

**ANTIBODY VARIABLE DOMAINS AND ANTIBODIES HAVING DECREASED
IMMUNOGENICITY**

FIELD OF THE INVENTION

[0001] The present invention relates to antibody variable domains, which exhibit a reduced binding to pre-existing anti-drug antibodies (ADA), and to antibodies comprising one or more of said antibody variable domains. The present invention further relates to nucleic acids encoding said antibody variable domains or said antibodies, vector(s) comprising said nucleic acids, host cell(s) comprising said nucleic acids or said vector(s), and a method of producing said antibody variable domains or said multispecific antibodies. Additionally, the present invention relates to pharmaceutical compositions comprising said antibodies and to methods of use thereof.

BACKGROUND OF THE INVENTION

[0002] In the past forty years since the development of the first monoclonal antibodies ("mAbs"; Köhler & Milstein, Nature, 1975, Vol.256, pp. 495-497), antibodies have become an increasingly important class of biomolecules for research, diagnostic and therapeutic purposes. Initially, antibodies were exclusively obtained by immunizing animals with the corresponding antigen of interest. While antibodies of non-human origin can be used in research and diagnostics, in therapeutic approaches the human body typically recognize non-human antibodies as foreign and raise an immune response against the non-human antibody drug substance, rendering it less or not effective. Even if the administered antibody therapeutics have been humanized, e.g. by grafting of CDRs of non-human origin into human immunoglobulin frameworks to minimize the non-human component, they can still elicit an immune response, which compromises the efficacy and/or safety of these therapeutics.

[0003] Such an immune response typically involves the binding of anti-drug antibodies (ADAs) to the therapeutic agent. These ADAs can be antibodies, which are already present in human serum (so called pre-existing ADAs) and/or antibodies, which are formed during the course of the therapy.

[0004] The risk of ADA binding can be significantly enhanced for therapeutic antibodies that comprise or are built of portions of a naturally occurring human antibody, e.g. Fab or Fv antibody fragments. It is believed that one of the main reasons for this increase in ADA binding is that in antibody fragments, typically a significant number of amino acids that are formerly

shielded by the contact to other antibody portions or domains, become exposed to the solvent and are present to the immune system as potential epitopes.

[0005] According to literature, antibody responses in patients are dependent on the presence of both B-cell epitopes and T-cell epitopes. When a B-cell receptor recognizes and binds an antigen such as an administered therapeutic antibody, the antigen is internalized into the B cell by receptor-mediated endocytosis and undergoes proteolytic processing. The resulting peptides are subsequently presented by MHC class II molecules. Upon recognition of the T cell epitope by a T helper cell, the latter stimulates the corresponding B cells to proliferate and differentiate into antibody producing plasma cells.

[0006] Several strategies have been provided in the prior art to further lower the response of the immune system of a patient to the administered antibodies.

[0007] For example, Zhao, L. and Li, J. (2010), BMC Struct. Biol., 10, S6, disclose a method for the prediction of potential B-cell epitopes on a protein surface, based on the structural information of antibody-antigen complexes. The authors identified common structural elements that are often present in B-cell epitopes. In particular they found that in antigen epitopes recognized by antibodies, polar amino acids with flexible side chains such as arginine (R), lysin (K), asparagine (N), glutamine (Q), and histidine (H) are significantly overrepresented. Knowledge of these critical structural elements forms the basis for strategies to avoid them.

[0008] Nataga, S. and Pastan, I. (2009), Adv Drug Deliv Rev, p. 977-985, Onda, M. et al (2008), PNAS Vol 105(32): 1 1311-11316 and Mazor, R. et al (2016), Immunol Rev. Vol. 270(1): 152-64, disclose a method for reducing the immunogenicity of foreign proteins by identifying B-cell epitopes on the protein and eliminating them by mutagenesis. The authors substituted bulky hydrophilic residues such as arginine (R), glutamine (Q), glutamic acid (E) or lysin (K) within solvent exposed areas by small amino acids (such as alanine, glycine and serine).

[0009] WO2011/075861 discloses a method for decreasing the immunogenicity of antibody variable domains, in particular scFvs, by mutating one or more amino acids located in the interface between the variable chain and the constant chain of a corresponding full-length antibody. It is further disclosed that (i) residues that are present in turn regions of secondary structures, (ii) residues that have large, flexible side chains or a bulky side chain, or (iii) residues that are hydrophobic, are prone to be B-cell epitopes and thus elicit an immunogenic reaction, and that removal of such amino acids interrupts B-cell epitopes. It is further specified that the one or more amino acid residues to be substituted are Leucine (L), Valine (V), Aspartic acid (D), Phenylalanine (F), Arginine (R) and/or Glutamic Acid (E). WO2011/075861 discloses one example of an scFv having the heavy chain point mutations

L12S, L103T and L144T (AHo numbering) that, compared to the unmutated version, exhibits reduced binding to pre-existing ADAs present in human sera. Thus, WO2011/075861 teaches a method for decreasing the immunogenicity of antibody variable domains towards pre-existing ADAs by replacing small hydrophobic residues such as L and V located in the interface between the variable chain with small and weakly hydrophilic amino acids (such as S and T) and by avoiding large and bulky hydrophilic residues located in said interface.

[0010] Although the currently available methods provide useful indications, how the immunogenicity of antibody variable domains can be reduced, the chance of success is case dependent and the antibody variable domains obtained by these methods often exhibit a significant residual immunogenicity. Thus, there is still a large unmet need for antibody variable domains, which exhibit low immunogenicity, and which can generally be applied in the construction of antibody fragments. More specifically, it would be desirable to have antibody variable domains at hand, which exhibiting low immunogenicity, in particular with regard to reduced binding to pre-existing ADAs, and which can generally be applied in the construction of antibody fragments. It is furthermore desirable that these antibody variable domains provide a high stability, when integrated in the final antibody format, which would allow their application in the construction of stable antibody fragments and fragment-based multispecific antibodies suitable for therapeutic development.

SUMMARY OF THE INVENTION

[0011] It is an object of the present invention to provide antibody variable domain variants, which exhibit reduced immunogenicity, more specifically, antibody variable domain variants that are significantly less recognized by pre-existing ADAs when compared to their unmodified variants. Furthermore, these antibody variable domain variants should be highly stable to allow their application in the construction of antibody fragments and multispecific antibodies suitable for pharmaceutical development.

[0012] The inventors have now surprisingly found that antibody variable domains, which are substituted at their heavy chain framework positions 12 and/or 144 by hydrophilic amino acids with large flexible and bulky side chains, i.e. an R at position 12 and/or a Q at position 144 (according to AHo numbering), and optionally are substituted at their heavy chain framework position 103 by a T, when being in scFv format, exhibit a reduced binding to pre-existing anti-drug antibodies (ADA) present in human sera when compared to their unsubstituted versions. Furthermore, these antibody variable domains could successfully be applied in the construction of various scFvs and fragment-based multispecific antibodies, which exhibit low immunogenicity and excellent stability.

[0013] Accordingly, in a first aspect, the present invention relates to an antibody variable domain, which specifically binds to a target antigen, comprising:

- (i) a variable heavy chain (VH) comprising from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from a human VH framework subtype, and wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
- an arginine (R) at amino acid position 12;
 - a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144;
 - a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
- (ii) a variable light chain (VL), wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein
- a. said variable light chain framework regions LFW1, LFW2, LFW3 and LFW4 are selected from a human antibody Vk framework subtype; or
 - b. said variable light chain framework regions LFW1, LFW2 and LFW3 are selected from a human antibody Vk framework subtype, and said variable light chain framework region LFW4 is selected from a Vλ framework subtype.

[0014] In a second aspect, the present invention relates to an antibody comprising one or more antibody variable domains of the present invention.

[0015] In a third aspect, the present invention relates to an antibody variable domain of the present invention, wherein said antibody variable domain specifically binds to CD137, and comprises:

- a) a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5;
- b) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;
- c) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
- d) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10.

[0016] In a fourth aspect, the present invention relates to an antibody variable domain of the present invention, wherein said antibody variable domain specifically binds to PDL1, and comprises:

- a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15;
- b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;
- c) a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
- d) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22.

[0017] In a fifth aspect, the present invention relates to an antibody variable domain of the present invention, wherein said antibody variable domain specifically binds to human serum albumin, and comprises:

- a) a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27;
- b) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;
- c) a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
- d) a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34.

[0018] In a sixth aspect, the present invention relates to an antibody variable domain of the present invention, wherein said antibody variable domain specifically binds to human serum albumin, and comprises

- a) a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
- b) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 39;
- c) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
- d) a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 40;
- e) a VH sequence of SEQ ID NO: 42 and a VL sequence of SEQ ID NO: 45;
- f) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 46;
- g) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 48; or
- h) a VH sequence of SEQ ID NO: 44 and a VL sequence of SEQ ID NO: 47.

[0019] In a seventh aspect, the present invention relates to a nucleic acid or two nucleic acids encoding the antibody variable domain or the antibody of the present invention.

[0020] In an eighth aspect, the present invention relates to a vector or two vectors comprising the nucleic acid or the two nucleic acids of the present invention.

[0021] In a ninth aspect, the present invention relates to a host cell or host cells comprising the vector or the two vectors of the present invention.

[0022] In a tenth aspect, the present invention relates to a method for producing the antibody variable domain of the present invention or the antibody of the present invention, comprising (i) providing the nucleic acid or the two nucleic acids of the present invention, or the vector or the two vectors of the present invention, expressing said nucleic acid or said two nucleic acids, or said vector or vectors, and collecting said antibody variable domain or said antibody from the expression system, or (ii) providing a host cell or host cells of the

present invention, culturing said host cell or said host cells; and collecting said antibody variable domain or said antibody from the cell culture.

