 SEQ ID NO: 400 shows the amino acid sequence of the F_c stump (F_{c3}) of the so-called CODV-Fab-OL1a "hz20G6xhz7G3" antibody-like binding protein.

FIGURES

Figure 1: Sequence alignments of the VH regions of the so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies.

Figure 2: Sequence alignments of the VL regions of so-called “20G6-F3”, “4B4-D7”, “4E7-C9”, “18F5-H10”, “11D7-C3”, “11H3-E5”, “13H2-C2”, “13C1-F6”, “1E6-C9”, “10F4-C10”, “10E6-G6”, “18G9-H11”, “11F3-B9”, “12G3-E8”, “5B1-G2”, “16F8-A7”, “11F9-F8”, “20E5-F10”, “20B5-F10”, “3H6-D2” anti-CD3 antibodies.

Figure 3: Sequence alignments of the VH and VL regions of the so-called “1E1-G5”, “6D6-B8”, “8B11-B7”, “9F6-G3” anti-CD123 antibodies.

Figure 4: Sequence alignments of the VH and VL regions of so-called “6C10-C4”, “9B8-G6”, “9D7-C8” anti-CD123 antibodies.

Figures 5 and 7: Fully human CODV-Fab-TL1 “hz20G6xhz7G3” IV Q3d in presence of human T cells inhibits Molm13 tumor growth in whole body at all tested doses.

Figures 6 and 8: Fully human CODV-Fab-TL1 “hz20G6xhz7G3” IV Q3d in presence of human T cells is associated with tumor regression in long bones at all tested doses.

Figures 9 and 11: Fully human CODV-Fab “hz20G6xhz7G3” IV in presence of human T cells inhibited tumor growth.

Figures 10 and 12: Fully human CODV-Fab “hz20G6xhz7G3” IV in presence of human T cells was associated with tumor regression in long bones.

Figures 13 and 15: Fully human CODV-Fab “hz20G6xhz7G3” CIP in presence of human T cells inhibited whole body tumor growth at dosage of 0.13 nmol/Kg/day or above.

Figures 14 and 16: Fully human CODV-Fab “hz20G6xhz7G3” CIP in presence of human T cells inhibited tumor growth in long bones at dosage of 0.13 nmol/Kg/day or above.

Figure 17: Diagrammatic representation of the structure of the CODV-Ig, CODV-Fab-TL and CODV-Fab-OL (further showing LALA mutations (when Fc of IgG1 backbone is used) and Knob-into-Hole mutations).

EXAMPLES

Example 1: Antibody generation

1.1 Construction of hCD3ε/δ-hFc fusion expression plasmid (CD3ed-Fc)

5 Using cDNA containing plasmids as a template, human and *Macaca fascicularis* CD3ε and CDδ fusion proteins were generated, as described herein below in detail, in reading frame with heavy chain constant region including the hinge region, CH2 and CH3 domains of human immunoglobulin IgG additionally carrying a 8 x His or Strep-II tag for optional tandem purification.

10 Using human genomic DNA as template, human CD3ε and human CDδ subunit extracellular domains were amplified, including the signal sequence. The resulting amplified cleaved and purified PCR products were combined by ligation PCR and ligated into mammalian expression vector pXL by InFusion method using NheI and HindIII site. Each subunit was cloned on one plasmid. The sequence of the resulting mature human
15 CD3ε His-tagged Fc-fusion protein is herein disclosed under SEQ ID NO: 3. Amino acids 1 to 104 of SEQ ID NO: 3 correspond to amino acids 23 to 126 of the wild-type full-length human CD3ε (herein disclosed under SEQ ID NO: 1, available in Uniprot database under accession number P07766) protein and thus the extracellular domain of human CD3ε.

20 Using cynomolgus monkey genomic DNA as template, *Macaca fascicularis* CD3ε and CD3δ extracellular domains were amplified, including the signal sequence. The resulting amplified cleaved and purified PCR products were combined by ligation PCR and ligated into mammalian expression vector pXL by InFusion method using NheI and HindIII. Each subunit was cloned on one plasmid. The resulting sequences for mature
25 *Macaca fascicularis* CD3ε Fc-fusion protein is disclosed under SEQ ID NO: 4. Amino acids 1 to 95 of SEQ ID NO: 3 correspond to amino acids 23 to 117 of the full-length *Macaca fascicularis* CD3ε protein and thus comprises the extracellular domain of wild-type full-length *Macaca fascicularis* CD3ε (herein disclosed under SEQ ID NO: 2, available in Uniprot database under accession number Q95LI5). The cloned fusion
30 protein further contains one Alanine to Valine exchange at the amino acid position 35 in comparison to amino acid position 57 of the wild-type sequence.

1.2 Expression and purification of human and cyno CD3 ϵ -Fc

Freestyle HEK293 cells growing in F17 serum free suspension culture (Life) were transiently transfected with the expression plasmid. Co-transfection of both plasmids representing the CD3 ϵ and CD3 δ extracellular domain (ECD) subunit were performed using Cellfectin transfection reagent (Life). The cells were cultured at 37°C for 7 days. The culture supernatant containing recombinant protein was harvested by centrifugation and was clarified by filtration (0.22 μ m).

For purification, the Fc-fusion protein variants were captured on protein A matrix (GE) and were eluted by pH shift. After polishing the protein by size exclusion chromatography (SEC) using a Superdex 200 (GE) and a final ultrafiltration concentration step the protein was used for further assays.

The human heterodimer was additionally applied on His-Trap column (GE) after capture on protein A and desalted. The eluted protein was applied to a Strepavidin column (GE) and eluted with d-desthiobiotin before final polishing by SEC using a Superdex 200 (GE). This strategy was used to isolate heterodimers from homodimers.

1.3 Generation of human/cynomolgous monkey cross-reactive anti-CD3 Antibodies

Human and *Macaca fascicularis* CD3 ϵ and CD3 δ cDNAs were cloned into Aldevron proprietary immunization vectors (pB8 and VV8) respectively, and used for genetic immunization of rats. Rats of immunization group MR12-266 ("CD3-cyno") were initially immunized with human CD3 ϵ and CD3 δ cDNAs, followed by further two immunizations with a mixture of human and *Macaca fascicularis* CD3 ϵ and CD3 δ cDNAs. The immune serum was taken at day 24 of the immunization protocol, after 4 genetic applications (IS24d-4). Sera, diluted in PBS 1% BSA, were tested by flow cytometry using mammalian cells transiently transfected with the target cDNAs in co-transfection experiments to obtain human and *Macaca fascicularis* CD3 ϵ and CD3 δ TCR complexes. In addition the immune sera were tested on the following cell lines: Jurkat E6-1 (expressing human TCR), Jurkat-RT-T3.5 (negative for TCR) and cyno HSC-F (expressing cyno TCR); no negative cell line for the cyno TCR was available. A goat anti-rat IgG R-phycoerythrin conjugate (Southern Biotech, #3030-09) at 10 μ g/ml was used as a secondary antibody.

Specific reactivity of the immune sera especially against cells transfected with combinations of CD3 ϵ and CD3 δ cDNAs could be detected in the immunized animals when compared to cells transfected with an irrelevant cDNA. The same is valid for the test on the TCR-positive Jurkat cell line (E6- 1), when compared to the TCR-negative cell line (RT-T3.5), much lesser signals but a still significant signals were detected on the cynomologues HSC-F cell line (see table 1).

Table 1: Detection of the specific reactivity of the immune sera against TCR-complex in each animal by flow cytometry. Data is presented as geometric means of the relative fluorescence intensities (rfu).

		pXL-CD3e-hum + pXL-CD3d-hum	pXL-CD3e-cyno + pXL-CD3d-cyno	pB1_ irrelevant	Jurkat-E6-1	Jurkat-RT-T3.5	cyno HSC-F
	w/o primary ab	5	5	4	7	10	6
	OKT3	-	-	-	858	25	-
	FN18	-	-	4	7	10	1256
	Mouse-anti-HA	6	6	-	-	-	-
	Rat-anti-myc	9	7	1033	10	14	9
rat #1	1:1000	290	273	5	186	36	55
	1:5000	79	86	5	65	21	20
rat #2	1:1000	499	401	5	390	55	75
	1:5000	147	110	4	126	28	25
rat #3	1:1000	239	247	5	108	30	31
	1:5000	89	92	4	32	17	9
rat #4	1:1000	280	283	5	176	37	73
	1:5000	97	110	4	55	22	23
rat #5	1:1000	208	137	4	99	31	11
	1:5000	61	65	4	47	19	7

Rats of immunization group MR12-265 ("CD3-hum") were co-immunized with human CD3 ϵ and CD3 δ cDNAs cloned into the corresponding expression vectors. The immune serum was taken at day 24 of the immunization protocol, after 4 genetic applications (IS24d-4). Sera, diluted in PBS 1% BSA, were tested by flow cytometry using
5 mammalian cells transiently transfected with the above mentioned target cDNAs in co-transfection experiments to obtain human and *Macaca fascicularis* CD3 ϵ and CD3 δ TCR complexes. A goat anti-rat IgG R-phycoerythrin conjugate (Southern Biotech, #3030-09) at 10 μ g/ml was used as a secondary antibody. In addition the immune sera were tested on the following cell lines: Jurkat E6-1 (expressing human TCR), Jurkat-RT-T3.5
10 (negative for TCR) and cyno HSC-F (expressing cyno TCR); no negative cell line for the cyno TCR was available. A goat anti-rat IgG R-phycoerythrin conjugate (Southern Biotech, #3030-09) at 10 μ g/ml was used as a secondary antibody.

Specific reactivity of the immune sera especially against cells transfected with
15 combinations of CD3 ϵ and CD3 δ cDNAs could be detected in the immunized animals when compared to cells transfected with an irrelevant cDNA. The same is valid for the test on the TCR-positive Jurkat cell line (E6-1), when compared to the TCR-negative cell line (RT-T3.5), but much lesser signals (however in the positive rats still significant) were detected on the cyno HSC-F cell line (see table 2)

Table 2: Detection of the specific reactivity of the immune sera against TCR-complex in each animal by flow cytometry. Data is presented as geometric means of the relative fluorescence intensities (rfu).

		pXL-CD3e-hum + pXL-CD3d-hum	pXL-CD3e-cyno + pXL-CD3d-cyno	pB1_irrelevant	Jurkat-E6-1	Jurkat-RT-T3.5	cyno HSC-F
	w/o primary ab	5	5	4	7	10	6
	OKT3	-	-	-	858	25	-
	FN18	-	-	4	7	10	1256
	Mouse-anti-HA	6	6	-	-	-	-
	Rat-anti-myc	9	7	1033	10	14	9
rat #1	1:1000	230	132	5	170	33	50
	1:5000	78	40	4	54	19	15
rat #2	1:1000	216	136	6	216	48	32
	1:5000	75	40	4	66	24	11
rat #3	1:1000	73	54	5	56	25	7
	1:5000	25	20	4	23	15	6
rat #4	1:1000	70	56	5	41	25	8
	1:5000	25	18	4	15	15	6
rat #5	1:1000	65	37	5	42	20	7
	1:5000	24	17	4	17	14	6

Rats with positive sera were sacrificed and B cells were fused with mouse myeloma cells. The resulting hybridomas were screened on HEK293 cells transfected with human or cynomolgus CD3 ϵ and CD3 δ expression plasmids, on Jurkat E6.1 (CD3+) and Jurkat T3.5 (CD3-) by flow cytometry. Supernatant of Hybridoma clones was used to assess by surface plasmon resonance single point kinetic against human and *Macaca fascicularis* CD3 ϵ / δ complex by fixing the analyte at 25nM (data presented in table 3).

Table 3: Detection of the specific reactivity of different hybridoma clone supernatants by flow cytometry and Biacore analysis. Supernatants were tested on different cell lines (Jurkat E6-1, Jurkat-RT-T3.5 and cyno HSC-F). Biacore analysis was performed against CD3 ϵ / δ complexes from human and cynomolgous monkey respectively.

Sample-ID	Biacore human CD3 ϵ / δ [RU] at 25nM	Biacore cyno CD3 ϵ / δ [RU] at 25nM
3G5-E10	27	3
18G9-H11	7	-7
12D2-E5	27	5
6C9-C9	26	32
11F9-F8	11,5	16
8H2-F3	32,5	40
4B4-D7	11	14
4E7-C9	2,5	10
13C1-F6	17	15,5
11D7-C3	17	10
13H2-C3	16	7
10E6-G6	10,5	0
12G3-E8	24	17
11F3-B9	22	17
20E3-B5	11	5
10F4-C10	22	27
11H3-E5	7	9
18F5-H10	n.a.	n.a.
18H11-F10	35	55
20E5-F10	12	8

Sample-ID	Biacore human CD3 ϵ / δ [RU] at 25nM	Biacore cyno CD3 ϵ / δ [RU] at 25nM
3H6-D2	9,5	7
8C2-F7	4,5	3
5B1-G2	1	0,5
7B7-F10	28	42
20B5-F10	1	9,5
16D3-E4	-2	-2
1E6-B8	n.a.	n.a.
16F8-A7	17,5	22,5
11A8-D6		
3E8-G1		
20G6-F3		
9D7-B5	n.a.	n.a.
9G5-G10		
17A11-D3		

Positive clones were expanded and respective cDNAs for variable heavy and light chains were isolated by RT-PCR. VH and VL sequences were cloned into expression vectors in fusion with either the human CH1, IGHG1-backbone or kappa chain in order to express Fab-fragments as well as full IgGs.

1.4 Expression of IgGs and Fab-fragments

The expression plasmids encoding the heavy and light chain of the IgGs and Fab-fragments were propagated in *E. Coli* NEB 10-beta (DH10B derivative). Plasmids used for transfection were prepared from *E. Coli* using the QIAGEN Plasmid Plus Kit (Cat. No.: 12991).

HEK 293-FS cells growing in Freestyle Medium (Invitrogen) were transfected with indicated LC and HC plasmids encoding the heavy chains and light chains using 293fectin (Invitrogen) transfection reagent as described by the manufacturer. Cells were cultivated at 37°C in a Kuhner ISF1-X shaking incubator at 110rpm with 8% CO₂. After 7 days of cultivation cells were removed by centrifugation, 10 % Vol/Vol 1M Tris HCl pH 8,0 was added and the supernatant was filtered via a 0,2 μ M bottle top filter to remove

particles. CODV-IgG1 constructs were purified by affinity chromatography on Protein A columns (HiTrap Protein A HP Columns, GE Life Sciences). After elution from the column with 0,1M Citrat, pH 3.0, the CODV-IgG1 constructs were desalted using HiPrep 26/10 Desalting Columns, formulated in PBS (Gibco 14190-136).

5 Bispecific CODV-Fab constructs were purified by HisTrap High Performance columns (GE Healthcare, Cat. No.: 17-5248-02). After elution from the column (Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4), the protein containing fractions were pooled and desalted using HiPrep 26/10 Desalting Columns, formulated in PBS (Gibco 14190-136).

10 To separate monomers from aggregates a high resolution fractionation step in PBS (Gibco 14190-136) for both constructs, the CODV-IgG and the CODV-Fab fragment, was performed, using a HiLoad Superdex 200 26/60 320ml column (GE Healthcare Cat. No.: 29-9893-36). Monomeric fractions were pooled and concentrated up to 1mg/ml, using Vivaspin 20 centrifugation columns (VS2002 Sartorius Stedim
15 biotech) and filtered using a 0.22 µm membrane (Millex® Syringe Filters SLGV033RS). Protein concentration was determined by measurement of absorbance at 280 nm. Each batch was analyzed by SDS-PAGE under reducing and non-reducing conditions to determine the purity and molecular weight of each subunit and of the monomer.

1.5 Assessment of affinities of the anti-CD3 antibodies

20 1.5.1 Assessment of affinities to both human and *Macaca fascicularis* CD3ε/δ

Binding affinities of anti-CD3 binding Fabs or CODV-Fabs were measured by surface plasmon resonance (SPR) using a Biacore3000 instrument (GE Healthcare). Assay buffer was HBS-EP (BR-1001-88, GE Healthcare). Capture of CD3ε/δ-Fc fusion proteins was achieved using the human antibody capture kit (GE Healthcare). The
25 capture antibody was coupled to CM5 chips (BR-1001-88, GE Healthcare) to approx. 12.000 RU using the amine coupling kit (BR-100-50, GE Healthcare). The CD3εδ-Fc fusions proteins were captured at 10µl/min to approx. 70 RU to yield Rmax values of 30 RU. Binding kinetics with the anti-CD3 Fabs or CODV-Fabs was measured at 30µl/min for 240 sec. and 600 sec. for association and dissociation phase, respectively. Twofold
30 dilutions of Fabs from 3 to 400nM in assay buffer were used. All Fab concentrations were run in duplicate together with duplicate buffer blanks for double referencing. Regeneration of the capture surface was performed with a 1min injection of 3M MgCl2

solution at 30µl/min. For data analysis the BIAevaluation software v.4.1 (GE Healthcare) was used. Data were fit globally using a 1:1 Langmuir model with mass transfer.

Measurement of binding affinities of anti-CD3 IgGs and CODV-Fc proteins was performed analogous to the binding assay for Fabs and CODV-Fabs with the exception of the capture antibody. In this case the His Capture Kit (28-9950-56, GE Healthcare) was used to capture human CD3-Fc protein via His-tag. For binding assay of with *Macaca fascicularis* CD3-Fc the Strep-MAB classic antibody (2-1507-001, IBA) was used as the capture antibody. In this case the regeneration solution was 10mM Glycine buffer pH2.0.

10

Table 4: Affinities of selected CD3 antibodies against human and *Macaca fascicularis* CD3ε/δ complexes measured by Biacore.

CD3 εδ	Clone (Fab fragment)	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)	Chi2	Ratio KD(cy)/ KD(hu)
human	12D2-E5	5,4E+06	6,2E-03	35	1,2E-09	0,34	4
cyno	12D2-E5	steady state	steady state	8	4,8E-09	0,129	
human	10E6-G6	1,4E+05	2,0E-03	37	1,4E-08	0,218	1
cyno	10E6-G6	1,9E+05	2,4E-03	18	1,3E-08	0,218	
human	4E7-C9	8,9E+04	1,6E-03	29	1,7E-08	0,244	1
cyno	4E7-C9	9,8E+04	1,5E-03	20	1,5E-08	0,232	
human	12G3-E8	1,1E+05	1,1E-03	30	9,3E-09	0,307	1
cyno	12G3-E8	1,7E+05	1,8E-03	15	1,0E-08	0,349	
human	5B1-G2	6,0E+04	3,9E-04	36	6,5E-09	0,218	1
cyno	5B1-G2	3,4E+04	1,5E-04	20	4,3E-09	2,1	
human	18F5-H10	2,0E+05	1,8E-04	28	8,9E-10	0,235	1
cyno	18F5-H10	1,4E+05	1,3E-04	15	8,9E-10	0,224	
human	18G9-H11	2,5E+05	1,1E-03	31	4,4E-09	0,975	6
cyno	18G9-H11	5,1E+04	1,2E-03	18	2,4E-08	0,617	
human	4B4-D7	1,1E+05	2,9E-03	22	2,7E-08	0,147	1
cyno	4B4-D7	1,1E+05	2,7E-03	12	2,4E-08	0,188	
human	1E6-B8	9,3E+04	3,9E-03	26	4,1E-08	0,157	1
cyno	1E6-B8	1,0E+05	4,2E-03	15	4,2E-08	0,122	

CD3 εδ	Clone (Fab fragment)	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)	Chi2	Ratio KD(cy)/ KD(hu)
human	13H2-C3	1,7E+05	4,9E-03	20	2,9E-08	0,317	1
cyno	13H2-C3	1,8E+05	5,9E-03	12	3,2E-08	0,166	
human	20G6-F3	3,5E+04	2,7E-04	21	7,7E-09	0,208	1
cyno	20G6-F3	2,7E+04	2,2E-04	15	8,2E-09	0,184	
human	11F9-F8	7,7E+04	4,0E-04	22	5,2E-09	0,137	1
cyno	11F9-F8	8,3E+04	3,6E-04	13	4,4E-09	0,109	
human	20E5-F10	9,9E+04	4,3E-04	25	4,3E-09	0,125	1
cyno	20E5-F10	1,2E+05	3,4E-04	14	2,9E-09	0,104	
human	SP-34	1,1E+06	3,0E-03	34	2,7E-09	2,3E-01	5
cyno	SP-34	2,2E+05	2,8E-03	34	1,3E-08	4,7E-01	

SP-34 was used as IgG

1.5.2 Binding of anti-CD3 antibodies to huCD3ε, huCD3δ, and huCD3ε/δ expressed on the surface of HEK293F cells by flow cytometry

5 To analyze binding of antibodies to human CD3ε and human CD3δ expressed on the surface of cells, HEK293F cells were transfected with both constructs either alone or in co-transfection and signals were measured by flow cytometry. For transfection procedure FuGENE HD Transfection Reagent (Promega, #E2311) was used according to manufacturer's protocol.

10 HEK293F cells were seeded in Freestyle293 medium (Gibco) at 6E6 cells per tube in 50 ml Cellstar Cellreactor tubes with filter (Greiner bio-one). Transfections were done according to the FuGENE protocol. Complex preparation was done in OptiMEM without phenol red (Gibco) at ratio 3:1 (Protocol for transfection of 293F cells grown in 8,000 µl of medium in T-25 flasks using a FuGENE®HD:DNA ratio of 3.0:1, <http://www.promega.com/techserv/tools/FugeneHdTool/default.aspx>).

15 Cells were incubated on a shaker at 37°C and 5% CO₂. At day one to three after transfection cells were harvested and binding of antibodies was analyzed by flow cytometry.

20 Antibodies for staining were seeded at 1 µg in 50 µl per well Stain Buffer with FBS (BD Pharmingen) in 96-well U-bottom suspension culture plates (Greiner bio-one).

Harvested transfected cells were resuspended in Stain Buffer with FBS and were added at 50 μ l per well to the antibodies. Cells were incubated at 4°C in the dark for 30 min and were washed twice. 0.5 μ g secondary antibody Goat F(ab')₂ Anti-Human IgG-FITC (Beckman Coulter, #732598) or Goat F(ab')₂ Anti-Human kappa-PE (Southern Biotech, #206209), respectively, in combination with 0.5 μ g 7-AAD per well was added in 100 μ l Stain Buffer with FBS. Cells were incubated at 4°C in the dark for 15 min and were washed twice. For measurement, cells were resuspended in 200 μ l Stain Buffer with FBS. Cells were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer, respectively. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of 7-AAD negative single cells positive for antibody staining (data presented in table 5).

1.5.3 Binding of anti-CD3 Fabs to huCD3 ϵ/δ and huCD3 ϵ/γ by SPR

Binding was tested by SPR using a BIAcore3000 instrument run with HBS-EP buffer. Recombinant huCD3 proteins (ϵ/δ (PB01226), ϵ/γ (PB01225)) were captured at 10 μ l/min via Fc-tag by anti-human Fc capture antibody MAB1302 (Millipore) immobilized on a CM5 sensor chip. Anti-CD3 Fabs were used as analytes at 100nM with association and dissociation times of 240sec and 300sec, respectively at 30 μ l/min. After each cycle the surfaces were regenerated by a 2 min pulse of 10mM glycine buffer pH2.5.

When only huCD3 δ was expressed on the surface of HEK293F cells no signal could be detected by flow cytometry. In contrast, nearly all antibodies could bind to cells transfected with huCD3 ϵ exclusively or in co-transfection with huCD3 δ indicating that huCD3 ϵ is necessary as epitope. In Biacore assays binding to huCD3 ϵ was shown irrespective of whether the δ or the γ chain was used for the recombinant protein suggesting that huCD3 ϵ is sufficient as antigen. The antibody 12D2 exceptionally bound only to huCD3 ϵ when a co-chain was present. There may be an indirect effect of co-expression of the g or d chain regarding the conformational structure of the protein to display the epitope for this antibody. The same effect was shown for the published antibody OKT3. This antibody is described to interact with a conformational epitope formed after association of huCD3 ϵ with huCD3 δ or γ , respectively (Salmeron et al., 1991, The Journal of Immunology). It was also shown that it binds to the huCD3 ϵ subunit exclusively (Kjer-Nielsen et al., 2004, PNAS). Because of the similar behavior of 12D2

and OKT3 an interaction with huCD3 ϵ is supposable for 12D2. Taken all together, huCD3 ϵ seems to be the antigenic structure for all analyzed antibodies (data presented in table 5).

5 **Table 5: Affinities of selected CD3 antibodies against huCD3 δ , huCD3 ϵ , huCD3 ϵ/δ and huCD3 ϵ/γ complexes measured by Flow cytometry and Biacore.**

	Flow cytometry			Biacore	
	% antibody positive transfected cells (n=3-6)			RU of Fab binding at 100nM	
Clone	huCD3 δ	huCD3 ϵ (+/- SEM)	huCD3 ϵ/δ (+/-SEM)	huCD3 ϵ/δ	huCD3 ϵ/γ
10E6	n.b.	96,3 +/- 2,2	93,3 +/- 4,7	11	8
11F9	n.b.	97,0 +/- 1,5	96,0 +/- 2,5	10	7
12D2	n.b.	n.b.	96,3 +/- 2,2	11	5
12G3	n.b.	96,3 +/- 2,2	96,7 +/- 1,9	10	7
13H2	n.b.	93,3 +/- 3,3	93,0 +/- 4,2	9	6
18F5	n.b.	70,6 +/- 11,9	70,4 +/- 9,9	13	7
18G9	n.b.	95,0 +/- 2,5	97,0 +/- 1,5	10	3
1E6	n.b.	87,7 +/- 4,4	91,7 +/- 4,9	10	7
20E5	n.b.	96,7 +/- 1,9	95,3 +/- 3,2	11	8
20G6	n.b.	68,6 +/- 12,1	67,4 +/- 10,4	7	4
4B4	n.b.	71,5 +/- 10,8	69,5 +/- 8,7	11	3
4E7	n.b.	69,0 +/- 12,3	66,4 +/- 10,0	8	6
5B1	n.b.	97,0 +/- 1,5	97,0 +/- 1,5	10	7
OKT3	n.b.	n.b.	70,2 +/- 9,6	4	3

n.b. = no binding (<20% of binding of huCD3 ϵ +huCD3 δ)

10 1.6 Binding of CD3 Fab to human T cells

The binding capacity of the CD3-Fabs was determined by flow cytometry. Primary human T cells were used as target cells. Therefore, peripheral blood mononuclear cells (PBMCs) were isolated from 200 ml peripheral blood of healthy donors treated with EDTA by Ficoll density centrifugation. 15 ml Histopaque (Sigma-
 15 Aldrich) was preloaded on a 50 ml Leucosep-Tube (Greiner bio-one). Blood was diluted with autoMACS Rinsing Buffer + 1% BSA (Miltenyi Biotec) and loaded on the membrane of a total of ten prepared tubes. Tubes were centrifuged without brake for 10 min at 1000 xg. PBMCs were collected and washed with autoMACS Rinsing Buffer + 1% BSA three

times. Finally, PBMCs were resuspended in autoMACS Running Buffer (Miltenyi Biotec) for isolation of T lymphocytes by autoMACSpro technology using the Pan T Cell isolation Kit (Miltenyi Biotec) according to manufacturer's instructions. Purity of separated T cells was analyzed by MACSQuant flow cytometry using the human 7-Color Immunophenotyping Kit (Miltenyi Biotec). Isolated T cells were resuspended in Stain Buffer with FBS (BD Pharmingen) and 1E5 cells in 100 µl per well were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one). Fab antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 30000 ng/ml. The assay was incubated for 45 min at 4°C. Cells were washed twice with Stain Buffer with FBS and 1 µg secondary antibody Goat F(ab')₂ Anti-Human kappa-FITC (Beckman Coulter, #732621) per well was added in 100 µl Stain Buffer with FBS per well. The assay was incubated for 20 min at 4°C and washed twice afterwards. Cells were resuspended in 150 µl Stain Buffer with FBS per well and were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of cells positive for antibody binding. Cells treated only with the secondary but no primary antibody were used to set the gates. EC₅₀ Curves were calculated by XLfit (Algorithm 205), EC₅₀ values were calculated as inflection point of the slope (data are shown in table 6).

Table 6: Affinities of CD3 Fab to human T cells measured by Flow cytometry.
Presented are mean EC50 values calculated from curves.

Antibody	Binding to human T cells EC50 [nM] mean +/- SEM		
12D2-E5-Fab	3,8	+/-	0,2
4B4-D7-Fab	15,3	+/-	3,0
1E6-C9-Fab	18,7	+/-	3,5
10E6-G6-Fab	4,7	+/-	0,9
4E7-C9-Fab	6,2	+/-	0,8
12G3-E8-Fab	4,4	+/-	0,3
5B1-G2-Fab	11,1	+/-	3,6
18F5-H10-Fab	4,1	+/-	0,0
18G9-H11-Fab	1,7	+/-	0,5
13H2-C2-Fab	14,3	+/-	0,9
20G6-F3-Fab	14,0		(n=1)
11F9-F8-huFab	11,6		(n=1)
20E5-F10-huFab	8,2		(n=1)

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1.7 Safety of CD3 Fab

1.7.1 Safety of CD3 Fab measured by CD25+ and CD69+ expression on human T cells
The effect of CD3 Fab antibodies on activation status of T cells as safety read out was analyzed by flow cytometry based detection of the expression of activation marker CD25 and CD69 on the surface of primary human T cells.