[0023] In an eleventh aspect, the present invention relates to a pharmaceutical composition comprising the antibody of the present invention and a pharmaceutically acceptable carrier.

[0024] In an twelfth aspect, the present invention relates to the pharmaceutical composition of the present invention for use as a medicament.

[0025] The aspects, advantageous features and preferred embodiments of the present invention summarized in the following items, respectively alone or in combination, further contribute to solving the object of the invention:

1. An antibody variable domain, which specifically binds to a target antigen, comprising:
 - (i) a variable heavy chain (VH) comprising from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from a human VH framework subtype, and wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
 - an arginine (R) at amino acid position 12;
 - a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144;
 - a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
 - (ii) a variable light chain (VL), wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein
 - a. said variable light chain framework regions LFW1, LFW2, LFW3 and LFW4 are selected from a human antibody Vk framework subtype; or

- b. said variable light chain framework regions LFW1, LFW2 and LFW3 are selected from a human antibody Vk framework subtype, and said variable light chain framework region LFW4 is selected from a Vλ framework subtype.
2. The antibody variable domain of item 1, wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
- an arginine (R) at amino acid position 12;
 - an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
 - an arginine (R) at amino acid position 12 and a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
- preferably wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
- an arginine (R) at amino acid position 12;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
- particularly wherein said HFW1, HFW2, HFW3 and HFW4 have the following substitutions (AHo numbering): an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103, and a glutamine (Q) at amino acid position 144.
3. The antibody variable domain of any one of items 1 or 2, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from the human VH framework subtypes VH1a, VH1b, VH3 or VH4, in particular from the human VH framework subtype VH3.
4. The antibody variable domain of any one of the preceding items, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from
- a. the combination of framework regions HFW1, HFW2, HFW3 and HFW4 (*i. e.* the non-italicized residues in Tables 1, 2 and 4) of any one of the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117; and
 - b. the combination of framework regions HFW1, HFW2, HFW3 and HFW4 (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96,

99, 100, 116 and 117 having 1, 2 or 3 mutations within the framework region at positions different from 12, 103 and 144 (AHO numbering).

5. The antibody variable domain of any one of the preceding items, wherein the human antibody Vk framework subtype is selected from the Vk1 framework subtype.
6. The antibody variable domain of any one of the preceding items, wherein LFW1, LFW2 and LFW3, and optionally also LFW4, if LFW4 is selected from a human antibody Vk framework subtype, are selected from
 - a. the combination of framework regions LFW1, LFW2, LFW3 and optionally LFW4 (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119; and
 - b. the combination of framework regions LFW1, LFW2, LFW3 and optionally LFW4 (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119 having 1, 2 or 3 mutations within the framework region.
7. The antibody variable domain of any one of the preceding items, wherein said variable light chain framework region LFW4 is selected from a Vλ framework subtype, particularly has a sequence selected from the group consisting of SEQ ID NOs: 123, 124, 125, 126, 127, 128, 129, 130 and 131.
8. The antibody variable domain of any one of the preceding items, wherein the format of said antibody variable domain is selected from an Fv, a dsFv and an scFv, particularly from an Fv and scFv.
9. The antibody variable domain of any one of the preceding items, wherein said antibody variable domain, when being in scFv format, exhibits a reduced binding to pre-existing anti-drug antibodies (ADA) present in human sera when compared to a version of said antibody variable domain that does not comprise the substitutions defined in item 1, as determined in a pre-existing ADA binding assay.
10. The antibody variable domain of any one of the preceding items, wherein said antibody variable domain, when being in scFv format, is further characterized by one or more of the following features:
 - a. has a melting temperature (T_m), determined by differential scanning fluorimetry (DSF), of at least 65°C, when formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;

- b. has a loss in monomer content, after storage for 28 days at 4°C, of less than 5 %, when formulated at a concentration of 10 mg/ml in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
 - c. has a loss in protein content, after storage for 28 days at 4°C, of less than 5 %, when formulated at a concentration of 10 mg/ml in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.
11. An antibody comprising one or more antibody variable domains as defined in any one of items 1 to 10.
12. The antibody of item 11, wherein the antibody is
- (i) monospecific and monovalent, bivalent or trivalent for its target antigen;
 - (ii) bispecific and, independently of each other, monovalent or bivalent for each target antigen;
 - (iii) trispecific and monovalent for each target antigen;
 - (iv) trispecific and bivalent for one of the target antigens and monovalent for the other target antigens;
 - (v) trispecific and bivalent for two of the target antigens and monovalent for the third target antigen;
 - (vi) tetraspecific and monovalent for each target antigen;
 - (vii) tetraspecific and bivalent for one of the target antigens and monovalent for the other target antigens; or
 - (viii) tetraspecific and bivalent for two of the target antigens and monovalent for the other target antigens.
13. The antibody of item 11, wherein the antibody comprises
- (i) a first and a second antibody variable domain as defined in any of items 1 to 10, wherein said first and said second antibody variable domains have specificity for two different target antigens; and
 - (ii) one, two, three or four further antibody variable domains as defined in any of items 1 to 10, having, independently of each other, either specificity for one of the target antigens of the first and second variable domains, or specificity for a target antigen different from the target antigens of the first and second variable domains.
14. The antibody of any one of items 11 to 13, wherein the format of said antibody is selected from bivalent bispecific IgG formats, trivalent bispecific IgG formats and tetravalent bispecific IgG formats;
- more particularly wherein the format of said antibody is selected from KiH-based IgGs; DVD-Ig; CODV-IgG and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv

fusion (Morrison-L)), even more particularly from DVD-Ig and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)).

15. The antibody of item 14, wherein the IgG is selected from the IgG subclasses IgG1 and IgG4, in particular IgG4.
16. The antibody of any one of items 11 to 13, wherein said antibody does not comprise an immunoglobulin Fc region.
17. The antibody of item 16, wherein said antibody is in a format selected from the group consisting of: a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a tandem tri-scFv, a tribody (Fab-(scFv)₂), a Fab-Fv₂, a triabody, an scDb-scFv, a tetrabody, a didiabody, a tandem-di-scFv and a MATCH.
18. The antibody of item 16, wherein said antibody does not comprise CH1 and/or CL regions.
19. The antibody of item 18, wherein said antibody is in a scDb-scFv, a triabody, a tetrabody or a MATCH format, in particular wherein said antibody is in a MATCH or scDb-scFv format, more particularly wherein said antibody is in a MATCH format, more particularly a MATCH3 or a MATCH4 format.
20. The antibody of item 18, wherein the antibody is trispecific and monovalent for each target antigen, and wherein the antibody comprises:
 - 1) one binding domain, which specifically binds to CD137 (CD137-BD) comprising
 - a) a VH sequence of SEQ ID NO: 1 and a VL sequence of SEQ ID NO: 5;
 - b) a VH sequence of SEQ ID NO: 2 and a VL sequence of SEQ ID NO: 5;
 - c) a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5;
 - d) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;
 - 2) one binding domain, which specifically binds to PDL1 (PDL1-BD) comprising
 - a) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 15;
 - b) a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 16;
 - c) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15; or
 - d) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;
 - 3) one human serum albumin binding domain (hSA-BD) comprising
 - a) a VH sequence of SEQ ID NO: 23 and a VL sequence of SEQ ID NO: 27;
 - b) a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 28;
 - c) a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27; or
 - d) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;

with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d);

or wherein the antibody comprises:

- 1) one binding domain, which specifically binds to CD137 (CD137-BD) comprising
 - a) a VH sequence of SEQ ID NO: 6 and a VL sequence of SEQ ID NO: 10;
 - b) a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 10;
 - c) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
 - d) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10;
- 2) one binding domain, which specifically binds to PDL1 (PDL1-BD) comprising
 - a) a VH sequence of SEQ ID NO: 17 and a VL sequence of SEQ ID NO: 21;
 - b) a VH sequence of SEQ ID NO: 18 and a VL sequence of SEQ ID NO: 22;
 - c) a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
 - d) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22; and
- 3) one human serum albumin binding domain (hSA-BD) comprising
 - a) a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 33;
 - b) a VH sequence of SEQ ID NO: 30 and a VL sequence of SEQ ID NO: 34;
 - c) a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
 - d) a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34;

with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d).