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Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I (Gibco) + 10% FCS (Invitrogen) and 2.5E5 cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one) in 100 µl per well.

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5µl Fab CD3 antibodies were added to the cells at a final concentration of 30 000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO₂.

After incubation time cells were spun down and stained for 15 min at 4°C in 100 µl Stain Buffer with FBS (BD Pharmingen) per well with following labeled antibodies: CD25-V450, CD69-APC

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Cells were washed twice after staining, resuspended in 150 µl Stain Buffer with FBS, and 5000 cells were measured using the LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CD25pos and CD69pos T cells (table 7).

Table 7: Safety of CD3 Fab measured by CD25+ and CD69+ expression on human T cells

Antibody	CD25+% Activation of human T cells normalized to PBS C = 30000ng/ml n=1	CD69+% Activation of human T cells normalized to PBS C = 30000ng/ml n=1
11H3-E5-Fab	0,7	0,5
12D2-E5-Fab - SEC	3,2	11,1
4B4-D7-Fab	2,5	5,4
1E6-B8/B9-C9-Fab	1,8	3,2
10E6-G6-Fab	0,8	2,6
4E7-C9-Fab	0,4	0,1
12G3-E8-Fab	0,0	1,5
5B1-G2-Fab	0,4	4,2
6F4-D10-Fab	2,1	6,4
18F5-H10-Fab	0,0	0,1
18G9-H11-Fab	0,7	2,2
13H2-C2-Fab	0,5	0,4
13C1-F6-Fab	0,4	0,2
1E6-B8/B9-C9-Fab	0,6	1,7
20G6-F3-Fab	0,2	3,2
1E6-B8/B9-C9-Fab	2,5	7,8
1E6-B8/B9-C9-Fab	0,1	1,0
11F9-F8-huFab	1,0	0,8
20E5-F10-huFab	1,5	1,3

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1.7.2 Safety of CD3 Fab measured by CD4+/CD69+, CD4+/CD25+, CD8+/CD69+ and CD8+/CD25+ expression on human T cells

The effect of CD3 Fab antibodies on activation status of T cells as safety read out was analyzed by flow cytometry based detection of the expression of activation marker CD25 and CD69 on the surface of primary human T cells. Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I (Gibco) + 10% FCS (Invitrogen) and 2.5E5 cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-

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one) in 50 µl per well. Either T cells exclusively were tested and wells were filled-up with 50 µl RPMI + GlutaMAX I + 10% FCS, or target cells (i.e. THP-1 cell line) were added at 2.5E4 cells per well in 50 µl RPMI + GlutaMAX I + 10% FCS. Bispecific antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 30 000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO₂. After incubation time cells were spun down and stained for 15 min at 4°C in 100 µl Stain Buffer with FBS (BD Pharmingen) per well with following labeled antibodies: CD4-PE, CD8-APC-Cy7, CD25-APC, CD69-PE-Cy7. As Fluorescence Minus One (FMO) control activated T cells were stained as described above but CD25 was replaced by its isotype (Isotype APC-IG1k) in one tube and CD69 was replaced by its isotype (Isotype PE-Cy7-IG1k) in a second tube. Cells were washed twice after staining, resuspended in 150 µl Stain Buffer with FBS, and 5000 cells were measured using the LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CD4posCD25pos, CD4posCD69pos, CD8posCD25pos, and CD8posCD69pos T cells. Gates were set according to FMO controls (see table 8).

Table 8: Safety of CD3 Fab measured by CD4+/CD69+, CD4+/CD25+, CD8+/CD69+ and CD8+/CD25+ expression on human T cells

Antibody	CD4+/CD69+ % Activation normalized to PBS C=100nM mean+/- SEM			CD8+/CD69+ % Activation normalized to PBS C=100nM mean+/- SEM			CD4+/CD25+ % Activation normalized to PBS C=100nM mean+/- SEM			CD8+/CD25+ % Activation normalized to PBS C=100nM mean+/- SEM		
20G6-F3-Fab	0,1	+/-	0,1	0,0	+/-	0,0	0,4	+/-	0,1	0,0	+/-	0,1
4B4-D7-Fab	3,2	+/-	1,1	0,3	+/-	0,3	1,3	+/-	0,6	0,0	+/-	0,0

Example 2: CD123 sequences

2.1 Construction of CD123 (IL3RA) -hFc fusion expression plasmids (CD123-Fc)

Using cDNA containing plasmids as a template, human and *Macaca fascicularis* CD123 fusion proteins were generated in reading frame with heavy chain constant region including a GS-linker (used in Macaca protein), the hinge region, CH2 and CH3

domains of human immunoglobulin IgG additionally carrying a Strep-II Tag (only in human protein version).

Using human genomic DNA as template, human CD123 (IL3RA) extracellular domain was amplified, including the signal sequence. The resulting amplified cleaved and purified PCR products were combined by ligation PCR and ligated into mammalian expression vector pXL by InFusion method using NheI and HindIII site. The sequence of the resulting mature human CD123 Strep-II tagged Fc-fusion protein is disclosed under SEQ ID NO: 196. Amino acids 1 to 284 correspond to the amino acids 22 to 305 of the full-length wild-type human CD123 protein (herein disclosed under SEQ ID NO: 194, available from the NCBI database under the accession number NP_002174.1) and thus the extracellular domain of human CD123.

To clone *Macaca fascicularis* CD123cDNA was made from blood of a *Macaca fascicularis* population. Using this isolated cDNA as template, *Macaca* CD123 (IL3ra) extracellular domain was amplified, including the signal sequence. The resulting amplified cleaved and purified PCR products were combined by ligation PCR and ligated into mammalian expression vector pXL by InFusion method using NheI and HindIII. The sequence of the resulting mature human CD123 Strep-II tagged Fc-fusion protein is disclosed under SEQ ID NO: 197. Amino acids 1 to 284 correspond to the amino acids 22 to 305 of the full-length wild-type *Macaca fascicularis* CD123 protein (herein disclosed under SEQ ID NO: 195, available from the NCBI database under the accession number NP_002174.1) and thus the extracellular domain of human CD123.

2.2 Expression and purification of human and *Macaca fascicularis* CD123-Fc

Freestyle HEK293 cells growing in F17 serum free suspension culture (Life) were transiently transfected with the expression plasmid. Transfection was performed using Cellfectin transfection reagent (Life) The cells were cultured at 37°C for 7 days. The culture supernatant containing recombinant protein was harvested by centrifugation and was clarified by filtration (0.22µm).

For purification the Fc-fusion protein variants were captured on protein A matrix (GE) and eluted by pH shift. After polishing the protein by SEC in PBS using a Superdex 200 (GE) and a final ultrafiltration concentration step, the protein was used for further assays.

2.3 Assessment of affinities to both human and *Macaca fascicularis* CD123Rat IgGs from hybridoma

Screening of anti-CD123 rat IgGs for binding affinities to human CD123 and cross-reactivities to cyno CD123 was performed with hybridoma supernatants using a Proteon XPR36 (Biorad) in a one-shot kinetics approach. A capture assay was established using a goat anti-rat IgG (112-005-071, Jackson Immuno Research). The capture antibody was coated on GLC chips (176-5011, Biorad) to approx. 8000 RU in the vertical direction using the amine coupling kit (176-2410, Biorad). Capture of the rat IgGs to approx. 200 RU in vertical direction resulted in Rmax values of up to 100 RU for CD123-Fc. Binding kinetics with human and cyno CD123-Fc fusion protein was measured at 100 μ l/min in the horizontal direction with 120 sec. and 600 sec. for association and dissociation, respectively. The CD123-Fc proteins were used in twofold dilutions from 6 nM to 100 nM. PBSET buffer (176-2730, Biorad) was used as assay buffer. Regeneration was achieved by injection of 10mM Glycine buffer pH 1.5 for 18 sec at 30 μ l/min. Data processing and analysis was performed using ProteonManager software v3.0. Fitting of the sensorgrams was done with a 1:1 Langmuir model. Clones were selected based on affinities for human CD123 with KD < 1 nM and crossreactivity to cyno CD123.

Fabs and CODV-Fabs

Binding affinities of anti-CD123 binding Fabs or CODV-Fabs were measured using a Biacore3000 instrument (GE Healthcare). Assay buffer was HBS-EP (BR-1001-88, GE Healthcare). Capture of CD123-Fc fusion proteins was achieved using the human antibody capture kit (GE Healthcare). The capture antibody was coupled to CM5 chips (BR-1001-88, GE Healthcare) to approx. 12.000 RU using the amine coupling kit (BR-100-50, GE Healthcare). The CD123-Fc fusions proteins were captured at 10 μ l/min to approx. 70 RU to yield Rmax values of 30 RU. Binding kinetics with the anti-CD123 Fabs or CODV-Fabs was measured at 30 μ l/min for 240 sec. and 600 sec. for association and dissociation phase, respectively. Twofold dilutions of Fabs from 3 to 200nM in assay buffer were used. All Fab concentrations were run in duplicate together with duplicate buffer blanks for double referencing. Regeneration of the capture surface was performed with a 1 min injection of 3M MgCl₂ solution at 30 μ l/min. For data analysis the BIAevaluation software v.4.1 (GE Healthcare) was used. Data were fit globally using a 1:1 Langmuir model with mass transfer.

IgGs and CODV-Fc proteins

Measurement of binding affinities of anti-CD123 IgGs and CODV-Fc proteins was performed analogous to the binding assay for Fabs and CODV-Fabs with the exception of the capture antibody. In this case the Strep-MAB classic antibody (2-1507-001, IBA) was used to capture human CD123-Fc via its StrepII-tag. Here the regeneration solution was 10mM Glycine buffer pH2.0.

2.4 Generation of human and *Macaca fascicularis* cross-reactive anti-CD123 antibodies

Human and *Macaca fascicularis* CD123 cDNAs were cloned into Aldevron proprietary immunization vectors (pB8 and VV8) respectively. Three rats of immunization group MR13-296 were immunized with the immunization vector IL3RA-hum.-ECD (aa19-305). The immune serum was taken at day 24 of the immunization protocol, after 4 genetic applications (IS24d-4). Sera, diluted in PBS 3% FBS, were tested by flow cytometry using mammalian cells transiently transfected with the human and cyno IL3RA cDNA variants IL3RA-hum.ECD and IL3RA-hum.D3.

Specific reactivity of the immune sera against cells transfected with pB1-IL3RA-hum.ECD, as well as with IL3RA-cyno (pFF1262) and the THP-1 cells could be detected in all immunised animals when compared to cells transfected with an irrelevant cDNA.

Rats with positive sera were sacrificed and B cells were fused with mouse myeloma cells. The resulting hybridomas were screened on HEK293 cells transfected with human or cynomolgus CD123 expression plasmids, on different cell lines expressing CD123 by flow cytometry (data shown in table 10).

Target cells were seeded at 5E4 cells in 50 µl Stain Buffer with FBS (BD Pharmingen) per well in 96-well U-bottom suspension culture plates (Greiner bio-one). Hybridoma supernatants were diluted 1:3 in serial in PBS (Invitrogen) and 50 µl each were added to the cells at a final maximum concentration of 1 µg/ml. The assay was incubated for 45 min at 4°C.

Cells were washed twice with Stain Buffer with FBS and 1 µg secondary antibody Goat Anti-Rat IgG (H+L)-Alexa Fluor 488 (Invitrogen-Life Technologies, # MH10520)

was added in 100 µl Stain Buffer with FBS per well. The assay was incubated for 15 min at 4°C and washed twice afterwards.

Cells were resuspended in 200 µl Stain Buffer with FBS per well and were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of cells positive for antibody binding. Cells treated only with the secondary but no primary antibody were used to set the gates. Curves were calculated by XLfit (Algorithm 205).

Specific binding of clones to CD123 could be shown on the surface of transfected HEK293 in comparison to untransfected HEK293 cells where no signal could be detected (data not shown). Binding of antibodies was concentration dependent with an EC50 value ranging between 0.4 and 17.7 ng/ml (table 9).

Table 9: Specific CD123 binding of rat IgG clones in hybridoma supernatants detected by flow cytometry. Concentration dependent binding of antibodies was measured using CD123 transfected HEK293 as target cells and a goat anti-rat IgG (H+L)-Alexa Fluor 488 secondary antibody. Presented are calculated EC50 values of the curves.

Antibody	EC50 [ng/ml]
BFX - 1A6	6,4
BFX - 1E1	2,2
BFX - 2B8	5,5
BFX - 2F4	4,5
BFX - 2F8	3,4
BFX - 2H7	17,7
BFX - 3B10	2,0
BFX - 3E3	2,0
BFX - 5A5	10,1
BFX - 6B10	5,0
BFX - 6C10	6,1
BFX - 6D6	0,9
BFX - 8B11	0,4
BFX - 9B8	1,2
BFX - 9D7	0,9
BFX - 9F6	1,3
BFX - 9H2	14,9

Table 10: Binding data of CD123 antibodies to recombinant CD123 and CD123 expressing cells. Binding data showing affinities of CD123 antibodies against recombinant CD123 protein from human and cynomolgous monkey. Binding on cell surface was detected by flow cytometry. SP2 refers to cell expressing N-terminal truncated (D1 region) variant of CD123. Antibodies were tested by Proteon XPR36 on their ability to compete with the IL3 binding to CD123.

Clone	ka (1/Ms)	kd (1/s)	KD (nM) Human	KD (nM) Cyno	Molm-13	THP-1	OCI-AML3	SP2	Basophils	IL3 Blocking
1E1	4,6E+05	1,1E-04	0,23	0,72	+	+	+	+	+	-
2B8	6,6E+05	1,2E-05	0,018	0,77	+	+	+	+	+	-
2F8	1,3E+05	6,6E-05	0,53	0,51	+	+	+	-	+	+
3B10	2,2E+05	0,5E-05	0,21	0,27	+	+	+	-	+	+
3E3	1,6E+05	6,4E-05	0,4	0,12	+	+	+	-	+	+
5A5	1,3E+05	1,2E-04	1,1	0,05	+	+	+	+	+	-
6B10	2,3E+05	1,5E-05	0,15	0,1	+	+	+	+	+	-
6C10	1,8E+05	1,4E-04	0,75	0,79	+	+	+	+	+	-
8B11	4,7E+05	6,2E-06	0,01	0,1	+	+	+	+	+	-
9B8	1,5E+06	2,8E-04	0,19	0,17	+	+	+	+	+	+
9D7	1,0E+06	3,2E-04	0,3	0,07	+	+	+	+	+	+
9F6	1,8E+05	6,7E-06	0,04	0,5	+	+	+	+	+	-

2.5 Humanization of anti-CD123 rat antibody sequences

Humanization of rat antibodies was performed by CDR-grafting or by the 4D method (US20110027266). For the rat-anti-CD3 antibody 3E3 the closest *Rattus* germline sequence identified was IGHV2S48*01 and IGHJ3*01 for the heavy chain variable region and IGLV3S2*01 and IGKJ3*01 for the light chain variable region. The calculated rat germinality index (frameworks sequences only) is 94.51% for the VH and 98.9% for the VL.