21. The antibody of item 20, wherein said antibody is a single chain protein in the scDb-scFvs (scMATCH3) format.

22. The antibody of item 21, wherein said antibody is a single-chain protein, wherein said single-chain protein comprises an amino acid sequence consisting of:

- (i) a first VL region comprising a sequence selected from SEQ ID NOs: 15 and 16;
- (ii) a first polypeptide linker;
- (iii) a first VH region comprising a sequence selected from SEQ ID NOs: 1, 2, 3 and 4;
- (iv) a second polypeptide linker;
- (v) a second VL region comprising the sequence of SEQ ID NO: 5,
- (vi) a third polypeptide linker; and
- (vii) a second VH region comprising either a sequence selected from SEQ ID NOs: 11 and 13, in case the first VL region has the sequence of SEQ ID NO: 15, or a sequence selected from SEQ ID NOs: 12 and 14, in case the first VL region has the sequence of SEQ ID NO: 16;

arranged one after another in the stated order;

wherein said first VL region associates with said second VH region to form said PDL1-BD, and said second VL region associates with said first VH region to form said CD137-BD;

and wherein said single-chain protein further comprises

(viii) a hSA-BD, which is formed by a third VL region, comprising a sequence selected from SEQ ID NOs: 27 and 28; and a third VH region, comprising either a sequence selected from SEQ ID NOs: 23 and 25, in case the third VL region has the sequence of SEQ ID NO: 27, or a sequence selected from SEQ ID NOs: 24 and 26, in case the third VL region has the sequence of SEQ ID NO: 28; wherein said third VL region and said third VH region are connected via a fourth polypeptide linker; and wherein said hSA-BD is fused C-terminally or N-terminally via a fifth polypeptide linker to said amino acid sequence;

or wherein said single-chain protein comprises an amino acid sequence consisting of:

- (i) a first VL region comprising a sequence selected from SEQ ID NOs: 21 and 22;
- (ii) a first polypeptide linker;
- (iii) a first VH region comprising a sequence selected from SEQ ID NOs: 6, 7, 8 and 9;
- (iv) a second polypeptide linker;
- (v) a second VL region comprising the sequence of SEQ ID NO: 10,
- (vi) a third polypeptide linker; and
- (vii) a second VH region comprising either a sequence selected from SEQ ID NOs: 17 and 18, in case the first VL region has the sequence of SEQ ID NO: 21, or a sequence selected from SEQ ID NOs: 18 and 20, in case the first VL region has the sequence of SEQ ID NO: 22;

arranged one after another in the stated order;

wherein said first VL region associates with said second VH region to form said PDL1-BD, and said second VL region associates with said first VH region to form said CD137-BD;

and wherein said single-chain protein further comprises

(viii) a hSA-BD, which is formed by a third VL region, comprising a sequence selected from SEQ ID NOs: 33 and 34; and a third VH region, comprising either a sequence selected from SEQ ID NOs: 29 and 31, in case the third VL region has the sequence of SEQ ID NO: 33, or a sequence selected from SEQ ID NOs: 30 and 32, in case the third VL region has the sequence of SEQ ID NO: 34; wherein said third VL region and said third VH region are connected via a fourth polypeptide linker; and wherein said hSA-BD is fused C-terminally or N-terminally via a fifth polypeptide linker to said amino acid sequence.

23. The antibody of any one of the items 20 to 22, wherein said antibody is selected from the scMATCH3 antibodies of SEQ ID NOs: 50, 51, 52, 53, 54, 55, 56, 57 and 58.
24. The antibody of any one of the items 20 to 23, wherein said antibody exhibits a reduced binding to pre-existing anti-drug antibodies (ADA) present in human sera, in particular reduced binding to pre-existing ADAs when compared to reference antibody NM21-1480, as determined in a pre-existing ADA binding assay.
25. An antibody variable domain as defined in any one of the items 1 to 10, wherein said antibody variable domain specifically binds to CD137, and comprises:
- a) a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5;
 - b) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;
 - c) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
 - d) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10.
26. An antibody variable domain as defined in any one of the items 1 to 10, wherein said antibody variable domain specifically binds to PDL1, and comprises:
- a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15;
 - b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;
 - c) a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
 - d) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22.
27. An antibody variable domain as defined in any one of the items 1 to 10, wherein said antibody variable domain specifically binds to human serum albumin, and comprises:
- a) a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27;
 - b) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;
 - c) a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
 - d) a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34.
28. An antibody variable domain as defined in any one of the items 1 to 10, wherein said antibody variable domain specifically binds to human serum albumin, and comprises
- a) a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
 - b) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 39;
 - c) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
 - d) a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 40;
 - e) a VH sequence of SEQ ID NO: 42 and a VL sequence of SEQ ID NO: 45;
 - f) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 46;

- g) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 48; or
 - h) a VH sequence of SEQ ID NO: 44 and a VL sequence of SEQ ID NO: 47.
29. The antibody variable domain of item 28, wherein said antibody variable domain, when being in scFv format
- a. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by surface plasmon resonance (SPR);
 - b. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR; and
 - c. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05g to 5 nM, at a pH value of 5.5, as measured by SPR.
30. The antibody variable domain of item 29, wherein said antibody variable domain, when being in scFv format, is further characterized by one or more of the following features:
- d. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by surface plasmon resonance (SPR);
 - e. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by SPR; and/or
 - f. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 20 nM, particularly with a KD of 0.1 to 20 nM, particularly of 0.1 to 15 nM, at a pH value of 7.4, as measured by SPR.
31. The antibody variable domain of item 29 or 30, wherein said antibody variable domain, when being in scFv format, is further characterized by one or more of the following features:
- g. has a melting temperature (T_m), determined by differential scanning fluorimetry (DSF), of at least 70°C, preferably at least 75°C, wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
 - h. has a loss in monomer content, after storage for 28 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;

- i. has a loss in protein content, after storage for 28 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
 - j. has a loss in protein content, after storage for 28 days at 40°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or
 - k. has a loss in monomer content, after storage for 14 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 50 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.
32. The antibody variable domain of any one of the items 28 to 31, wherein the hSA that is bound to said antibody variable domain is still capable of binding to FcRn.
33. A nucleic acid or two nucleic acids encoding the antibody variable domain of any one of items 1 to 10 or 25 to 32, or the antibody of any one of items 11 to 24.
34. A vector or two vectors comprising the nucleic acid or the two nucleic acids of item 33.
35. A host cell or host cells comprising the vector or the two vectors of item 34.
36. A method for producing the antibody variable domain of any one of items 1 to 10 or 25 to 32, or the antibody of any one of items 11 to 24, comprising (i) providing the nucleic acid or the two nucleic acids of item 33, or the vector or the two vectors of item 34, expressing said nucleic acid sequence or nucleic acids, or said vector or vectors, and collecting said antibody variable domain or said antibody from the expression system, or (ii) providing a host cell or host cells of item 35, culturing said host cell or said host cells; and collecting said antibody variable domain or said antibody from the cell culture.
37. A pharmaceutical composition comprising the antibody of any one of items 11 to 24 and a pharmaceutically acceptable carrier.
38. The antibody of any one of items 11 to 24, or the pharmaceutical composition of item 36, for use as a medicament.

39. A method for modifying an antibody, where the antibody is fragment-based or is an antibody comprising one or more scFv fragments, the method comprises the step of introducing the following substitutions (AHo numbering) in the VH sequence(s) of said fragment-based antibody or in the VH sequence(s) of the scFv fragment(s) of said antibody:

- an arginine (R) at amino acid position 12;
- a glutamine (Q) at amino acid position 144;
- an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
- an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144;
- a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
- an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144

to obtain a modified antibody;

wherein the modified antibody exhibits a decreased binding to pre-existing anti-drug-antibodies (ADAs) present in human sera from healthy donors when compared to its unmodified version, and wherein the decrease in binding is determined by an ELISA-based pre-existing anti-drug-antibody binding assay.

40. The method of item 39, wherein said modified antibody comprises an antibody variable domain as defined in any one of the items 1 to 32.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] **FIG. 1** shows the absorption levels of pre-existing ADAs in 20 human serum samples for PRO1922 (A), PRO2230 (C), PRO2922 (E) and PRO2925 (G), determined by the ELISA-based pre-existing ADA binding assay described in example 3. The measurements were performed with spiked and unspike serum samples (confirmation assay setup). Further shown are the corresponding reduction of absorbance level (inhibition (%)) of spiked human serum samples for PRO1922 (B), PRO2230 (D), PRO2922 (F) and PRO2925 (H). NC: negative control. PC: positive control, *i. e.* a human/rabbit chimeric antibody exhibiting high binding to pre-existing antibodies.

[0027] **FIG. 2** shows the absorption levels of pre-existing ADAs in 40 human serum samples for NM21-1480 (A) and PRO2764 (B), determined by the ELISA-based pre-existing

ADA binding assay described in example 3. The measurements were performed with spiked and unspike serum samples (confirmation assay setup). NC: negative control.

[0028] **FIG. 3** shows the absorption levels of pre-existing ADAs in 20 human serum samples for PRO2741 (A) and PRO2660 (B), determined by the ELISA-based pre-existing ADA binding assay described in example 3. The measurements were performed with spiked and unspike serum samples (confirmation assay setup). Further shown are the corresponding reduction of absorbance level (Inhibition (%)) of spiked human serum samples said molecules (C).

[0029] **FIG. 4** shows the absorption levels of pre-existing ADAs in 20 human serum samples (19 in case of PRO2589) for PRO2268 (A), PRO2269 (B), PRO2510 (C) and PRO2589 (D), determined by the ELISA-based pre-existing ADA binding assay described in example 3. The measurements were performed with spiked and unspike serum samples (confirmation assay setup). Further shown are the corresponding reduction of absorbance level (Inhibition (%)) of spiked human serum samples said molecules (E).

DETAILED DESCRIPTION OF THE INVENTION

[0030] The inventors have now surprisingly found that antibody variable domains, which are substituted at their heavy chain framework positions 12 and/or 144 by hydrophilic amino acids with large flexible and bulky side chains, i.e. an R at position 12 and/or a Q at position 144 (according to AHo numbering), and optionally are substituted at their heavy chain framework position 103 by a T, when being in scFv format, exhibit a reduced binding to pre-existing anti-drug antibodies (ADA) present in human sera when compared to their unsubstituted versions. Furthermore, these antibody variable domains could successfully be applied in the construction of various scFvs and fragment-based multispecific antibodies, which exhibit low immunogenicity and excellent stability.

[0031] Despite that fact that many method and strategies have been developed to reduce the immunogenicity of antibody variable domains, the immunogenicity reductions achievable with these methods are often not as strong as would be desirable from a pharmaceutical development perspective. Thus, there is still a large unmet need for antibody variable domains, which exhibit low immunogenicity. More specifically, it would be desirable to have antibody variable domains at hand, which exhibiting low immunogenicity, in particular with regard to reduced binding to pre-existing ADAs, and which can generally be applied in the construction of antibody fragments. It is furthermore desirable that these antibody variable domains provide a high stability, when integrated in the final antibody format, which would

allow their application in the construction of stable antibody fragments and fragment-based multispecific antibodies suitable for therapeutic development.

[0032] The present invention provides novel antibody variable domains, which are substituted with an R at heavy chain position 12 and/or with a Q at heavy chain position 144 (according to AHo numbering), and optionally are substituted with a T at heavy chain framework position 103. These novel antibody variable domain variants, when being in scFv format, exhibit a significantly reduced binding to pre-existing ADAs when compared to their unsubstituted versions. Furthermore, these antibody variable domains could successfully be applied in the construction of various scFvs and fragment-based multispecific antibodies, which exhibit low immunogenicity and excellent stability.

[0033] To the best knowledge of the inventors, there exist no antibody variable domains in the prior art that have such advantageous properties.

[0034] The antibody variable domain of the present invention could be successfully used for the construction of several scFvs and antibody fragment-based multispecific antibody formats. For example, the antibody variable domains of the present invention have been successfully applied in the construction of antibody fragment-based multispecific antibodies that target human mesothelin (MSLN), CD3 and human serum albumin (hSA) (MATCH4 format). The design, manufacturing and the functional and biophysical properties of these antibody fragment-based anti-MSLNxCD3xhSA antibodies are disclosed in detail in the patent application PCT/EP2021/064427. Specific examples are PRO2741, PRO2745 and PRO2746.

[0035] Furthermore, the antibody variable domains of the present invention have been successfully incorporated in antibody fragment-based multispecific antibodies that target ROR1, CD3 and hSA (scMATCH3 and MATCH4 format). The design, manufacturing and the functional and biophysical properties of fragment-based anti-ROR1xCD3xhSA antibodies are disclosed in detail in the experimental part and in the priority document EP21154786.4. Specific examples are PRO2667, PRO2668, PRO2669 and PRO2670.

[0036] Furthermore, the antibody variable domains of the present invention have been successfully incorporated in Morrison-based multispecific antibodies that target IL-4R and IL-31 (Morrison-H format). The design, manufacturing and the functional and biophysical properties of the Morrison-based anti-IL4RxIL31 antibodies are disclosed in detail in the priority document EP20216957.9. Specific examples are PRO2198 and PRO2199.

[0037] Furthermore, the antibody variable domains of the present invention have been successfully incorporated into the antibody fragment-based multispecific antibody NM21-1480 (scMATCH3 format), which targets PD-L1, CD137 and hSA. Several variants of NM21-1480 have been prepared. The design, manufacturing and the functional and biophysical

properties of the parental fragment-based anti-PDL1xCD137xhSA antibodies are disclosed in detail in the experimental part and in the patent application WO 2019/072868. Specific examples are PRO2758, PRO2759, PRO2760, PRO2761, PRO2762, PRO2763, PRO2764, PRO2765 and PRO3351.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains.

The terms "comprising" and "including" are used herein in their open-ended and non-limiting sense unless otherwise noted. With respect to such latter embodiments, the term "comprising" thus includes the narrower term "consisting of".

[0039] The terms "a" and "an" and "the" and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

[0040] In a first aspect, the present invention relates to an antibody variable domain, which specifically binds to a target antigen, comprising:

- (i) a variable heavy chain (VH) comprising from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from a human VH framework subtype, and wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHO numbering):
- an arginine (R) at amino acid position 12;
 - a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
 - a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12 and a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;

- (ii) a variable light chain (VL), wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein
 - a. said variable light chain framework regions LFW1, LFW2, LFW3 and LFW4 are selected from a human antibody Vk framework subtype; or
 - b. said variable light chain framework regions LFW1, LFW2 and LFW3 are selected from a human antibody Vk framework subtype, and said variable light chain framework region LFW4 is selected from a Vλ framework subtype.

[0041] The term “antibody” and the like, as used herein, includes whole antibodies or single chains thereof; and any antigen-binding variable domain (*i. e.*, “antigen-binding portion”) or single chains thereof; and molecules comprising antibody CDRs, VH regions or VL regions (including without limitation multispecific antibodies). A naturally occurring “whole antibody” is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), flanked by regions that are more conserved, termed framework regions (FRs). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e. g.*, effector cells) and the first component (C1q) of the classical complement system.

[0042] The term “antibody variable domain”, as used herein, refers to one or more parts of an intact antibody that have the ability to specifically bind to a given antigen (*e. g.*, PDL1, CD137, ROR1, MSLN, CD3, IL-4R, IL-31 or hSA). This can be any antigen-binding fragment (*i. e.*, “antigen-binding portion”) of an intact antibody or single chains thereof; and molecules comprising antibody CDRs, VH regions or VL regions. Specifically, in case of the multispecific antibodies of the present invention, the term “antibody variable domain”, as used herein, refers to a Fab fragment, *i. e.* a monovalent fragment consisting of the VL, VH, CL and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm

of an antibody (Fv); a disulfide stabilized Fv fragment (dsFv); a single chain Fv fragment (scFv); and a single chain Fv fragment having an additional light chain constant domain (C_L) fused to it (scAB). Preferably, the antibody variable domain of the present invention is selected from a Fab fragment, an Fv fragment, a disulfide stabilized Fv fragment (dsFv) and an scFv fragment. More preferably, the antibody variable domain of the present invention is selected from a Fab fragment, an Fv fragment, a disulfide stabilized Fv fragment (dsFv) and an scFv fragment. In particular embodiments, the antibody variable domain of the present invention is an Fv fragment, an scFv fragment or a disulfide stabilized Fv fragment (dsFv). In other particular embodiments, the antibody variable domain of the present invention is a single-chain Fv fragment (scFv). In other particular embodiments, the VL and VH domains of the scFv fragment are stabilized by an interdomain disulfide bond, in particular said VH domain comprises a single cysteine residue in position 51 (A_{Ho} numbering) and said VL domain comprises a single cysteine residue in position 141 (A_{Ho} numbering).

[0043] The term "Complementarity Determining Regions" ("CDRs") refers to amino acid sequences with boundaries determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme); Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering scheme); ImMunoGenTics (IMGT) numbering (Lefranc, M.-P., The Immunologist, 7, 132-136 (1999); Lefranc, M.-P. et al., Dev. Comp. Immunol., 27, 55-77 (2003)) ("IMGT" numbering scheme); and the numbering scheme described in Honegger & Plückthun, J. Mol. Biol. 309 (2001) 657-670 ("A_{Ho}" numbering). For example, for classic formats, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in human VH and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in human VL. Under IMGT the CDR amino acid residues in the VH are numbered approximately 26-35 (HCDR1), 51-57 (HCDR2) and 93-102 (HCDR3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (LCDR1), 50-52 (LCDR2), and 89-97 (LCDR3) (numbering according to "Kabat"). Under IMGT, the CDRs of an antibody can be determined using the program IMGT/DomainGap Align.

[0044] In the context of the present invention, the numbering system suggested by Honegger & Plückthun ("AHO") is used (Honegger & Plückthun, J. Mol. Biol. 309 (2001) 657-670), unless specifically mentioned otherwise. In particular, the following residues are defined as CDRs according to AHO numbering scheme: LCDR1 (also referred to as CDR-L1): L24-L42; LCDR2 (also referred to as CDR-L2): L58-L72; LCDR3 (also referred to as CDR-L3): L107-L138; HCDR1 (also referred to as CDR-H1): H27-H42; HCDR2 (also referred to as CDR-H2): H57-H76; HCDR3 (also referred to as CDR-H3): H108-H138. Correspondingly, in the context of the present invention, the following residues are defined as light chain frameworks 1 to 4 (LFW1-LFW4) and heavy chain frameworks 1 to 4 (HFW1-HFW4), respectively, according to AHO numbering scheme: LFW1: L1-L23; LFW2: L43-L57; LFW3: L73-L106; LFW4: L139-L149; HFW1: H1-H26; HFW2: H43-H56; HFW3: H77-H107; HFW4: H139-H149. For the sake of clarity, the numbering system according to Honegger & Plückthun takes the length diversity into account that is found in naturally occurring antibodies, both in the different VH and VL subfamilies and, in particular, in the CDRs, and provides for gaps in the sequences. Thus, in a given antibody variable domain usually not all positions 1 to 149 will be occupied by an amino acid residue.

[0045] The term "binding specificity" as used herein refers to the ability of an individual antibody to react with one antigenic determinant and not with a different antigenic determinant. As used herein, the term "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In its most general form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between the target of interest and an unrelated molecule, as determined, for example, in accordance with specificity assay methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA, RIA, ECL, IRMA, SPR (Surface plasmon resonance) tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (*e. g.* secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogen peroxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (= negative reaction) may be about 0.1 OD; typical positive reaction may be about 1 OD. This means the ratio between a positive and a negative score can be 10-fold or higher. In a further example, an SPR assay can be carried out, wherein at least 10-fold, particularly at least 100-fold difference between

a background and signal indicates on specific binding. Typically, determination of binding specificity is performed by using not a single reference molecule, but a set of about three to five unrelated molecules, such as milk powder, transferrin or the like.