Potential exposed problematic residues were checked and one residue in CDRH2 was modified.

Using the grafting method, a variety of humanized variants were generated based on the closest human germline sequences identified: IGHV4-59*05 and IGHJ4*01 for the VH (germinality index on frameworks: 75.82%); IGLV6-57*01 and IGLJ3*01 for VL (germinality index on frameworks: 72.22%).

In addition to CDR grafting, the 4D humanization protocol (US20110027266) was used to humanize the Rat anti-CD123 3E3 variable light (VL) and heavy (VH) domains. A molecular dynamics (MD) simulation was performed on the minimized 3D homology model (done with MOE ; PDB used: 1FLR) of Rat anti-CD123 3E3 and compared to the 49 Human models derived from the seven representative light chains (vk1, vk2, vk3, vk4, vlambd1, vlambd2, vlambd3) and the seven representative heavy chains (vh1a, vh1b, vh2, vh3, vh4, vh5, vh6) designed by LGCR/SDI and available within MOE.

Two Models have been selected for the “4D humanization”: VI3-vh4 with and VL3-VH2 with the best both hydrophobic and electrostatic components and sequence identity outside CDRs. For the pairwise association between the Rat anti-CD123 3E3 variable domain and the two selected models, the sequences were aligned based on the optimal 3D superposition of the alpha carbons of the corresponding homology models.

Example 3: Antibodies in the bispecific CODV-Fab format

3.1 Cloning of selected CD3 sequences in combination with anti-CD123 mAb 7G3 in the bispecific CODV-Fab format to study their T-cell engagement activity

Selected CD3 antibody sequences, such as I2C, mAb2 (Macrogenics) and the so-called “20G6-F3”, “4E7-C9”, “4B4-D7” and “18F5-H10” were expressed as monospecific anti-CD3 Fabs, Said selected sequences were as well expressed as bispecific CD3xCD123 CODV-Fabs using sequences of the monoclonal antibody 7G3, resulting in the CODV-Fab constructs “I2Cx7G3” and the so-called “7G3x20G6”, “7G3x4E7”,

“7G3x4B4” and “7G3x18F5”, as further described in the section “antibody-like binding proteins” herein above. Purified proteins were used in a Biacore assay to compare the affinity against CD3 ϵ / δ complexes (data presented in table 11). No changes in affinities could be detected by Biacore analysis when CD3 sequences were introduced into the bispecific CODV-Fab format.

Table 11: Comparison of affinities between Fab-fragments and CODV-Fabs against CD3 ϵ / δ complexes from human and cynomolgous monkey

	Molecule	hu kon 1/Ms	hu koff 1/s	hu KD nM	cy KD nM
Fab- fragments	I2C	1,1E+07	1,3E-03	0,13	0,5
	mAb2	3,1E+05	4,3E-03	13,7	16,3
	BDW-20G6-F3	3,5E+04	2,7E-04	8	8
	BDX-4E7-C9	8,9E+04	1,5E-03	17	15
	BDW-4B4-D7	1,1E+05	2,9E-03	27	24
	BDX-18F5-H10	2,0E+05	1,8E-04	1	1
CODV-Fabs	7G3xI2C	4,0E+06	2,0E-03	0,5	0,4
	7G3x20G6	7,2E+04	5,1E-04	7	7
	7G3x4E7	1,2E+05	1,9E-03	16	10
	7G3x4B4	2,7E+05	4,4E-03	17	18

3.2 Bispecific CODV-Fabs directed against CD123 and CD3 mediate potent redirected T-Cell Killing

Such bispecific CODV-Fabs have the ability to localize a T-cell (by binding such T-cell to the CD3-binding portion of a CD3-binding CODV-Fab) to the location of a tumor cell (by binding such cancer cell to the CD123 portion of the CODV-Fab). The localized T-cell can then mediate the killing of the tumor cell in a process termed herein "redirected" killing. Bispecific CODV-Fab specific for CD123 and CD3 were constructed having the anti-CD123 variable domains of monoclonal antibody 7G3 and anti-CD3 variable domains of selected CD3 antibodies generated in example 1.

Therefore, peripheral blood mononuclear cells (PBMCs) were isolated from 200 ml peripheral blood of healthy donors treated with EDTA by Ficoll density centrifugation.

15 ml Histopaque (Sigma-Aldrich) was preloaded on a 50 ml Leucosep-Tube (Greiner bio-one). Blood was diluted with autoMACS Rinsing Buffer + 1% BSA (Miltenyi Biotec) and loaded on the membrane of a total of ten prepared tubes. Tubes were centrifuged without brake for 10 min at 1000 xg. PBMCs were collected and washed with autoMACS Rinsing Buffer + 1% BSA three times. Finally, PBMCs were resuspended in autoMACS Running Buffer (Miltenyi Biotec) for isolation of T lymphocytes by autoMACSpro technology using the Pan T Cell isolation Kit (Miltenyi Biotec) according to manufacturer's instructions. Purity of separated T cells was analyzed by MACSQuant flow cytometry using the human 7-Color Immunophenotyping Kit (Miltenyi Biotec).

T-cell engaging effect of bispecific antibodies was analyzed by a flow cytometry based cytotoxic assay. Target cells (i.e. THP-1 cell line) were stained for 15 min at 37°C with 1 µM CFSE in 1 ml RPMI + GlutaMAX I (Gibco) per 1E7 cells. Afterwards, cells were washed twice and resuspended in RPMI + GlutaMAX I + 10% FCS (Invitrogen). 2.5E4 target cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one) in 50 µl medium per well.

Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I + 10% FCS and were added at indicated effector-to-target ratio in 50 µl per well to the target cells (in general E:T=10:1).

Bispecific antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 3 000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO₂.

To detect dead target cells, all cells were stained with 7-AAD. Therefore, 5 µg/ml 7-AAD diluted in Stain Buffer with FBS (BD Pharmingen) were added to each well and were incubated for 15 min at 4°C in the dark. Cells were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer, respectively. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CFSE and 7-AAD double positive cells.

The results of these investigations shown for example in tables 20 to 22 demonstrate the ability of the CD123xCD3 CODV-Fabs to mediate redirected killing of tumor cells.

3.3 Safety assessment of redirected T-Cell Killing

The effect of bispecific antibodies on activation status of T cells as safety read out was analyzed by flow cytometry based detection of the expression of activation marker CD25 and CD69 on the surface of primary human T cells.

5 Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I (Gibco) + 10% FCS (Invitrogen) and 2.5E5 cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one) in 50 µl per well.

10 Either T cells exclusively were tested and wells were filled-up with 50 µl RPMI + GlutaMAX I + 10% FCS, or target cells (i.e. THP-1 cell line) were added at 2.5E4 cells per well in 50 µl RPMI + GlutaMAX I + 10% FCS.

Bispecific antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 30 000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO₂.

15 After incubation time cells were spun down and stained for 15 min at 4°C in 100 µl Stain Buffer with FBS (BD Pharmingen) per well with following labeled antibodies: CD4-PE, CD8-APC-Cy7, CD25-APC, CD69-PE-Cy7

As Fluorescence Minus One (FMO) control activated T cells were stained as described above but CD25 was replaced by its isotype (Isotype APC-IG1k) in one tube and CD69 was replaced by its isotype (Isotype PE-Cy7-IG1k) in a second tube.

20 Cells were washed twice after staining, resuspended in 150 µl Stain Buffer with FBS, and 5000 cells were measured using the LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CD4posCD25pos, CD4posCD69pos, CD8posCD25pos, and CD8posCD69pos T cells. Gates were set according to FMO controls (see table 12).

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Table 12: Activity of CD123 x CD3 CODV-Fabs measured in a cytotoxic assay and safety measured by the expression of CD69

Molecule	Activity EC50 [ng/ml]	Safety CD69 expression %cells/%max
7G3 x I2C	20 ± 9	25,8 ± 7,6
7G3 x OKT3	16 ± 5	25,5 ± 8,0
7G3 x 20G6	18 ± 5	8,7 ± 0,8
7G3 x 4E7	12 ± 3	12,3 ± 2,5
7G3 x 4B4	11 ± 2	11,6 ± 0,8
7G3 x 18F5	61 ± 15	9,8 ± 1,1

5 3.4 Humanization of anti-CD3 rat antibody sequences and bispecific antibodies

Humanization of rat antibodies was performed by CDR-grafting or by the 4D method (US20110027266).

For the rat-anti-CD3 antibody “20G6” the closest Rattus germline sequences was identified as IGHV6S17*01 and IGHJ2*01 (for the heavy chain variable region and
10 IGKV1S21*01 and IGKJ4*01 for the light chain variable region). The calculated rat germinality index (frameworks sequences only) is 97,80% for the VH and 95,5% for the VL.

A variety of humanized variants using grafting method were generated based

- 1) on the closest human germline sequences identified IGHV3-30-01_IGHJ4-01 for
15 the VH with a germinality index on framework of 77%; IGK2D-29-02_IGKJ4-01 for the VL with a germinality index on the framework of 80%), or
- 2) or based on the a closest germline sequence identified having a lower PI IGVH3-48*02-IGHJ4-01 for the VH with a germinality index on the framework of 75% ; IGKV2-28*01-IGKJ4-01 for the VL with a geminality index on the framework
20 of 77,5%, or
- 3) consisting on a more distant human germline sequence (change germline clade) (IGVH1-46*01-IGHJ4*01 for the VH with a germinality index on the framework of 56%; IGKV4*01-IGKJ4*01 for the VL with a germinality index on the framework of 67,5%).

The Humanized sequences were then introduced into the CODV-Fab format of the so-called so-called CODV-Fab “7G3 x 20G6” in combination with the anti-CD123 sequence from antibody 7G3 as described before. Purified CD123 x CD3 CODV-Fabs were used in a Biacore assay to assess the affinity to CD3 ϵ/δ (see table 13).

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Table 13: Affinity of selected humanized variants of the anti-CD3 antibody 20G6 to recombinant CD3 ϵ/δ complex.

Humanized sequence combinations used in the CODV-Fab “7G3 x 20G6”	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)
parental sequence	2,17E+04	5,71E-04	46	2,63E-08
VL1A_VH1A	2,86E+04	4,34E-03	46	1,52E-07
VL1B_VH1A	2,99E+04	4,14E-03	45	1,38E-07
VL1C_VH1D	2,30E+04	1,01E-03	58	4,40E-08
VL1D_VH1B	1,86E+04	1,09E-03	62	5,87E-08
VL1D_VH1C	2,22E+04	7,13E-04	71	3,21E-08
VL1D_VH1D	2,40E+04	7,46E-04	59	3,10E-08

10 For the rat-anti-CD3 antibody 4B4-D7 the closest Rattus germline sequence was identified as IGHV6S17*01 (identity of 93%) and IGHJ2*01 (identity of 87.5%) for the heavy chain variable region and IGKV1S21*01 (identity of 93%) and IGKJ4*01 (identity of 100%) for the light chain variable region.

15 The calculated percentage of identity of the identified Rattus V-sequences to Human Germinality (frameworks sequences only) is 79% for the VH and 77.53% for the VL.

A variety of humanized variant pairs for VH and VL were generated by grafting with additional sequence engineering, using the closest human germline sequences (IGHV3-30*01_IGHJ6*02; IGKV2-30*02/IGKV2D-39*02_IGKJ2*01). The calculated percentages

of Human Germinality (4 IMGT frameworks sequences only) for the humanized V-sequences are listed in table 14.

5 **Table 14: Percentages of Human Germinality for humanized V-sequences obtained by grafting of the so-called “B4-D7” antibody.** The percentages were calculated based on the 4 IMGT frameworks sequences only.

Grafted “4B4—D7” variants	% Human germinality index
VL1Cmodif1	92.1
VL1Cmodif2	91.0
VL1Cmodif3	91.0
VL1Amodif1	97.8
VL1Amodif2	96.6
VL1Amodif3	95.5
VL2C	95.5
VL2D	96.7
VL2F	96.7
VH6Bmodif1	92.3
VH6Bmodif2	90.1
VH6Amodif1	94.5
VH6Amodif2	92.3
VH6Amodif3	91.2
VH6C	95.5
VH6D	93.5

10 In addition to CDR grafting, the 4D humanization protocol as described in the US patent application US20110027266 was used to humanize the Rat anti-CD3 4B4-D7 variable light (VL) and heavy (VH) domains. A molecular dynamics (MD) simulation was performed on the minimized 3D homology model (done with MOE ; PDB used: 1FLR) of Rat anti-CD3 4B4-D7 and compared to the 49 Human models derived from the seven representative light chains (vk1, vk2, vk3, vk4, vlamba1, vlamba2, vlamba3) and the
15 seven representative heavy chains (vh1a, vh1b, vh2, vh3, vh4, vh5, vh6) designed by LGCR/SDI and available within MOE

Two Models were selected for the “4D humanization”. vk1-vh6 with the highest 4D similarity, whit both hydrophobic and electrostatic components. vk2-vh3 with the highest

sequence identity outside CDR. For the pairwise association between the Rat anti-CD3 4B4-D7 variable domain and the two selected models, the sequences were aligned based on the optimal 3D superposition of the alpha carbons of the corresponding homology models. A variety of other humanized variant pairs for VH and VL were further optimized.

5 The calculated percentages of Human Germinality (4 IMGT frameworks sequences only) for the humanized V-sequences are listed in Table 15:

10 **Table 15: Percentages of Human Germinality for humanized V-sequences obtained by 4D humanization of the so-called “B4-D7” antibody.** The percentages were calculated based on the 4 IMGT frameworks sequences only.

4D “4B4—D7” variants	% Human germinality index
VL1A	75
VL1B	75
VL2A	83
VL2B	83
VH1A	80
VH1B	80
VH2A	80
VH2B	84.5

Humanized sequences were expressed as Fab-fragments and purified followed by a Biacore assay to assess the affinity to CD3 ϵ/δ (data shown in table 16).

15

Table 16: Affinity of selected humanized variants of the anti-CD3 antibody 4B4-D7 to recombinant CD3 ϵ / δ complex.