[0046] The antibody variable domains of the present invention bind to a target antigen, which can be any target antigen. Examples of target antigens include, but are not limited to: a transmembrane molecule, a receptor, a ligand, a growth factor, a growth hormone, a clotting factor, an anti-clotting factor, a plasminogen activator, a serum albumin, a receptor for a hormone or a growth factor, a neurotrophic factor, a nerve growth factor, a fibroblast growth factor, transforming growth factor (TGF), a CD protein, an interferon, a colony stimulating factor (CSF), an interleukin (IL), a T-cell receptor, a T-cell co-stimulatory receptor, such as CD137, a surface membrane protein, a viral protein, a tumor associated antigen, an integrin or an interleukin, VEGF; a renin; a human growth hormone; a bovine growth hormone; a growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; a lipoprotein; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factor VIIIc; clotting factor IX; tissue factor (TP); von Willebrands factor; Protein C; atrial natriuretic factor; a lung surfactant; urokinase; human urine; tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha or -beta; enkephalinase; RANTES (Regulated on Activation Normally T-cell Expressed and Secreted); human macrophage inflammatory protein (MIP-I)-alpha; human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA); CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); protein A or D; a rheumatoid factor; bone-derived neurotrophic factor (BDNF); neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6); NGF-beta; platelet-derived growth factor (PDGF); aFGF; bFGF; epidermal growth factor (EGF); TGF-alpha; TGF-beta, including TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, or TGF-beta5; insulinlike growth factor-I or -II (IGF-I or IGF-II); des(I-3)-IGF-I (brain IGF-I), an insulin-like growth factor binding protein, erythropoietin; an osteoinductive factor; an immunotoxin; a bone morphogenetic protein (BMP); interferon-alpha, -beta, or -gamma; M-CSF, GM-CSF or G-CSF; IL-1 to IL-10; superoxide dismutase; decay accelerating factor; an AIDS envelope protein; a transport protein; a homing receptor; an addressin; a regulatory protein; CD3, CD4, CD8, CD11a, CD11b, CD11c, CD18, CD19, CD20, CD34, CD40, or CD46, an ICAM, VLA-4 or VCAM; or HER2, HER3 or HER4 receptor; a member of the ErbB receptor family; an EGF receptor; HER2, HER3 or HER4 receptor; a cell adhesion molecule; LFA-1, Mac1, p150.95, VLA-4, ICAM-1, VCAM, alpha4/beta7 integrin or alpha5/beta3 integrin; an alpha or

beta subunit of a cell adhesion molecule; antibodies); a growth factor, VEGF; tissue factor (TF); TGF-beta; alpha interferon (alpha-IFN); IL-8; IgE; blood group antigens Apo2; death receptor, such as PD-1; death receptor ligands, such as PD-L1; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA4 or protein C.

[0047] The term "tumor-associated antigen (TAA)" refers to an antigen that is expressed on the surface of a tumor cell. In particular embodiments, a TAA is an antigen that is preferentially expressed on a tumor cell when compared to non-tumor cells, particularly wherein expression of the TAA on a tumor cell is at least more than 5-fold, at least more than 10-fold, at least more than 20-fold, at least more than 50-fold, or at least more than 100-fold higher than on non-tumor cells from the same organism or patient.

[0048] Examples of tumor associated antigen targets include, but are not limited to: ADRB3, AFP, ALK, BCMA, beta human chorionic gonadotropin, CA-125 (MUC16), CAIX, CD123, CD133, CD135, CD135 (FLT3), CD138, CD171, CD19, CD20, CD22, CD24, CD276, CD33, CD38, CD44v6, CD79b, CD97, CDH3 (cadherin 3), CEA, CEACAM6, CLDN6, CLEC12A (CLL1), CSPG4, CYP1B1, EGFR, EGFRvIII, EPCAM, EPHA2, Ephrin B2, ERBBs (e. g. ERBB2), FAP, FGFR1, folate receptor alpha, folate receptor beta, Fos-related antigen, GA733, GD2, GD3, GFRalpha4, globoH, GPC3, GPR20, GPRC5D, HAVCR1, Her2/neu (HER2), HLA-A2, HMWMAA, HPV E6 or E7, human telomerase reverse transcriptase, IL-11Ra, IL-13Ra2, intestinal carboxyl esterase, KIT, Legumain, LewisY, LMP2, Ly6k, MAD-CT-1, MAD-CT-2, ML-IAP, MN-CA IX, MSLN, MUC1, mut hsp 70-2, NA-17, NCAM, neutrophil elastase, NY-BR-1, NY-ESO-1, o-acetyl-GD2, OR51E2, PANX3, PDGFR-beta, PLAC1, Polysialic acid, PSCA, PSMA, RAGE1, ROR1, sLe, sperm protein 17, SSEA-4, SSTR2, sTn antigen, sTn-Oglycopeptides, TAG72, TARP, TEM1/CD248, TEM7R, thyroglobulin, Tn antigen, Tn-O-Glycopeptides, TPBG (5T4), TRP-2, TSHR, UPK2 and VEGFR2. Preferred examples are: CD138, CD79b, TPBG (5T4), HER2, MSLN, MUC1, CA-125 (MUC16), PSMA, BCMA, CD19, EpCAM, CLEC12A (CLL1), CD20, CD22, CEA, CD33, EGFR, GPC3, CD123, CD38, CD33, CD276, CDH3 (cadherin 3), FGFR1, SSTR2, CD133, EPHA2, HLA-A2, IL13RA2, ROR1, CEACAM6, CD135, GD-2, GA733, CD135 (FLT3), CSPG4 and TAG-72. Particular examples are: CD138, CD79b, CD123, MSLN, PSMA, BCMA, CD19, CD20, CEA, CD38, CD33, CLEC12a, and ROR1.

[0049] In preferred embodiments, the HFW1, HFW2, HFW3 and HFW4 comprised in the antibody variable domains of the invention have one of the following substitutions (AHO numbering):

- an arginine (R) at amino acid position 12;
- an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144; or

- an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144.

[0050] In particular embodiments, the HFW1, HFW2, HFW3 and HFW4 comprised in the antibody variable domains of the invention have the following substitutions (AHO numbering): an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103, and a glutamine (Q) at amino acid position 144.

[0051] Suitably, the VH domains of the binding domains of the invention belong to a human antibody VH family. In preferred embodiments, the VH domains of the binding domains of the invention belong to VH framework subtypes VH1a, VH1b, VH3 or VH4. In particular embodiments, the binding domains of the invention comprises a VH domain belonging to the VH framework subtype VH3.

[0052] In the context of the present invention, the term "belonging to or selected from a VHx framework subtype (or Vk/Vλ framework subtype)" means that the framework sequences HFW1 to HFW4 (or LF1 to LFW4) show the highest degree of homology to said human antibody VH or VL framework subtype. A specific example of a VH domain belonging to the VH3 framework subtype is represented by SEQ ID NO: 114 or 115, and specific examples of a VH domain belonging to the VH1a, VH1b or VH4 framework subtype are represented by SEQ ID NO: 120, 121 and 122 (Table 8, framework regions are marked in non-bold). Alternative examples of VH1a, VH1b, VH3 and VH4 sequences, and examples of other VHx sequences, may be found in Knappik et al., J. Mol. Biol. 296 (2000) 57-86 or in WO 2019/057787.

[0053] In particular embodiments, the variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 of the antibody variable domain of the present invention are selected from the combination of framework regions (the non-italicized residues in Tables 1 to 7, *i. e.* all residues that are not marked as CDR residues) of any one of the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117; and from the combination of framework regions (the non-italicized residues in Tables 1 to 7) of variants of SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions at positions different from 12, 103 and 144 (AHO numbering) have been mutated. In this connection, the term "mutation" means, as various non-limiting examples, an addition, substitution or deletion. The VH regions further include VH domains comprising at least positions 5 to 140 (AHO numbering), particularly at least positions 3 to 145, more particularly at least positions 2 to 147 of one of the sequences

shown in the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117, provided that such VH domains exhibit the functional features defined above in items 9 and 10.

[0054] In preferred embodiments, the variable light chain frameworks LFW1, LFW2, LFW3 and LFW4 of the antibody variable domain of the present invention are selected from a human antibody Vk framework subtype (e.g. a Vk1, Vk2, Vk3 or Vk4 framework subtype), particularly are of the Vk1 framework subtype. A specific example of a Vk1 framework subtype is represented by SEQ ID NO: 118 or 119 (Table 8, framework regions are marked in non-bold). Alternative examples of Vk1 sequences, and examples of Vk2, Vk3 or Vk4 sequences, may be found in Knappik et al., J. Mol. Biol. 296 (2000) 57-86.

[0055] In other preferred embodiments, the variable light chain frameworks LFW1, LFW2 and LFW3 are selected from a human antibody Vk framework subtype, preferably a Vk1 framework subtype, and the variable light chain framework LFW4 is selected from a Vλ framework subtype. In particular embodiments, the variable light chain framework LFW4 of the antibody variable domain of the present invention is selected from the group consisting of the Vλ framework 4 sequences of SEQ ID NOs: 123, 124, 125, 126, 127, 128, 129, 130 and 131. Vλ framework 4 sequence of SEQ ID NO: 129 comprises a single cysteine residue at the variable light (VL) chain position 144 (AHO numbering) and is in particular applied in cases where a second single cysteine is present in the corresponding variable heavy (VH) chain, particularly in position 51 (AHO numbering) of VH, for the formation of an inter-domain disulfide bond.

[0056] In particular embodiments, the variable light chain frameworks LFW1, LFW2, LFW3 and LFW4 of the antibody variable domain of the present invention are selected from the combination of framework regions (the non-italicized residues in Tables 1 to 7, *i. e.* all residues that are not marked as CDR residues) of any one of the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119; and from the combination of framework regions (the non-italicized residues in Tables 1 to 7) of variants of SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions have been mutated. In this connection, the term "mutation" means, as various non-limiting examples, an addition, substitution or deletion. The VL regions further include VL domains comprising at least positions 5 to 140 (AHO numbering), particularly at least positions 3 to 145, more particularly at least positions 2 to 147 of one of the sequences shown in the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34,

38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119, provided that such VL domains exhibit the functional features defined above in items 9 and 10.

[0057] In preferred embodiments, the antibody variable domain of the present invention is in a format selected from a Fab fragment, *i. e.* a monovalent fragment consisting of the VL, VH, CL and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a disulfide stabilized Fv fragment (dsFv); and a single chain Fv fragment (scFv). Preferably, the antibody variable domain of the present invention is selected from an Fv fragment, a disulfide stabilized Fv fragment (dsFv) and a single-chain Fv fragment (scFv). In particular embodiments, the antibody variable domain of the present invention is selected from an Fv fragment and a single-chain Fv fragment (scFv). In other particular embodiments, the VL and VH domains of the scFv fragment are stabilized by an interdomain disulfide bond, in particular said VH domain comprises a single cysteine residue in position 51 (AHO numbering) and said VL domain comprises a single cysteine residue in position 141 (AHO numbering).

[0058] The antibody variable domain of the present invention, when being in scFv format, exhibits a reduced immunogenicity, when compared to a version of said antibody variable domain that does not comprise the above defined substitutions in the VH framework regions. More specifically, the antibody variable domain of the present invention, when being in scFv format, exhibits a reduced binding to pre-existing anti-drug antibodies (ADA) present in human sera, in particular reduced binding to pre-existing ADAs when compared to a version of said antibody variable domain that does not comprise the above defined substitutions in the VH framework regions, as determined in a pre-existing ADA binding assay, in particular as determined in a pre-existing ADA binding assay as defined in Example 3.

[0059] Immunogenicity, *i.e.* the tendency of a therapeutic protein to induce an antibody response within the patient's body, can *e.g.* be predicted by its capacity to be recognized by anti-drug antibodies (ADAs) that are already present in human sera of healthy and untreated individuals, herein referred to as "pre-existing ADAs".

[0060] Thus, for the purpose of the present invention, the term "immunogenicity", as used herein, refers to the capacity of a therapeutic protein, *e.g.* an antibody, an antibody fragment or an antibody binding domain, to be recognized by pre-existing ADAs in human serum samples. Without being bound to theory, it is believed that pre-existing ADA binding as well as the induction of the formation of ADAs during therapeutic treatment is linked with the occurrence of B cell and/or T cell epitopes on a therapeutic protein. The extent of such immunogenicity can be determined by an ELISA assay and can be expressed by the percentage of human serum samples, which contain measurable amounts of pre-existing

ADAs and/or ADAs formed during therapeutic treatment, that recognize, *i. e.* bind to, the therapeutic protein in question, relative to the total number of tested human sera (percentage of positive serum samples). A reduction of immunogenicity between a therapeutic protein and a corresponding therapeutic protein being modified with the goal to reduce its immunogenicity can be measured by comparing the percentage of positive serum samples against the modified therapeutic protein, with the percentage of positive serum samples against the original therapeutic protein. A lower number or percentage of positive serum samples for the modified therapeutic protein indicates a reduction of immunogenicity relative to the original therapeutic protein.

[0061] A serum sample is deemed to contain measurable amounts of pre-existing ADAs, when the ELISA signal surpasses a certain threshold. This threshold is herein also referred to as the screening cut-point (SCP). The SCP can be calculated as defined below or set to an arbitrary value relative to the maximum ELISA signal obtained for the tested sera (e.g. 20 %, 15 %, 10 % or 5 % of the maximum ELISA signal obtained for the tested sera). Preferably, the SCP is calculated as defined below.

[0062] The antibody variable domains of the present invention, when being in scFv format, further have advantageous biophysical properties, in particular an excellent stability.

Suitably, the antibody variable domain of the present invention, when being in scFv format, is further characterized by one or more of the following features:

- a. has a melting temperature (T_m), determined by differential scanning fluorimetry (DSF), of at least 65°C, when formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- b. has a loss in monomer content, after storage for 28 days at 4°C, of less than 5 %, when formulated at a concentration of 10 mg/ml in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- c. has a loss in protein content, after storage for 28 days at 4°C, of less than 5 %, when formulated at a concentration of 10 mg/ml in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

[0063] DSF is described earlier (Egan, et al., MAbs, 9(1) (2017), 68-84; Niesen, et al., Nature Protocols, 2(9) (2007) 2212-2221). The midpoint of transition for the thermal unfolding of the scFv constructs is determined by Differential Scanning Fluorimetry using the fluorescence dye SYPRO® Orange (see Wong & Raleigh, Protein Science 25 (2016) 1834-1840). Samples in phosphate-citrate buffer at pH 6.4 are prepared at a final protein concentration of 50 µg/ml and containing a final concentration of 5x SYPRO® Orange in a total volume of 100 µl. Twenty-five microliters of prepared samples are added in triplicate to white-walled AB gene PCR plates. The assay is performed in a qPCR machine used as a

thermal cycler, and the fluorescence emission is detected using the software's custom dye calibration routine. The PCR plate containing the test samples is subjected to a temperature ramp from 25°C to 96°C in increments of 1°C with 30 s pauses after each temperature increment. The total assay time is about 2 h. The T_m is calculated by the software GraphPad Prism using a mathematical second derivative method to calculate the inflection point of the curve. The reported T_m is an average of three measurements.

[0064] The loss in monomer content is as determined by area under the curve calculation of SE-HPLC chromatograms. SE-HPLC is a separation technique based on a solid stationary phase and a liquid mobile phase as outlined by the US Pharmacopeia (USP), chapter 621. This method separates molecules based on their size and shape utilizing a hydrophobic stationary phase and aqueous mobile phase. The separation of molecules is occurring between the void volume (V_0) and the total permeation volume (V_T) of a specific column. Measurements by SE-HPLC are performed on a Chromaster HPLC system (Hitachi High-Technologies Corporation) equipped with automated sample injection and a UV detector set to the detection wavelength of 280 nm. The equipment is controlled by the software EZChrom Elite (Agilent Technologies, Version 3.3.2 SP2) which also supports analysis of resulting chromatograms. Protein samples are cleared by centrifugation and kept at a temperature of 4-6°C in the autosampler prior to injection. For the analysis of scFv samples the column Shodex KW403-4F (Showa Denko Inc., #F6989202) is employed with a standardized buffered saline mobile phase (50 mM sodium-phosphate pH 6.5, 300 mM sodium chloride) at the recommended flow rate of 0.35 ml/min. The target sample load per injection was 5 µg. Samples are detected by an UV detector at a wavelength of 280 nm and the data recorded by a suitable software suite. The resulting chromatograms are analyzed in the range of V_0 to V_T thereby excluding matrix associated peaks with >10 min elution time.

[0065] In a second aspect, the present invention relates to an antibody comprising one or more antibody variable domains of the present invention.

[0066] In certain embodiments, the antibody of the present invention further comprises antibody variable domains that differ from the antibody variable domains of the present invention. More specifically, the antibody of the present invention further comprises antibody variable domains that do not have the substitutions in the framework regions as defined herein.

[0067] In preferred embodiments, the antibody of the present invention exclusively comprises antibody variable domains of the present invention. More specifically, in these preferred embodiments, the antibody of the present invention exclusively comprises antibody variable domains that have the substitutions in the framework regions as defined herein.

[0068] The term “monovalent antibody” or “antibody that is monovalent for its target antigen”, as used herein, refers to an antibody that binds to a single target molecule, and more specifically to a single epitope on a target molecule. Also, the term “binding domain” or “monovalent binding domain”, as used herein, refers to a binding domain that binds to a single epitope on a target molecule.

[0069] The term “bivalent antibody” or “antibody that is bivalent for its target antigen”, as used herein, refers to a single antibody with two valencies, where “valency” is described as the number of antigen-binding moieties that binds to epitopes on a specific target molecule. As such, the single antibody can bind to two binding sites on a target molecule and/or to two target molecules due to the presence of two copies of the corresponding antigen-binding moieties.

[0070] Likewise, the term “trivalent antibody” or “antibody that is trivalent for its target antigen”, as used herein, refers to a single antibody with three valencies. As such, the single antibody can bind to three binding sites on a target molecule and/or can bind up to three target molecules due to the presence of three copies of the corresponding antigen-binding moieties.

[0071] In case the antibodies of the invention comprise two or three binding domains, said two or three binding domains either bind the same epitope or different epitopes on the target molecules. Preferably, the two or three binding domains bind the same epitope on the target molecule.

[0072] The term “same epitope”, as used herein, refers to an individual protein determinant on the protein capable of specific binding to more than one antibody, where that individual protein determinant is identical, *i. e.* consist of identical chemically active surface groupings of molecules such as amino acids or sugar side chains having identical three-dimensional structural characteristics, as well as identical charge characteristics for each of said antibodies.

[0073] The term “different epitope”, as used herein in connection with a specific protein target, refers to individual protein determinants on the protein, each capable of specific binding to a different antibody, where these individual protein determinants are not identical for the different antibodies, *i. e.* consist of non-identical chemically active surface groupings of molecules such as amino acids or sugar side chains having different three-dimensional structural characteristics, as well as different charge characteristics. These different epitopes can be overlapping or non-overlapping.