Fab	VL	VH	Ligand	ka	kd	KD
4B4			huCD3	3,26E+05	2,85E-03	8,79E-09
			cyCD3	1,90E+05	2,37E-03	1,26E-08
4B4_1	VL1a 75%	VH1B 80%	huCD3	6,28E+05	1,74E-03	2,77E-09
			cyCD3	5,06E+05	1,58E-03	3,13E-09
4B4_2	VL1b 75%	VH1B 80%	huCD3	2,86E+05	2,14E-03	7,48E-09
			cyCD3	2,77E+05	2,10E-03	7,57E-09
4B4_5	VL2C 95,5%	VH6D 93,5%	huCD3	2,33E+05	4,23E-03	1,82E-08
			cyCD3	2,53E+05	4,20E-03	1,66E-08
4B4_6	VL1a 75%	VH1A 80%	huCD3	1,50E+06	1,85E-03	1,24E-09
			cyCD3	1,49E+06	1,77E-03	1,19E-09
4B4_7	VL1b 75%	VH1A 80%	huCD3	3,06E+05	2,48E-03	8,10E-09
			cyCD3	3,01E+05	2,19E-03	7,28E-09
4B4_9	VL2B 83%	VH2A 80%	huCD3	2,18E+05	2,91E-03	1,34E-08
			cyCD3	2,85E+05	3,44E-03	1,21E-08
4B4_10	VL1_CM2 91%	VH6_BM2 90%	huCD3	4,34E+05	3,99E-03	9,19E-09
			cyCD3	4,30E+05	3,84E-03	8,94E-09
4B4_11	VL1_CM3 91%	VH6_BM2 90%	huCD3	5,66E+05	7,74E-03	1,37E-08
			cyCD3	2,38E+05	4,86E-03	2,04E-08
4B4_17	VL2A 83%	VH2B 84,5%	huCD3	3,10E+05	2,76E-03	8,91E-09
			cyCD3	2,98E+05	2,70E-03	9,07E-09
4B4_20	VL2F 96,5%	VH6C 95,5%	huCD3	5,09E+04	1,71E-03	3,35E-08
			cyCD3	4,56E+04	1,98E-03	4,34E-08

3.5 Binding CODV hu-Fab CD123 x CD3 to THP-1 and TF-1 cells

5 Sequences of selected CD123 antibodies were cloned into the CODV-Fab format in combination with a CD3 binding sequence and proteins were expressed and purified.

Their binding capacity to cells naturally expressing CD123 was determined by flow cytometry. THP-1 cell line or TF-1 cell line were used as target cells.

Target cells were blocked with FcR-Blocker (Sigma). Therefore, target cells were resuspended in Stain Buffer with FBS (BD Pharmingen) and were blocked with 100 µl blocking reagent per ml for 1 h at 4°C. Cells were filled-up with Stain Buffer with FBS and 1E5 cells in 50 µl per well were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one).

Antibodies were added at 3 µg in 50 µl Stain Buffer with FBS per well. The assay was incubated for 30 min at 4°C.

Cells were washed twice with Stain Buffer with FBS and 1 µg secondary antibody Goat F(ab')₂ Anti-Human kappa-FITC (Beckman Coulter, #732621) per well was added in 100 µl Stain Buffer with FBS per well. The assay was incubated for 20 min at 4°C and washed twice afterwards.

Cells were resuspended in 150 µl Stain Buffer with FBS per well and were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of cells positive for antibody binding. Cells treated only with the secondary but no primary antibody were used to set the gates.

Binding of CD123xCD3 CODV-Fabs to CD123 was shown with two different cell lines expressing CD123 either with co-expression of CD131 on TF-1 cell line or on the surface of THP-1 cells lacking CD131 expression. Exemplarily shown are five different clones binding to target cells. As negative control (specificity control) a CD19xCD3 CODV-Fab was used as and a reference CD123xCD3 CODV-Fab as positive control (table 17).

Table 17: Specific binding of CD123 targeting sequences cloned into CODV-Fab backbone in combination with a CD3 binding sequence to THP-1 and TF-1 cell lines. Binding of antibodies was detected using CD123 expressing THP-1 and TF-1 as target cells. 3 µg antibody was added and detected by a secondary goat anti-human kappa-FITC antibody. Shown are percentages of antibody positive target cells.

Antibody	% FITC positive Cells	
	THP-1 cells	TF-1 cells
medium control	0,10	0,00
sec. AB control	0,00	0,20
specificity control CD19 x CD3	0,10	0,20
CODV-Fab CD123 x CD3	99,90	82,50
CODV-Fab BFX-2F8-D6 x CD3	24,00	9,30
CODV-Fab BFX-3E3-D3 x CD3	99,00	31,60
CODV-Fab BFX-9B8-G6 x CD3	48,80	31,10
CODV-Fab BFX-9D7-C8 x CD3	88,70	50,20
CODV-Fab BFX-9F6-G3 x CD3	99,70	60,70

3.6 Cytotoxic effect to THP-1 cells mediated by CODV-Fab CD123 x CD3

T-cell engaging effects of bispecific antibodies consisting of new generated CD123 sequence and the same CD3 binding sequence was analyzed by a flow cytometry based cytotoxic assay. Effector cells were primary T cells isolated from whole blood of healthy donors. THP-1 cells were used as CD123 expressing target cells.

Peripheral blood mononuclear cells (PBMCs) were isolated from 200 ml peripheral blood of healthy donors treated with EDTA by Ficoll density centrifugation. 15 ml Histopaque (Sigma-Aldrich) was preloaded on a 50 ml Leucosep-Tube (Greiner bio-one). Blood was diluted with autoMACS Rinsing Buffer + 1% BSA (Miltenyi Biotec) and loaded on the membrane of a total of ten prepared tubes. Tubes were centrifuged without brake for 10 min at 1000 xg. PBMCs were collected and washed with autoMACS Rinsing Buffer + 1% BSA three times. Finally, PBMCs were resuspended in autoMACS Running Buffer (Miltenyi Biotec) for isolation of T lymphocytes by autoMACSpro technology using the Pan T Cell isolation Kit (Miltenyi Biotec) according to manufacturer's instructions. Purity of

separated T cells was analyzed by MACSQuant flow cytometry using the human 7-Color Immunophenotyping Kit (Miltenyi Biotec).

Target cells (i.e. THP-1 cell line) were stained for 15 min at 37°C with 1 µM CFSE in 1 ml RPMI + GlutaMAX I + 10% FCS (Invitrogen). 2.5E4 target cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one) in 50 µl medium per well.

Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I + 10% FCS and were added at indicated effector-to-target ratio in 50 µl per well to the target cells (in general E:T=10:1).

Bispecific antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 3 000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO₂.

To detect dead target cells, all cells were stained with 7-AAD. Therefore, 5 µg/ml 7-AAD diluted in Stain Buffer with FBS (BD Pharmingen) were added to each well and were incubated for 15 min at 4°C in the dark. Cells were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer, respectively. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CFSE and 7-AAD double positive cells. Curves were calculated by XLfit (Algorithm 205).

As exemplarily shown in table 18 the bispecific antibodies were able to engage primary T cells and to lyse THP-1 target cells in vitro. An antibody concentration dependent increase in dead target cells could be detected after 20 h co-incubation. For the antibodies shown in here EC₅₀ values were calculated ranging between 12.2 and 429.3 ng/ml.

Table 18: T-cell engaging effect of bispecific CODV-Fab CD123 x CD3 detected in flow cytometry based cytotoxic assays. Presented are mean EC₅₀ values calculated from curves

Antibody	EC ₅₀ geomean (n=4)	[ng/ml] +/- SEM	EC ₅₀ geomean (n=4)	[nM] +/- SEM	n
CODV-Fab BFX-2B8-F3 x CD3	106,9	+/- 33,9	1420,5	+/- 450,2	4
CODV-Fab BFX-3E3-D3 x CD3	12,2	+/- 5,9	163,0	+/- 80,1	4
CODV-Fab BFX-6B10-E4 x CD3	39,7	+/- 17,9	529,1	+/- 237,7	4
CODV-Fab BFX-9B8-G6 x CD3	106,1	+/- 36,1	1410,2	+/- 479,8	4
CODV-Fab BFX-9D7-C8 x CD3	86,3	+/- 29,3	1148,6	+/- 390,5	4
CODV-Fab BFX-9F6-G3 x CD3	13,9	+/- 7,7	185,9	+/- 104,1	3

CODV-Fab BFX-3B10-E6 x CD3	429,3	+/-	82,6	5709,3	+/-	1099,0	2
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The CD123 clone 3E3 was combined with a humanized variant of anti-CD3 antibody 4B4 in the CODV-Fab format. Their T-cell engaging effect and their ability to activate T cells *in vitro* was analyzed.

5 Cytotoxic assays were performed as described above. The lytic effect of primary human T cells to THP-1 target cell mediated by these constructs is exemplarily shown in table 20 by CODV-Fab hz4B4(4D_A)x3E3. Cytotoxic activity could be induced reliably with a concentration dependent effect with T cells isolated from seven different healthy donors (table 19).

10

Table 19: T-cell engaging effect of bispecific CODV-Fab hz4B4(4D_A) x 3E3 detected in a flow cytometry based cytotoxic assay. Presented are mean EC50 values calculated from curves.

CODV-Fab	Cytotoxicity (THP-1)			Cytotoxicity (THP-1)		
	geomean EC50 [pM] +/- SEM n=7			geomean EC50 [ng/ml] +/- SEM n=7		
hz4B4(4D_A) x 3E3 CODV-Fab	26,5	+/-	5,9	2,0	+/-	0,5

15

3.7 T-cell activating effect of CD123xCD3 CODV-Fab or DART

The effect of bispecific antibodies on activation status of T cells as safety read out was analyzed by flow cytometry based detection of the expression of activation marker CD25 and CD69 on the surface of primary human T cells, as described before. The comparison included the single chain CD123 x CD3 bi-specific diabody in DART format (herein called "MGD006") which was described in WO2015026892 as comprising a first polypeptide chain of sequence SEQ ID NO:386 (which is SEQ ID NO:1 as shown in WO2015026892) and a second polypeptide chain of sequence SEQ ID NO:387 (which is SEQ ID NO:3 as shown in WO2015026892) covalently bonded to one another by a disulfide bond.

25

When the CODV-Fabs were incubated with isolated T cells alone no significant increase in expression of late activation marker CD25 could be detected on the surface of CD4 positive and CD8 positive T cells (data not shown). Equally, there was no

concentration dependent increase in expression level of early activation marker CD69 on both T-cell subsets (table 20). Therefore, the construct was evaluated as not active (NA). In contrast, a huge increase in expression level of both markers was measurable when THP-1 target cells were added (CD25 data not shown, CD69 data table 21).

5

Table 20: Effect of bispecific CD123 x CD3 CODV- Fab or DART on activation state of T cells detected by CD69 expression level in a flow cytometry based assay. Presented are mean percentages of activated CD8 and CD4 T cells at 100nM antibody concentration and the Min-Effect-Concentration in assays with T cells exclusively.

10

CODV-Fab	Safety - T cell activation w/o target cells n=3											
	CD4+/CD69+ % Activation normalized to PBS C=100nM mean+/- SEM			CD4+/CD69+ Min-Effect-Conc [pM] mean+/- SEM			CD8+/CD69+ % Activation normalized to PBS C=100nM mean+/- SEM			CD8+/CD69+ Min-Effect- Conc [pM] mean+/- SEM		
hz4B4(4D_A)x3E3 CODV-Fab	14	+/-	2		NA		13	+/-	2		NA	
hz20G6xhz7G3 CODV-Fab	22	+/-	2				22	+/-	2			
hz20G6xhz7G3 CODV-Fab-TL1	18	+/-	4				15	+/-	2			
hz20G6xhz7G3 CODV-Fab-OL1	6	+/-	2				9	+/-	2			
Single chain antibody DART format MGD006	82	+/-	9				83	+/-	4			

The results shown in Table 20 indicate that the single chain antibody (DART) causes significantly more T-cell activation in the absence of target cells under the conditions tested.

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Table 21: Effect of bispecific CODV- Fab hz4B4(4D_A) x 3E3 on activation state of T cells detected by CD69 expression level in a flow cytometry based assay. Shown are mean percentages of activated CD8 and CD4 T cells at maximal antibody concentration (Cmax) and at the antibody concentration at EC50 in cytotoxic assay. Assays were performed with co-incubation of THP-1 target cells and T cells.

CODV-Fab	Safety - T cell activation with THP-1 target cells n=3											
	CD4+/CD69+ % Activation normalized to PBS C=meanEC50 Cytotox mean+/- SEM			CD4+/CD69+ % Activation normalized to PBS Cmax mean+/- SEM			CD8+/CD69+ % Activation normalized to PBS C=meanEC50 Cytotox mean+/- SEM			CD8+/CD69+ % Activation normalized to PBS Cmax mean+/- SEM		
hz4B4(4D_A)x3E3 CODV-Fab	71	+/-	4	84	+/-	2	69	+/-	6	82	+/-	4

Table 22: Effect of bispecific fully humanized 7G3 containing CODV molecules and single chain Dart on activation state of T cells detected by CD69 expression level in a flow cytometry based assay. Shown are EC50 values of representative tests of activated CD8 and CD4 T cells in cytotoxic assay. Assays were performed with co-incubation of THP-1 target cells and T cells.

CODV-Fab	Safety - T cell activation with THP-1 target cells	
	CD4+ T cells (% CD69+ cells) EC50 (pM) n=1-6	CD8+ T cells (% CD69+ cells) EC50 (pM) n=1-6
hz20G6xhz7G3 CODV-Fab	1.4	3.1
hz20G6xhz7G3 CODV-Fab-TL1	3.2	9.9
hz20G6xhz7G3 CODV-OL1	1.0	3.3
Single chain antibody DART format MGD006	1.0	3.5

In order to assess the cytotoxic effects of new CD123 antibodies with humanized CD3 parts, the CODV-Fabs “hz20G6x7G3”, “7G3xhz4B4”, “hz4B4x3E3” were generated containing different combinations of Fvs. One Fc containing variant was also generated, the CODV-Fab “hz20G6x7G3-TL4” being Fc tagged at the light chain to form Fc heterodimers with the corresponding heavy chain (TL4 variant). Affinities to the CD3ε/δ-

complex and CD123 of the bispecific construct were measured by Biacore. Furthermore, a cytotoxic assay was performed as described above and CD4⁺ activation and CD8⁺ activation was measured.

5 **Table 23: Affinities and activities of bispecific CD123 x CD3 CODV-molecules.**

Bispecific molecule	KD (CD3ε/d) [nM]	KD (CD123) [nM]	Cytotoxic assay (THP cells) EC50 [pM]	CD69 expression @ EC50 cytotox	
				% CD4 ⁺ activation	% CD8 ⁺ activation
hz20G6 x 7G3 CODV-Fab	5,0	0,6	30,9 ± 3,6	73	66
7G3 x hz4B4 CODV-Fab	5,4	0,1	26,7 ± 2,9	65	55
hz4B4 x 3E3 CODV-Fab	7,0	4,8	26,5 ± 5,9	71	69
Fc-tagged variant TL4: hz20G6 x 7G3 CODV-Fab-TL4	13,9	1,7	16,7 ± 10,1	73	74

Cytotoxic effects of the CODV-Fab “hz20G6xhz7G3”, CODV-Fab-TL1 “hz20G6xhz7G3”, CODV-Fab-OL1 “hz20G6xhz7G3” and the single chain Dart MGD006 were also assessed. Affinities to the CD3ε/δ-complex and CD123 of each bispecific construct were measured by Biacore. Furthermore, a cytotoxic assay was performed as described above and CD4⁺ activation and CD8⁺ activation was measured.

10 **Table 24: Affinities and activities of bispecific CD123 x CD3 CODV-molecules and DART (MGD006)**

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Bispecific molecule	KD (CD3ε/d) [nM]	KD (CD123) [nM]	Cytotoxic assay (THP cells) EC50 [pM] n= 3
hz20G6xhz7G3 CODV-Fab	9	0.2	2.1+/-0.2
hz20G6xhz7G3 CODV-Fab-TL1	11	0.2	1.0+/-0.1
hz20G6xhz7G3 CODV-Fab-OL1	15	0,4	0.9+/-0.1

Single chain antibody DART format MGD006	9	0.2	0.3+/-0.04
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To assess the potential of the molecules to trigger T-cell activation in the presence (wanted) and absence (unwanted) of target cells, a new assay was implemented. NFAT-RE-luc2 Jurkat Cells (Promega #CS176403 cells) were incubated with freshly isolated human T-cells in an Effector target ration of 1:1 at 37°C and 5% CO₂ in RPMI 1640, with 2 g/L (11 mM) Glucose, with GlutaMAX, with 25 mM HEPES in 386 well plates. After 5 hrs the incubation was stopped and luminesce was measures using Bio-Glo Luciferase Assay System, Promega #G7940 in a Luminescence HTS Micro Plate Reader.

Table 25: T-cell activation as activation induced by CD123 x CD3 CODV-molecules and MGD006 measured in Jurkat-NFAT-Luc-reporter cell line.

Bispecific molecule	With THP1 cells EC50 (pM) n= 3	No target cells Activation at Cmax in relation to max. activation in assay with target cells (%) n= 3
hz20G6xhz7G3 CODV-Fab	561±0.2	0.2±0.1
hz20G6xhz7G3 CODV-Fab-TL1	444±0.2	0.4±0.3
hz20G6xhz7G3 CODV-Fab-OL1	320±0.2	0.4±0.3
Single chain antibody DART format MGD006	370±0.2	25.1±9.9

Results shown in Table 25 indicate that all antibodies induce reporter cell activation with EC50 values below nM in the presence of target cells. For T-cell engagement approaches, T-cell activation should be restricted to the presence of target cells. This is seen for the CODV molecules as there is no significant luminescence signal in the absence of target cells. In contrast, the single chain DART molecule induces a higher reporter cell line activation in the absence of target cells. These results are in agreement with the results obtained with primary T-cells.

3.8 *In vivo* anti-tumor activity of CD123xCD3 Bispecific CODV-Fab-TL1 and CD123xCD3 Bispecific CODV-Fab

MATERIALS AND METHODS

Human PBMC and T cell isolation from Whole blood

PBMCs were isolated from the whole blood of human healthy donors with a Ficoll gradient centrifugation. Whole blood was diluted 1:1 in sterile phosphate buffered saline (PBS). Then, two volumes of thirty-five mL of the diluted blood were put into two 50 mL Falcon Tubes in presence of 15 mL Ficoll-Paque. The tubes were centrifuged at 200g for 40 minutes at room temperature without brake. The two buffy coat layers were recovered and put in six 50 mL Falcon tubes with 45 mL of sterile PBS and centrifuged three times (in between each centrifugation, the supernatant was discarded and 45 mL of PBS was added) at 100g during ten minutes at room temperature without brake. After the last centrifugation, the two pellets were put together in a final volume of 50 mL completed by PBS in a 50 mL Falcon tube. The total viable PBMCs number was defined by Vicell counting. The pellet was then recovered in Automacs running buffer from Miltenyi Biotech (130-091-221) and T cells were isolated from PBMCs using the negative selection KIT from Miltenyi Biotech (130-091-156) and Automacs according to manufacturer instructions. The purified T cells were recovered and put in culture in Xvivo-15 5% HIS +peni-strepto1X medium at a concentration of 2.5×10^6 cells/mL.

Human T cell amplification

The human enriched T cell population was activated and expanded in vitro during 14 days using the T Cell Activation/Expansion kit from Miltenyi Biotech (130-091-441)

Human T cell preparation for in vivo administration

Cells and cell culture medium were centrifuged 10 minutes at 400g. The pellet was recovered at a concentration of 2×10^7 cells/ml in sterile PBS. Elimination of the activating beads from the amplified T cells was performed using the MACsiMAG separator from Miltenyi Biotech (130-092-168) according to manufacturer instructions. Enriched T cell populations were counted by Vicell counting and were recovered in 25 mL of sterile PBS in a 50 mL Falcon tube. After a step of centrifugation at 400g during 10 minutes at room temperature, the cell pellet was recovered in an adequate volume of sterile PBS to obtain a final concentration of 5×10^7 cells/mL.

Tumor Model

Molm-13 human Acute Myeloid Leukemia cells expressing CD123 were obtained from the Leibniz-institut DSMZ-German collection of microorganisms and cell cultures (DSMZ Braunschweig, Germany). Cells were grown in culture (37°C, 5% CO₂, 95% humidity) in RPMI1640 Glutamax medium (completed with foetal cow serum 20%). Molm-13 cells were infected with a Luciferase vector (SV40-PGL4-Puro – i.e. Luciferase vector consisting in Simian Virus 40 promoterlinked to the Luciferase 2 and the Puromycin resistance cassette sequences) carried by a non-replicative lentivirus.

The Molm13-luc⁺ tumoral cells were injected intravenously (IV) in NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ NSG mice (10E+6 cells per animal in 200µl PBS suspension). Twenty-four hours later, 10E+7 human T-cells were administered intraperitoneally (IP) to the same mice under a volume of 0.2 mL of sterile PBS.

Baseline bioluminescence imaging at day three post tumor implantation was performed using the IVIS100 imager (PerkinElmer, Waltham, MA, USA) with the Living Image 3.2 acquisition software (Perkin-Elmer, Waltham, M, USA). Animals were injected IP with Beetle luciferin potassium salt (batch 316019, Promega, Lyon, France) 120 mg/kg solution in PBS 15 minutes before image. Mice were anesthetized with ketamine®/Xylazine® (120 mg/kg; 6 mg/kg IM, 5 ml/kg) 5 minutes before image.

CODV-Fab-TL1 “hz20G6xhz7G3”, CODV-Fab “hz20G6xhz7G3”, CD123xCD3 bispecific DART competitor (Single chain antibody DART format MGD006 or a close analog herein called “DART-tool”) or PBS treatments by intravenous route (IV) or continuous intraperitoneal infusion (CIP) started at day four post tumor implantation on established tumors already detectable in bones, as outlined in table 26 (CODV-Fab-TL1 “hz20G6xhz7G3”), table 27 (CODV-Fab “hz20G6xhz7G3” IV) and table 28 (CODV-Fab “hz20G6xhz7G3” CIP).

Table 26: CD123xCD3 Bispecific CODV-Fab-TL1 intravenous (IV) evaluation study design

Treatment Group	Dose (nmol/Kg)	Volume/inj Route	Schedule	Animal number
Control	-	-	-	7
CODV-Fab-TL1 "hz20G6xhz7G3"	1.3	0.2ml IV	Q3d (4,7,10)	7
CODV-Fab-TL1 "hz20G6xhz7G3"	0.13	0.2ml IV	Q3d (4,7,10)	8
CODV-Fab-TL1 "hz20G6xhz7G3"	0.013	0.2ml IV	Q3d (4,7,10)	6
CODV-Fab "hz20G6xhz7G3"	1.3	0.2ml IV	Qd (4-13)	8
Single chain antibody DART format MGD006	1.3	0.2ml IV	Qd (4-13)	7

5 Table 27: CD123xCD3 Bispecific CODV-Fab intravenous (IV) evaluation study design

Treatment Group	Dose (nmol/Kg)	Volume/inj Route	Schedule	Animal number
Control	-	-	-	5
CODV-Fab "hz20G6xhz7G3"	1.3	0.2ml IV	Qd (4-13)	8
CODV-Fab "hz20G6xhz7G3"	0.13	0.2ml IV	Qd (4-13)	5
Single chain antibody DART format DART tool	1.3	0.2ml IV	Qd (4-13)	8
Single chain antibody DART format DART tool	0.13	0.2ml IV	Qd (4-13)	7
CODV-Fab hz20G6x7G3	1.3	0.2ml IV	Qd (4-13)	8

Table 28: CD123xCD3 Bispecific CODV-Fab continuous intraperitoneal infusion (CIP) evaluation study design

Treatment Group	Dose (nmol/Kg/day)	Volume/day Route	Schedule	Animal number
Vehicle	-	6µl/day ip Alzet 1002	Continuous infusion (4-14)	9
CODV-Fab "hz20G6xhz7G3"	3.9	6µl/day ip Alzet 1002	Continuous infusion (4-14)	9
CODV-Fab "hz20G6xhz7G3"	1.3	6µl/day ip Alzet 1002	Continuous infusion (4-14)	10
Single chain antibody DART format MGD006	3.9	6µl/day ip Alzet 1002	Continuous infusion (4-14)	9
Single chain antibody DART format MGD006	1.3	6µl/day ip Alzet 1002	Continuous infusion (4-14)	9
CODV-Fab "hz20G6xhz7G3"	1.3	0.2ml IV	Qd (4-13)	10
Vehicle	-	6µl/day ip Alzet 1002	Continuous infusion (4-14)	10
CODV-Fab "hz20G6xhz7G3"	0.13	6µl/day ip Alzet 1002	Continuous infusion (4-14)	8
CODV-Fab "hz20G6xhz7G3"	0.013	6µl/day ip Alzet 1002	Continuous infusion (4-14)	8
Single chain antibody DART format MGD006	0.13	6µl/day ip Alzet 1002	Continuous infusion (4-14)	8
Single chain antibody DART format MGD006	0.013	6µl/day ip Alzet 1002	Continuous infusion (4-14)	9
CODV-Fab "hz20G6xhz7G3"	1.3	0.2ml IV	Qd (4-13)	10

5 DATA collection and efficacy criteria

Animal body weight was monitored from day 3 to the end of assay in order to follow impact of therapy. A dosage producing a 20% weight loss or 15% weight loss for 3 consecutive days or 10% or more drug related deaths, was considered an excessively toxic dosage. Animal body weights included the tumor weights.

- 10 Tumor load was followed by non-invasive bioluminescence imaging (BLI). Baseline BLI was performed at day three post tumor implantation, 24 hours before start of treatments. Animals were dispatched in different groups based on all body bioluminescence signal. Tumor growth was followed in all body and long bones in posteriors legs by BLI signal measurements at days 7, 10 and 14 after tumor implantation.
- 15 Long bone signal was measured by segmentation and could be influenced by nearby loco-regional signal (eg residual signal in soft tissues in late time points). Treated groups

were compared to control animals bearing Molm13-luc+ disseminated tumor and Human T cells.

The primary efficacy end points were the ratio of tumor signal changes from baseline between treated and control groups (dT/dC), the number of partial tumor regressions (PR) and the number of complete tumor regression (CR).

Tumor growth based on bioluminescence signal curves (expressed in Phot/sec) in time was monitored for each animal of each treatment group and represented as median curve $\pm$ MAD, both for all body and bone segmented signals. Changes in tumor bioluminescence signal are calculated for each control (C) or treated (T) animal and for each day by subtracting the tumor signal on the day of first treatment (staging day) from the tumor signal on the specified observation day. The median T is calculated for the treated group and the median C is calculated for the control group.

Then the ratio T/C is calculated and expressed as a percentage:

$$dT/dC = [(\text{median T day obs} - \text{median T day 3}) / (\text{median C day obs} - \text{median C day 3})] \times 100$$

The dose is considered as therapeutically active when dT/dC at the end of the experiment (day 14) is lower than 42% and very active when dT/dC is lower than 10%.

Percent tumor regression is defined as the % of tumor signal decrease in the treated group at a specified observation day compared to its signal on the first day of treatment. At a specific time point and for each animal, % regression is calculated as:

$$\% \text{ regression (at } t) = \frac{\text{Signal}_{t_0} - \text{Signal}_t}{\text{Signal}_{t_0}} \times 100$$

Given the risk of signal variability due to luciferin kinetics and possible IP miss-injection, signal regression for an animal is considered as a true tumor regression only when observed at least at two consecutive time points.

Partial regression (PR): Regressions are defined as partial if the tumor signal decreases below the signal at the start of treatment for two consecutive time points, one remaining superior to 50% of baseline signal.

Complete regression (CR): Regressions are defined as complete if the tumor signal decreases more than 50% below the signal at the start of treatment for two consecutive time points.

Statistical analysis

IV route compounds evaluation

Individual bioluminescence signal of each group of treatment was compared to others using Bonferroni-Holm adjustment for multiplicity pairwise comparisons following Two way anova with repeated measures by day: $p > 0.05$: NS, $0.05 > p > 0.01$: *, $p < 0.01$: **. Statistical analysis is performed for both all body bioluminescence signals and long-bones bioluminescence signals

CIP route compounds evaluation

The CODV-Fab “hz20G6xhz7G3” CIP route evaluation results in data aggregation of two independent studies (1st study concerning compounds at high dosages, 2nd study for low dosages, both studies including a vehicle control group and CODV-Fab “hz20G6xhz7G3” 1.3nmol/kg IV Qd positive control group). Statistical analysis of bioluminescence signal of each mouse at each day was performed after data normalization by the mean of the bioluminescence signal of the vehicle group at the same day of the same experiment (pooled vehicle controls n=19; pooled positive controls n=20). Individual normalized bioluminescence signal of each group of treatment was compared to other groups using Bonferroni-Holm adjustment for multiplicity pairwise comparisons following Two way anova with repeated measures by day: $p > 0.05$: NS, $0.05 > p > 0.01$: *, $p < 0.01$: **. Statistical analysis is performed for both all body bioluminescence signals and long-bones bioluminescence signals.

RESULTS

CD123xCD3 Bispecific CODV-Fab-TL1 “hz20G6xhz7G3” IV

Fully human CODV-Fab-TL1 “hz20G6xhz7G3” IV Q3d in presence of human T cells inhibited Molm13 tumor growth at all tested doses (1.3, 0.13 and 0.013 nmol/Kg Q3d) with dT/dC of 20%, 14% and 38% respectively in whole body (Figures 5 and 7) and was associated with tumor regression in long bones at all tested doses with 4/7 CR, 6/8 CR and 2/6 CR respectively (Figures 6 and 8).

Fully human CODV-Fab-TL1 “hz20G6xhz7G3” maximal response was obtained in whole body and in bone at 0.13 nmol/kg Q3d. At this dose, the activity was not statistically different from DART 1.3 nmol/kg IV Qd (whole body dT/dC 29% with 1/7CR and 1/7PR in long bones), and equivalent to CODV-Fab “hz20G6xhz7G3” 1.3 nmol/kg IV Qd (whole body dT/dC 23% with 1/8CR and 1/8PR tumor regression in long bones). Data were confirmed by terminal histopathology analysis (not shown).