[0074] In one group of embodiments, the format of the antibody is selected from bivalent bispecific IgG formats, trivalent bispecific IgG formats and tetravalent bispecific IgG formats. In particular, the format of said antibody is selected from KiH-based IgGs, such as

DuoBodies (bispecific IgGs prepared by the Duobody technology) (MAbs. 2017 Feb/Mar;9(2):182-212. doi: 10.1080/19420862.2016.1268307); DVD-Ig; IgG-scFv fusions, such as CODV-IgG, Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)), bsAb (scFv linked to C-terminus of light chain), Bs1Ab (scFv linked to N-terminus of light chain), Bs2Ab (scFv linked to N-terminus of heavy chain), Bs3Ab (scFv linked to C-terminus of heavy chain), Ts1Ab (scFv linked to N-terminus of both heavy chain and light chain) and Ts2Ab (dsScFv linked to C-terminus of heavy chain). More particularly, the format of said antibody is selected from KiH-based IgGs, such as DuoBodies; DVD-Ig; CODV-IgG and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)), even more particularly from DVD-Ig and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)).

[0075] In this group of embodiments, the IgG is preferably selected from the IgG subclasses IgG1 and IgG4, in particular IgG4.

[0076] In particular embodiments, the format of said antibody is selected from a Morrison format, *i. e.* a Morrison-L and a Morrison-H format. The Morrison-L and Morrison-H format used in the present invention are tetravalent and bispecific molecular formats bearing an IgG Fc region, in particular an IgG4 Fc region. Two highly stable scFv binding domains, wherein the light chain comprises V_κ FR1 to FR3 in combination with a V_λ FR4, and which are based on the antibody variable domains of the present invention, are fused via a linker L1 to the heavy chain (Morrison-H) or light chain (Morrison-L) C-termini.

[0077] The linker L1 is a peptide of 2-30 amino acids, more particularly 5-25 amino acids, and most particularly 10-20 amino acids. In particular embodiments, said linker L1 comprises one or more units of four (4) glycine amino acid residues and one (1) serine amino acid residue (GGGGS)_n, wherein n=1, 2, 3, 4 or 5, particularly n=2.

[0078] The VH regions and the VL regions of the two scFv domains are connected by a linker L2. The linker L2 is a peptide of 10-40 amino acids, more particularly 15-30 amino acids, and most particularly 20-25 amino acids. Particularly, said linker L2 comprises one or more units of four (4) glycine amino acid residues and one (1) serine amino acid residue (GGGGS)_n, wherein n=1, 2, 3, 4, 5, 6, 7 or 8, particularly n=4.

[0079] In another group of embodiments, the antibody of the invention does not comprise an immunoglobulin Fc region.

[0080] The term "immunoglobulin Fc region" or "Fc region", as used herein, is used to define a C-terminal region of an immunoglobulin heavy chain, *i. e.* the CH2 and CH3 domains of the heavy chain constant regions. The term "Fc region" includes native-sequence Fc regions and variant Fc regions, *i. e.* Fc regions that are engineered to exhibit certain desired properties, such as for example altered Fc receptor binding function and/or reduced

or suppressed Fab arm exchange. An example of such an engineered Fc region is the knob-into-hole (KiH) technology (see for example Ridgway et al., Protein Eng. 9:617-21 (1996) and Spiess et al., J Biol Chem. 288(37):26583-93 (2013)). Native-sequence Fc regions include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4. "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. Particularly, the FcR is a native sequence human FcR, which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors including FcγRIIA (an "activating receptor") and FcγRI IB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain, (see M. Daeron, Annu. Rev. Immunol. 5:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol. 9: 457-92 (1991); Capet et al, Immunomethods 4: 25-34 (1994); and de Haas et al, J. Lab. Clin. Med. 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer et al., J. Immunol. 117: 587 (1976) and Kim et al., J. Immunol. 24: 249 (1994). Methods of measuring binding to FcRn are known (see, e. g., Ghetie and Ward, Immunol. Today 18: (12): 592-8 (1997); Ghetie et al., Nature Biotechnology 15 (7): 637-40 (1997); Hinton et al., J. Biol. Chem. TJI (8): 6213-6 (2004); WO 2004/92219 (Hinton et al). Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e. g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, e. g., Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).

[0081] In this group of embodiments, the antibody is preferably in a format selected from the group consisting of: a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a tandem tri-scFv, a tribody (Fab-(scFv)₂), a Fab-Fv₂, a triabody, an scDb-scFv, a tetrabody, a di-diabody, a tandem-di-scFv and a MATCH (described in WO 2016/0202457; Egan T., et al., MABS 9 (2017) 68-84).

[0082] In particular embodiments, the antibody of the invention does further not comprise CH1 and/or CL regions. In these particular embodiments, the antibody is in a scDb-scFv, a triabody, a tetrabody or a MATCH format, particularly in a MATCH or scDb-scFv format. More particularly, the antibody of the invention is in a MATCH3 or a MATCH4 format.

[0083] In specific embodiments, the antibody of the invention is trispecific and tetravalent.

[0084] In further specific embodiments, the antibody of the invention is trispecific and trivalent.

[0085] The antibody variable domains comprised in the bispecific, trispecific tetraspecific or pentaspecific, antibodies of the invention are capable of binding to their respective antigens or receptors simultaneously. The term “simultaneously”, as used in this connection refers to the simultaneous binding of one of the antibody variable domains, which for example specifically binds to ROR1, and of one or two further antibody variable domains, which for example have specificity for CD3 and hSA.

[0086] Suitably, the antibody variable domains comprised in the bispecific, trispecific tetraspecific or pentaspecific, antibodies of the invention are operably linked.

[0087] The term “operably linked”, as used herein, indicates that two molecules (e. g., polypeptides, domains, binding domains) are attached in a way that each molecule retains functional activity. Two molecules can be “operably linked” whether they are attached directly or indirectly (e. g., via a linker, via a moiety, via a linker to a moiety). The term “linker” refers to a peptide or other moiety that is optionally located between binding domains or antibody variable domains used in the invention. A number of strategies may be used to covalently link molecules together. These include, but are not limited to, polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. Choosing a suitable linker for a specific case where two polypeptide chains are to be connected depends on various parameters, including but not limited to the nature of the two polypeptide chains (e. g., whether they naturally oligomerize), the distance between the N- and the C-termini to be connected if known, and/or the stability of the linker towards proteolysis and oxidation. Furthermore, the linker may contain amino acid residues that provide flexibility.

[0088] In the context of the present invention, the term “polypeptide linker” refers to a linker consisting of a chain of amino acid residues linked by peptide bonds that is connecting two domains, each being attached to one end of the linker. The polypeptide linker should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In particular embodiments, the polypeptide linker has a continuous chain of between 2 and 30 amino acid residues (e. g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acid residues). In addition, the amino acid residues selected for inclusion in the polypeptide linker should exhibit properties that do not interfere significantly with the activity of the polypeptide. Thus, the linker peptide on the whole should

not exhibit a charge that would be inconsistent with the activity of the polypeptide, or interfere with internal folding, or form bonds or other interactions with amino acid residues in one or more of the monomers that would seriously impede the binding of receptor monomer domains. In particular embodiments, the polypeptide linker is non-structured polypeptide. Useful linkers include glycine-serine, or GS linkers. By "Gly-Ser" or "GS" linkers is meant a polymer of glycines and serines in series (including, for example, (Gly-Ser)_n, (SGGS)_n, (GGGS)_n and (GGGS)_n, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers such as the tether for the shaker potassium channel, and a large variety of other flexible linkers, as will be appreciated by those in the art. Glycine-serine polymers are preferred since oligopeptides comprising these amino acids are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Secondly, serine is hydrophilic and therefore able to solubilize what could be a globular glycine chain. Third, similar chains have been shown to be effective in joining subunits of recombinant proteins such as single-chain antibodies.

[0089] Suitably, the antibody variable domain of the invention is an isolated variable domain. Likewise, the antibodies of the invention are isolated antibodies. The term "isolated variable domain" or "isolated antibody", as used herein, refers to a variable domain or an antibody that is substantially free of other variable domains or other antibodies having different antigenic specificities (e. g., an isolated antibody variable domain that specifically binds mesothelin is substantially free of antibody variable domains that specifically bind antigens other than mesothelin). Moreover, an isolated antibody variable domain or isolated antibody may be substantially free of other cellular material and/or chemicals.

[0090] Suitably, the antibody variable domains and antibodies of the invention are monoclonal antibody variable domains and antibodies. The term "monoclonal antibody variable domains" or "monoclonal antibody" as used herein refers to variable domains or antibodies that have substantially identical amino acid sequences or are derived from the same genetic source. A monoclonal variable domain or antibody displays a binding specificity and affinity for a particular epitope, or binding specificities and affinities for specific epitopes.

[0091] The antibody variable domains and antibodies of the invention include, but are not limited to, chimeric, human and humanized antibody variable domains and antibodies.

[0092] The term "chimeric antibody" or "chimeric antibody variable domain", as used herein, refers to an antibody molecule or antibody variable domain in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen-binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species; or (b) the variable region, or a portion thereof, is altered, replaced or

exchanged with a variable region having a different or altered antigen specificity. For example, a mouse antibody can be modified by replacing its constant region with the constant region from a human immunoglobulin. Due to the replacement with a human constant region, the chimeric antibody can retain its specificity in recognizing the antigen while having reduced antigenicity in human as compared to the original mouse antibody.