Differences observed between whole body and long bones are linked to residual tumor growth in ovaries and abdominal fat consecutive to extra-medullar tumor dissemination after IV injection.

CD123xCD3 Bispecific CODV-Fab “hz20G6xhz7G3” IV

Fully human CODV-Fab “hz20G6xhz7G3” IV in presence of human T cells inhibited tumor growth at all tested doses (1.3 and 0.13 nmol/Kg Qd4-13) with dT/dC of 14% and 39% respectively (Figures 9 and 11) associated with tumor regression at 1.3 nmol/kg in long bones with 5/8 CR (Figures 10 and 12).

DART 1.3 nmol/kg IV Qd4-13 inhibited tumor growth with whole body dT/dC 29% and 3/8 CR tumor regression in long bones, not significantly different from CODV-Fab “hz20G6xhz7G3” 1.3 nmol/Kg IV. DART was inactive at inhibiting whole body tumor signal at 0.13 nmol/Kg IV Qd4-13 (dT/dC 62%) despite 1/7PR tumor regression in long bones. No significant difference was observed with the same dosage of fully human CODV-Fab “hz20G6xhz7G3” at the end of study. No statistical differences could be seen between fully human CODV-Fab “hz20G6xhz7G3” and partly humanised CODV-Fab hz20G6x7G3 compound when given at the same dosage of 1.3 nmol/kg iv QD4-13 : at this dose CODV-Fab hz20G6x7G3 inhibited whole body tumor growth with dT/dC of 34% associated with tumor regressions in long bones (1/8CR and 1/8PR).

Differences observed between whole body and long bones are linked to residual tumor growth in ovaries and abdominal fat consecutive to extra-medullar tumor dissemination after IV injection.

CD123xCD3 Bispecific CODV-Fab CIP

Fully human CODV-Fab “hz20G6xhz7G3” CIP, in presence of human T cells, inhibited whole body tumor growth at 3.9, 1.3 and 0.13 nmol/Kg/day CIP4-14 and was inactive at 0.013 nmol/kg/day with respective dT/dC of 2%, 3%, 21%, and 57% in whole body (Figures 13 and 15). It was associated with tumor regression at 3.9, 1.3, and 0.13 nmol/kg in long bones with 5/9CR 3/9PR, 6/10CR 1/10PR and 7/8CR respectively (Figures 14 and 16).

DART 3.9, 1.3, and 0.13 nmol/kg/day CIP4-14, but not 0.013 nmol/kg/day inhibited whole body tumor growth with dT/dC of 21%, 5%, 21% and 46% respectively (Figure 13) inducing tumor regression in long bones at 3.9, 1.3, and 0.13 nmol/kg with 3/9 CR 3/9PR ; 8/9CR; and 4/8CR 2/8PR respectively (Figure 14).

Full human CODV-Fab “hz20G6xhz7G3” 1.3 nmol/kg IV Qd4-13 inhibited tumor growth in whole body with dT/dC of 4% and 5% (1st and 2nd study resp.) associated with tumor regression in long bones (8/10CR 1/10PR vs 8/10 CR in 1st and 2nd study resp).

CLAIMS

1. An isolated antibody that binds to the extracellular domain of human CD3ε protein comprising:

- 5 a) a heavy chain variable domain comprising a CDR1-H consisting of sequence
 GFX₁X₂X₃X₄AW (SEQ ID NO: 331) wherein X₁ is T or S, X₂ is F or V, X₃ is S or T and
 X₄ is N, K, L or Y, or any combination thereof; and
 a CDR2-H consisting of sequence IKX₁X₂X₃NX₄YX₅T (SEQ ID NO: 332) wherein X₁ is
 A or D, X₂ is K or R, X₃ is S or A, X₄ is N or S and X₅ is A or E, or any combination
 10 thereof; and
 a CDR3-H consisting of sequence TWRHYSSHTMDA (SEQ ID NO: 69) or
 RALTYYGKRDAMDG (SEQ ID NO: 129) or RX₁X₂X₃YX₄X₅X₆X₇X₈X₉X₁₀X₁₁DX₁₂
 (SEQ ID NO: 333) wherein X₁ is Y, G or A, X₂ is V, T or L, X₃ is H, N, Y or Q, X₄ is G,
 R or A, X₅ is F or V or no amino acid, X₆ is R or no amino acid, X₇ is F, S or I or no
 15 amino acid, X₈ is F, L, N, M, Y, S, A or G, X₉ is Y, A, K, S, N, T, F or L, X₁₀ is A, P, G
 or T, X₁₁ is M, L, F or S and X₁₂ is A, V or Y, or any combination thereof, and
 a light chain variable domain comprising a CDR1-L consisting of sequence
 QX₁LX₂HX₃NGX₄TY (SEQ ID NO: 334) wherein X₁ is R or S, X₂ is V or E, X₃ is N, D or
 T, and X₄ is N or Y, or any combination thereof; and
 20 a CDR2-L consisting of sequence 'KVS'; and
 a CDR3-L consisting of sequence GQGX₁X₂YPFT (SEQ ID NO: 335) wherein X₁ is T,
 A or S and X₂ is H, E or Q, or any combination thereof; or
- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 30 or a
 sequence differing from SEQ ID NO: 30 by one amino acid substitution; CDR2-H of
 25 sequence SEQ ID NO: 31 or a sequence differing from SEQ ID NO: 31 by one or
 more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 32 or a sequence
 differing from SEQ ID NO: 32 by one amino acid substitution; and
 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 34 or a
 sequence differing from SEQ ID NO: 34 by one amino acid substitution; CDR2-L of
 30 sequence 'RDD' or a sequence differing from the sequence 'RDD' by one amino acid
 substitution and CDR3-L of sequence SEQ ID NO: 35 or a sequence differing from
 SEQ ID NO: 35 by one amino acid substitution; or
- b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 50 or a
 sequence differing from SEQ ID NO: 50 by one amino acid substitution; CDR2-H of

sequence SEQ ID NO: 51 or a sequence differing from SEQ ID NO: 51 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 52 or a sequence differing from SEQ ID NO: 52 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 54 or a sequence differing from SEQ ID NO: 54 by one amino acid substitution; CDR2-L of sequence 'NAN' or a sequence differing from the sequence 'NAN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 55 or a sequence differing from SEQ ID NO: 55 by one amino acid substitution; or

c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 90 or a sequence differing from SEQ ID NO: 90 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 91 or a sequence differing from SEQ ID NO: 91 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 32 or a sequence differing from SEQ ID NO: 32 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 93 or a sequence differing from SEQ ID NO: 93 by one amino acid substitution; CDR2-L of sequence 'GAS' or a sequence differing from the sequence 'GAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 94 or a sequence differing from SEQ ID NO: 94 by one amino acid substitution; or

d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 96 or a sequence differing from SEQ ID NO: 96 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 97 or a sequence differing from SEQ ID NO: 97 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 98 or a sequence differing from SEQ ID NO: 98 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100 or a sequence differing from SEQ ID NO: 100 by one amino acid substitution; CDR2-L of sequence 'NTN' or a sequence differing from the sequence 'NTN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 101 or a sequence differing from SEQ ID NO: 101 by one amino acid substitution; or

e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103 or a sequence differing from SEQ ID NO: 103 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 104 or a sequence differing from SEQ ID NO: 104 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 105 or a sequence differing from SEQ ID NO: 105 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or a sequence differing from SEQ ID NO: 10 by one amino acid substitution; CDR2-L of

sequence 'KVS' or a sequence differing from the sequence 'KVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 11 or a sequence differing from SEQ ID NO: 11 by one amino acid substitution; or

- f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 116 or
 5 a sequence differing from SEQ ID NO: 116 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 117 or a sequence differing from SEQ ID NO: 117 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 118 or a sequence differing from SEQ ID NO: 118 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100 or a
 10 sequence differing from SEQ ID NO: 100 by one amino acid substitution; CDR2-L of sequence 'VTN' or a sequence differing from the sequence 'VTN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 120 or a sequence differing from SEQ ID NO: 120 by one amino acid substitution; or

- g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 122 or
 15 a sequence differing from SEQ ID NO: 122 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 123 or a sequence differing from SEQ ID NO: 123 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 124 or a sequence differing from SEQ ID NO: 124 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 126 or a
 20 sequence differing from SEQ ID NO: 126 by one amino acid substitution; CDR2-L of sequence 'RDD' or a sequence differing from the sequence 'RDD' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 127 or a sequence differing from SEQ ID NO: 127 by one amino acid substitution.

25 2. The isolated antibody according to claim 1, comprising:

- a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 6,
 CDR2-H of sequence SEQ ID NO: 7, CDR3-H of sequence SEQ ID NO: 8 and a
 light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10 or
 SEQ ID NO: 142, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID
 30 NO: 11; or

- b) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13,
 CDR2-H of sequence SEQ ID NO: 14, CDR3-H of sequence SEQ ID NO: 15 and
 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17 or
 SEQ ID NO: 184, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID
 35 NO: 11; or

- c) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 20 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 22, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 5 d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 24, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 25 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 27, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- 10 e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 30, CDR2-H of sequence SEQ ID NO: 31, CDR3-H of sequence SEQ ID NO: 32 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 34, CDR2-L of sequence 'RDD' and CDR3-L of sequence SEQ ID NO: 35; or
- 15 f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 38 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- 20 g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 41 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 25 h) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 44 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 30 i) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 47 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- j) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 50, CDR2-H of sequence SEQ ID NO: 51, CDR3-H of sequence SEQ ID NO: 52 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 54, CDR2-L of sequence 'NAN' and CDR3-L of sequence SEQ ID NO: 55; or
- k) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 57, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 58 and

- a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- l) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 61 and
5 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- m) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 64, CDR2-H of sequence SEQ ID NO: 65, CDR3-H of sequence SEQ ID NO: 47 and
10 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 67, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 28; or
- n) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 69 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 71; or
- o) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 84 and
15 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 17, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- p) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 75, CDR2-H of sequence SEQ ID NO: 76, CDR3-H of sequence SEQ ID NO: 77 and
20 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- q) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80, CDR2-H of sequence SEQ ID NO: 76, CDR3-H of sequence SEQ ID NO: 81 and
25 a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- r) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 84 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
30
- s) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 47 and
a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 88; or

- t) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 90, CDR2-H of sequence SEQ ID NO: 91, CDR3-H of sequence SEQ ID NO: 32 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 93, CDR2-L of sequence 'GAS' and CDR3-L of sequence SEQ ID NO: 94; or
- 5 u) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 96, CDR2-H of sequence SEQ ID NO: 97, CDR3-H of sequence SEQ ID NO: 98 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100, CDR2-L of sequence 'NTN' and CDR3-L of sequence SEQ ID NO: 101; or
- 10 v) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103, CDR2-H of sequence SEQ ID NO: 104, CDR3-H of sequence SEQ ID NO: 105 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 15 w) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 80, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 108 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 20 x) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 37, CDR3-H of sequence SEQ ID NO: 111 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 113, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 114; or
- y) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 116, CDR2-H of sequence SEQ ID NO: 117, CDR3-H of sequence SEQ ID NO: 118 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 100, CDR2-L of sequence 'VTN' and CDR3-L of sequence SEQ ID NO: 120; or
- 25 z) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 122, CDR2-H of sequence SEQ ID NO: 123, CDR3-H of sequence SEQ ID NO: 124 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 126, CDR2-L of sequence 'RDD' and CDR3-L of sequence SEQ ID NO: 127; or
- 30 aa) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 13, CDR2-H of sequence SEQ ID NO: 19, CDR3-H of sequence SEQ ID NO: 129 and a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 10, CDR2-L of sequence 'KVS' and CDR3-L of sequence SEQ ID NO: 11; or
- 35 bb) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 103, CDR2-H of sequence SEQ ID NO: 104, CDR3-H of sequence SEQ ID NO: 105; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 133, CDR2-L of sequence 'LVS' and CDR3-L of sequence SEQ ID NO: 134.

3. An antibody-like binding protein that binds specifically to human CD3ε comprising two polypeptide chains that form two antigen-binding sites, wherein a first polypeptide has a structure represented by the formula [I]:



and a second polypeptide has a structure represented by the formula [II]:



10 wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

15 C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the first and the second polypeptide form a cross-over light chain-heavy chain pair, and

20 wherein the V_{D1} and V_{D4} , or V_{D2} and V_{D3} comprise a heavy chain variable domain and a light chain variable domain of an antibody as defined in claim 1.

4. The antibody like binding protein according to claim 3, wherein the polypeptide of formula [II] further comprises a F_c domain.

25

5. The antibody like binding protein according to claim 3 or 4, comprising two polypeptide chains that form two antigen-binding sites, wherein one polypeptide chain has a structure represented by the formula [I]:



30 and one polypeptide chain has a structure represented by the formula [III]:



wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

35 V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the polypeptide formula I and the polypeptide of formula III form a cross-over light chain-heavy chain pair, and

wherein the V_{D1} and V_{D4} , or V_{D2} and V_{D3} comprise a heavy chain variable domain and a light chain variable domain of an antibody as defined in claim 1.

6. The antibody like binding protein according to claim 4 or 5, wherein the polypeptide of formula [I] further comprises a F_c domain (F_{c2}).

7. The antibody like binding protein according to any one of claims 4 to 6, comprising two polypeptide chains that form two antigen-binding sites, wherein one polypeptide chain has a structure represented by the formula [IV]:



and one polypeptide chain has a structure represented by the formula [III]:



wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

F_{c2} is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

L_1 , L_2 , L_3 , L_4 and L_5 are amino acid linkers;

wherein the polypeptide of formula [IV] and the polypeptide of formula [III] form a cross-over light

8. The antibody like binding protein according to claim 4 or 5, which comprises a third polypeptide chain comprising a F_c domain (F_{c3}).

9. The antibody like binding protein according to any one of claims 4, 5 and 8, comprising three polypeptide chains that form two antigen-binding sites, wherein a first polypeptide has a structure represented by the formula [I]:



a second polypeptide has a structure represented by the formula [III]:



10 a third polypeptide F_{c3} which is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin; wherein

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

15 V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

20 F_c is the immunoglobulin hinge region and CH_2 , CH_3 immunoglobulin heavy chain constant domains of an immunoglobulin;

L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair,

25 wherein the polypeptide of formula [III] heterodimerizes with the third polypeptide through its F_c domain; and

wherein the V_{D1} and V_{D4} , or V_{D2} and V_{D3} comprise a heavy chain variable domain and a light chain variable domain of an antibody as defined in claim 1.

30 10. The antibody like binding protein according to any one of claims 3 to 9, wherein the antibody-like binding protein binds to at least one further target antigen.

11. The antibody like binding protein according to claim 10, wherein the at least one further antigen target is CD123.

12. The antibody like binding protein according to claim 11, wherein the antibody-like binding protein comprises

- 5 a) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 9 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 5 or a sequence at least 85% identical thereto, or
- 10 b) light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 21 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 18 or a sequence at least 85% identical thereto, or
- 15 c) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 16 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO 12 or a sequence at least 85% identical thereto, or
- 20 d) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 26 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the

amino acid sequence SEQ ID NO : 23 or a sequence at least 85% identical thereto,
or

e) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 308 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 143 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 138 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, or

f) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 158 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 308 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 312 or a sequence at least 85% identical thereto, and a heavy chain variable domain of a first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 171 or a sequence at least 85% identical thereto,

g) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 230 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 158 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 171 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4}) consisting of the amino acid sequence SEQ ID NO : 226 or a sequence at least 85% identical thereto,

h) a light chain variable domain of a first immunoglobulin (V_{D1}) consisting of the amino acid sequence SEQ ID NO: 385 or a sequence at least 85% identical thereto, a light chain variable domain of a second immunoglobulin (V_{D2}) consisting of the amino acid sequence SEQ ID NO : 141 or a sequence at least 85% identical thereto, a heavy chain variable domain of the second immunoglobulin (V_{D3}) consisting of the amino acid sequence SEQ ID NO : 138 or a sequence at least 85% identical thereto, and a heavy chain variable domain of the first immunoglobulin (V_{D4})

consisting of the amino acid sequence SEQ ID NO : 383 or a sequence at least 85% identical thereto.

13. The antibody like binding molecule according to claim 3, which comprises:

- 5 a) one polypeptide according to formula [I] consisting of the amino acid sequence SEQ ID NO: 388 which comprises V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310, or
a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences
10 SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of V_{D1} , and the 3 CDRs of sequences SEQ ID NO: 142, 'KVS' and SEQ ID NO: 11 of V_{D2} are unaltered ; and
- b) one polypeptide according to formula [II] consisting of the amino acid sequence SEQ ID NO: 390 which comprises V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid and C_{H1} of sequence SEQ ID NO:
15 313, or
a sequence at least 85% identical to SEQ ID NO: 390 in which the 3 CDRs of sequences SEQ ID NO: 381, SEQ ID NO: 384, and SEQ ID NO: 382 of V_{D4} , and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of V_{D3} are unaltered;
and wherein the polypeptide of formula [I] and the polypeptide of formula [II] form a cross-
20 over light chain-heavy chain pair.

14. The antibody like binding molecule according to claim 6 or 7, which comprises:

- a) one polypeptide according to formula [IV] consisting of the amino acid sequence SEQ ID NO: 391 which comprises V_{D1} of sequence SEQ ID NO: 385, L_1 of
25 sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389, C_L of sequence SEQ ID NO: 310, L_5 which contains 0 amino acid, and F_{c2} of sequence SEQ ID NO: 392, or
a sequence at least 85% identical to SEQ ID NO: 391 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of V_{D1} , and the 3 CDRs of sequences SEQ
30 ID NO: 142, 'KVS' and SEQ ID NO: 11 of V_{D2} are unaltered; and
- b) one polypeptide according to formula [III] consisting of the amino acid sequence SEQ ID NO : 393 which comprises V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and and F_c of sequence SEQ ID NO: 394, or

a sequence at least 85% identical to SEQ ID NO : 393 in which the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of V_{D4} , and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of V_{D3} are unaltered;.

and wherein the polypeptide of formula [IV] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair.

15. The antibody like binding molecule according to claim 8 or 9; which comprises:

a) one polypeptide according to formula [I] consisting of the amino acid sequence SEQ ID NO: 388 which comprises V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310, or

a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of V_{D1} , and the 3 CDRs of sequences SEQ ID NO:142, 'KVS' and SEQ ID NO:11 of V_{D2} are unaltered ; and

b) one polypeptide according to formula [III] consisting of the amino acid sequence SEQ ID NO : 395 which comprises V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c of sequence SEQ ID NO: 396, or

a sequence at least 85% identical to SEQ ID NO : 395 in which the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of V_{D4} , and the 3 CDRs of sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of V_{D3} are unaltered; and

c) one polypeptide F_{c3} which consists of sequence SEQ ID NO : 397, or a sequence at least 85% identical thereto, wherein said F_{c3} or the sequence at least 85% identical thereto heterodimerises with the F_c region of the polypeptide according to formula [III]; and

wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair.

16. The antibody like binding molecule according to claim 8 or 9; which comprises:

a) one polypeptide according to formula [I] consisting of the amino acid sequence SEQ ID NO: 388 which comprises V_{D1} of sequence SEQ ID NO: 385, L_1 of sequence SEQ ID NO: 389, V_{D2} of sequence SEQ ID NO: 141, L_2 of sequence SEQ ID NO: 389 and C_L of sequence SEQ ID NO: 310, or

a sequence at least 85% identical to SEQ ID NO: 388 in which the 3 CDRs of sequences SEQ ID NO: 378, 'WAS' and SEQ ID NO: 379 of V_{D1} , and the 3 CDRs of sequences SEQ ID NO:142, 'KVS' and SEQ ID NO:11 of V_{D2} are unaltered ; and

b) one polypeptide according to formula [III] consisting of the amino acid sequence
 5 SEQ ID NO: 399 which comprises V_{D3} of sequence SEQ ID NO: 138, L_3 is 0 amino acid, V_{D4} of sequence SEQ ID NO: 383, L_4 is 0 amino acid, C_{H1} of sequence SEQ ID NO: 313, and F_c of sequence SEQ ID NO: 400, or

a sequence at least 85% identical to SEQ ID NO: 399 in which the 3 CDRs of sequences SEQ ID NO:381, SEQ ID NO:384, and SEQ ID NO: 382 of V_{D4} , and the 3 CDRs of
 10 sequences SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 of V_{D3} are unaltered; and

c) one polypeptide F_{c3} which consists of sequence SEQ ID NO : 398, or a sequence at least 85% identical thereto, wherein said F_{c3} or the sequence at least 85% identical thereto heterodimerises with the F_c region of the polypeptide according to formula [III];
 and

15 wherein the polypeptide of formula [I] and the polypeptide of formula [III] form a cross-over light chain-heavy chain pair.

17. An isolated antibody that binds to the extracellular domain of human CD123 protein comprising:

20 a) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 227 or a sequence differing from SEQ ID NO: 227 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 228 or SEQ ID NO:353 or SEQ ID NO: 279, or a sequence differing from SEQ ID NO: 228 or SEQ ID NO:353 or SEQ ID NO: 279 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 229 or
 25 a sequence differing from SEQ ID NO: 229 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 231 or a sequence differing from SEQ ID NO: 231 by one amino acid substitution; CDR2-L of sequence 'RDD' or a sequence differing from 'RDD' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 232 or a sequence differing
 30 from SEQ ID NO: 232 by one amino acid substitution; or

b) a heavy chain variable domain comprising a CDR1-H consisting of sequence $X_1YTFTDX_2I$ (SEQ ID NO: 336) wherein X_1 is G or A and X_2 is H, Y or N, or any combination thereof; and

a CDR2-H consisting of sequence $INPYSX_1GX_2$ (SEQ ID NO: 337) wherein X_1 is G
 35 or D and X_2 is T or A, or any combination thereof; and

a CDR3-H consisting of sequence ALNYGSYYAMDA (SEQ ID NO 201), and
 a light chain variable domain comprising a CDR1-L consisting of sequence
 $X_1DIX_2X_3N$ (SEQ ID NO: 338) wherein X_1 is E or K, X_2 is F, H or Y and X_3 is N or
 S, or any combination thereof; and

5 a CDR2-L consisting of sequence 'DAN' or 'DAS'; and

a CDR3-L consisting of sequence X_1QYNX_2YPYT (SEQ ID NO: 339) wherein X_1 is
 H or Q and X_2 is I, K or N, or any combination thereof; or

c) a heavy chain variable domain comprising a CDR1-H consisting of sequence
 GFSLTSYX₁ (SEQ ID NO: 340) wherein X_1 is H or S; and

10 a CDR2-H consisting of sequence MWX₁DGDT (SEQ ID NO: 341) wherein X_1 is S
 or N; and

a CDR3-H consisting of sequence ARGX₁X₂X₃X₄X₅X₆X₇X₈X₉FX₁₀Y (SEQ ID NO:
 342) wherein X_1 is D, Y or H, X_2 is Y or R, X_3 is S or T, X_4 is S or P, X_5 is Y or no
 amino acid, X_6 is L, I or no amino acid, X_7 is Y or no amino acid, X_8 is L or no
 15 amino acid, X_9 is W or no amino acid, X_{10} is A or D, or any combination thereof,
 and

a light chain variable domain comprising a CDR1-L consisting of sequence
 QSFLSSGDX₁X₂NY (SEQ ID NO: 343) wherein X_1 is E or G and X_2 is R or K, or
 any combination thereof; and

20 a CDR2-L consisting of sequence 'WAS'; and

a CDR3-L consisting of sequence QQYYDTPLT (SEQ ID NO: 253), or

d) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 206
 or a sequence differing from SEQ ID NO: 206 by one amino acid substitution;
 CDR2-H of sequence SEQ ID NO: 207 or a sequence differing from SEQ ID NO:
 25 207 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:
 208 or a sequence differing from SEQ ID NO: 208 by one amino acid substitution;
 and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 210 or
 a sequence differing from SEQ ID NO: 210 by one amino acid substitution; CDR2-
 30 L of sequence 'ETS' or a sequence differing from 'ETS' by one amino acid
 substitution and CDR3-L of sequence SEQ ID NO: 211 or a sequence differing
 from SEQ ID NO: 211 by one amino acid substitution; or

e) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 213
 or a sequence differing from SEQ ID NO: 213 by one amino acid substitution;
 35 CDR2-H of sequence SEQ ID NO: 214 or a sequence differing from SEQ ID NO:

214 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 215 or a sequence differing from SEQ ID NO: 215 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 217 or a sequence differing from SEQ ID NO: 217 by one amino acid substitution; CDR2-L of sequence 'NTN' or a sequence differing from 'NTN' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 218 or a sequence differing from SEQ ID NO: 218 by one amino acid substitution; or

f) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 220 or a sequence differing from SEQ ID NO: 220 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 221 or a sequence differing from SEQ ID NO: 221 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 222 or a sequence differing from SEQ ID NO: 222 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 224 or a sequence differing from SEQ ID NO: 224 by one amino acid substitution; CDR2-L of sequence 'RVS' or a sequence differing from 'RVS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 225 or a sequence differing from SEQ ID NO: 225 by one amino acid substitution; or

g) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 234 or a sequence differing from SEQ ID NO: 234 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 235 or a sequence differing from SEQ ID NO: 235 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO: 236 or a sequence differing from SEQ ID NO: 236 by one amino acid substitution; and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 238 or a sequence differing from SEQ ID NO: 238 by one amino acid substitution; CDR2-L of sequence 'GAS' or a sequence differing from 'GAS' by one amino acid substitution and CDR3-L of sequence SEQ ID NO: 239 or a sequence differing from SEQ ID NO: 239 by one amino acid substitution; or

h) a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 241 or a sequence differing from SEQ ID NO: 241 by one amino acid substitution; CDR2-H of sequence SEQ ID NO: 242 or a sequence differing from SEQ ID NO: 242 by one or more amino acid substitutions; CDR3-H of sequence SEQ ID NO:

243 or a sequence differing from SEQ ID NO: 243 by one amino acid substitution;
and

a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 245 or
a sequence differing from SEQ ID NO: 245 by one amino acid substitution; CDR2-
L of sequence 'YAS' or a sequence differing from 'YAS' by one amino acid
substitution and CDR3-L of sequence SEQ ID NO: 246 or a sequence differing
from SEQ ID NO: 246 by one amino acid substitution.

18. An antibody-like binding protein that binds specifically to human CD123
comprising two polypeptide chains that form two antigen-binding sites, wherein a first
polypeptide has a structure represented by the formula [I]:



and a second polypeptide has a structure represented by the formula [II]:



wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of said second immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of said first immunoglobulin;

C_L is a light chain constant domain of an immunoglobulin;

C_{H1} is a C_{H1} heavy chain constant domain of an immunoglobulin;

L_1 , L_2 , L_3 , and L_4 are amino acid linkers;

and wherein the first and the second polypeptide form a cross-over light chain-heavy
chain pair, and

wherein the V_{D1} and V_{D4} or V_{D2} and V_{D3} comprise a heavy chain variable domain and a
light chain variable domain of an antibody as defined in claim 17.

19. A pharmaceutical composition comprising an antibody-like binding protein
according to any one of claims 3 to 16 or 18, or an anti-CD123 antibody according to
claim 17 or an anti-CD3 antibody according to claim 1 or 2 and a pharmaceutically
acceptable carrier.

20. An antibody-like binding protein according to any one of claims 3 to 16 or 18, or
an anti-CD123 antibody according claim 17, or an anti-CD3 antibody according claim 1 or
a pharmaceutical composition according to claim 19 for use as a medicament.

21. An antibody-like binding protein according to any one of claims 3 to 16 or 18, or an anti-CD123 antibody according claim 17, or a pharmaceutical composition thereof for use for the treatment of cancer.

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22. An anti-CD3 antibody according to claim 1 or 2 or a pharmaceutical composition thereof for use for use in preventing or treating a pathological immune response.

23. An antibody-like binding protein, an antibody or pharmaceutical composition for the use according to claim 21, wherein the cancer is a haematological cancer.

10

24. A method of treating or preventing a disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of an antibody-like binding protein according to any one of claims 3 to 16 or 18, or an anti-CD123 antibody according claim 17, or an anti-CD3 antibody according to claim 1 or 2 or a pharmaceutical composition according to claim 19.

15

25. An isolated nucleic acid comprising a sequence encoding an antibody-like binding protein according to any one of claims 3 to 16 or 18, an anti-CD123 antibody according to claim 17 or an anti-CD3 antibody according to claim 1 or 2.

20

26. A host cell which has been transformed by a nucleic acid according to claim 25.

27. A kit comprising comprising at least one antibody according to any one of claims 1, 2 or 17 at least one antibody-like binding protein according to any one of claims 3 to 16.

25

28. An antibody according to claim 17 for use for *ex vivo* or *in vivo* detecting CD123 expression in biological sample of a subject.

29. The antibody for the use according to claim 28, wherein said antibody is labelled with a detectable molecule or substance.

30

30. The antibody for the use according to any one of claims 28 to 29, wherein said use is for diagnosing the presence of a cancer in a subject, determining susceptibility of a patient having cancer to a therapeutic agent targeting CD123, or monitoring effectiveness

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of anti-CD123 cancer therapy or detecting cancer relapse after anti-CD123 cancer therapy.

- 5 31. The antibody for the use according to 30, wherein the anti-CD123 cancer therapy uses or antibody-like binding protein according to any one of claims 3 to 16 or 18 or an anti-CD123 antibody according to claim 17.