[0093] The term “human antibody” or “human antibody variable domain”, as used herein, is intended to include antibodies or antibody variable domains having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody or antibody variable domain contains a constant region, the constant region also is derived from such human sequences, *e. g.*, human germline sequences, or mutated versions of human germline sequences. The human antibodies and antibody variable domains of the invention may include amino acid residues not encoded by human sequences (*e. g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). This definition of a human antibody or antibody variable domain specifically excludes a humanized antibody or antibody variable domain comprising non-human antigen-binding residues. Human antibodies and antibody variable domains can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1992); Marks et al, *J. Mol. Biol.*, 222:581 (1991)). Also available for the preparation of human monoclonal antibodies and human monoclonal antibody variable domains are methods described in Cole et al, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boemer et al, *J. Immunol.*, 147(l):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies and human antibody variable domains can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies and antibody variable domains in response to antigenic challenge, but whose endogenous loci have been disabled, *e. g.*, immunized xenomice (see, *e. g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al, *Proc. Natl. Acad. Sci. USA*, 103:3557- 3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0094] The term “humanized” antibody or “humanized” antibody variable domain, as used herein, refers to an antibody or antibody variable domain that retains the reactivity of a non-human antibody or antibody variable domain while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody or antibody variable domain with their human counterparts (*i. e.*, the constant region as well as the framework portions of the variable region). Additional framework region modifications may be made within the human framework sequences as

well as within the CDR sequences derived from the germline of another mammalian species. The humanized antibodies and antibody variable domains of the invention may include amino acid residues not encoded by human sequences (*e. g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing). See, *e. g.*, Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855, 1984; Morrison and Oi, Adv. Immunol., 44:65-92, 1988; Verhoeyen et al., Science, 239: 1534-1536, 1988; Padlan, Molec. Immun., 28:489-498, 1991; and Padlan, Molec. Immun., 31: 169-217, 1994. Other examples of human engineering technology include but are not limited to the Xoma technology disclosed in US 5,766,886.

[0095] The term “recombinant humanized antibody” or “recombinant humanized antibody variable domain” as used herein, includes all human antibodies and human antibody variable domains that are prepared, expressed, created or isolated by recombinant means, such as antibodies and antibody variable domains isolated from a host cell transformed to express the humanized antibody or humanized antibody variable domain, *e. g.*, from a transfectoma, and antibodies and antibody variable domains prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences.

[0096] Preferably, the antibody variable domains and antibodies of the invention are humanized. More preferably, the antibody variable domains and antibodies of the invention are humanized and comprise rabbit-derived CDRs.

[0097] The term “bispecific antibody”, “trispecific antibody”, “tetraspecific antibody”, “pentaspecific antibody” or the more general term “multispecific antibody” as used herein, refers to an antibody that binds to two or more different epitopes on at least two or more different targets, for example 2 different targets (bispecific), 3 different targets (trispecific), 4 different targets (tetraspecific), or 5 different targets (pentaspecific). Preferably, the antibodies of the invention are bispecific, trispecific or tetraspecific, particularly bispecific or trispecific, more particularly trispecific. As indicated above, the term trispecific antibody refers to an antibody that binds to at least three different epitopes on three different targets (*e. g.*, mesothelin, CD3 and hSA or ROR1, CD3 and hSA).

[0098] The term “epitope” means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. “Conformational” and “linear” epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0099] The term “conformational epitope” as used herein refers to amino acid residues of an antigen that come together on the surface when the polypeptide chain folds to form the native protein.

[0100] The term “linear epitope” refers to an epitope, wherein all points of interaction between the protein and the interacting molecule (such as an antibody) occurring linearly along the primary amino acid sequence of the protein (continuous).

[0101] The term “recognize” as used herein refers to an antibody or antibody variable domain that finds and interacts (*e. g.*, binds) with its conformational epitope.

[0102] The inventors found that the antibody variable domain of the invention could be successfully applied in the construction of divers antibody fragments, *e. g.* scFv fragments, and multispecific antibodies, *e. g.* bispecific and trispecific antibodies, which exhibits significantly reduced immunogenicity, when compared to their unmodified counterparts, and which have an excellent stability.

[0103] One specific group of embodiments relates to an antibody comprising one or more antibody variable domains of the present invention, wherein the antibody is trispecific and monovalent for each target antigen, and wherein the antibody comprises:

- 1) one binding domain, which specifically binds to CD137 (CD137-BD);
- 2) one binding domain, which specifically binds to PDL1 (PDL1-BD); and
- 3) one human serum albumin binding domain (hSA-BD).

[0104] Preferably, in this specific group of embodiments,

the one binding domain, which specifically binds to CD137 (CD137-BD) comprises

- a. a VH sequence of SEQ ID NO: 1 and a VL sequence of SEQ ID NO: 5;
- b. a VH sequence of SEQ ID NO: 2 and a VL sequence of SEQ ID NO: 5;
- c. a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5; or
- d. a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;

the one binding domain, which specifically binds to PDL1 (PDL1-BD) comprises

- a. a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 15;
- b. a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 16;
- c. a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15; or
- d. a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;

the one human serum albumin binding domain (hSA-BD) comprises

- a. a VH sequence of SEQ ID NO: 23 and a VL sequence of SEQ ID NO: 27;
- b. a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 28;
- c. a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27; or
- d. a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;

with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d);

or

the one binding domain, which specifically binds to CD137 (CD137-BD) comprises

- a. a VH sequence of SEQ ID NO: 6 and a VL sequence of SEQ ID NO: 10;
- b. a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 10;
- c. a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
- d. a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10;

the one binding domain, which specifically binds to PDL1 (PDL1-BD) comprises

- a. a VH sequence of SEQ ID NO: 17 and a VL sequence of SEQ ID NO: 21;
- b. a VH sequence of SEQ ID NO: 18 and a VL sequence of SEQ ID NO: 22;
- c. a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
- d. a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22; and

the one human serum albumin binding domain (hSA-BD) comprises

- a. a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 33;
- b. a VH sequence of SEQ ID NO: 30 and a VL sequence of SEQ ID NO: 34;
- c. a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
- d. a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34;

with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d).

[0105] The above definition further includes variants of said VH and VL domains, *i. e.* variants of SEQ ID NOs: 3, 4, 5, 13, 14, 15, 16, 25, 26, 27 and 28 or variants of SEQ ID NOs: 8, 9, 10, 19, 20, 21, 22, 31, 32, 33 and 34, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions (the non-italicized residues in Table 1) at positions different from heavy chain positions 12, 103 and 144 (AHO numbering) have been mutated, provided that the VH and VL domains selected from these variant sequences still exhibit the respective binding properties to CD3, PDL1 or hSA as well as the functional properties as defined above in item 24. In this connection, the term "mutation" means, as various non-limiting examples, an addition, substitution or deletion.

[0106] The terms "binding domain" of an antibody, as used herein, or the terms "antigen-binding fragment thereof" or "antigen-binding portion" of an antibody, and the like, refer to one or more parts of an intact antibody that have the ability to specifically bind to a given antigen. Antigen-binding functions of an antibody can be performed by fragments of an intact antibody. Specifically, in case of the antibodies of the present invention, the terms "binding domain", as used herein, or the terms "antigen-binding fragment thereof" or "antigen-binding portion", and the like, refer to a Fab fragment, *i. e.* a monovalent fragment consisting of the

VL, VH, CL and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a disulfide stabilized Fv fragment (dsFv); and a single chain Fv fragment (scFv). Preferably, the binding domains of the antibodies of the present invention are independently of each other selected from an Fv fragment, a disulfide stabilized Fv fragment (dsFv) and a single-chain Fv fragment (scFv). In particular embodiments, the binding domains of the antibodies of the present invention are independently of each other selected from an Fv fragment and a single-chain Fv fragment (scFv). In other particular embodiments, the VL and VH domains of the scFv fragment are stabilized by an interdomain disulfide bond, in particular said VH domain comprises a single cysteine residue in position 51 (Aho numbering) and said VL domain comprises a single cysteine residue in position 141 (Aho numbering).

[0107] Preferably, the antibodies of said specific group of embodiments do not comprise an immunoglobulin Fc region. In particular, the antibodies of said specific group of embodiments do not comprise an immunoglobulin Fc region and do also not comprises CH1 and/or CL regions.

[0108] Preferably, at least two of said binding domains, particularly all three binding domains, are constructed from an antibody variable domain of the present invention, *i. e.* comprise heavy chain framework regions having the specific substitutions as defined herein for the antibody variable domains of the present invention.

[0109] Preferably, the antibodies of this specific group of embodiments comprise VH/VL sequences as defined herein, which can be found in Table 3.

[0110] Preferably, the antibodies of this specific group of embodiments are in a MATCH3 format, particularly have the scMATCH3 format.

[0111] Particularly, the antibodies of this specific group of embodiments are variants of the trispecific trivalent antibody NM21-1480. Specific examples are PRO2758, PRO2759, PRO2760, PRO2761, PRO2762, PRO2763, PRO2764, PRO2765 and PRO3351, whose sequences can be found in Table 3.

[0112] As mentioned above, details about the manufacturing and biophysical properties of these parental fragment-based anti-PDL1xCD137xhSA antibodies are disclosed herein. Further details, in particular further details on their design and their functional properties, are disclosed in the patent application WO 2019/072868.

[0113] The anti-PDL1xCD137xhSA antibodies of the present invention, in particular the NM21-1480 variants defined herein, exhibit a significantly reduced immunogenicity, *i. e.* exhibit a significantly reduced binding to pre-existing ADAs present in human serum samples of healthy untreated individuals when compared to NM21-1480, which does not comprise the substitutions as defined in item 1. The assay used for determining the binding of ADAs in

said serum samples to the anti-PDL1xCD137xhSA antibodies of the present invention, the NM21-1480 variants and NM21-1480 is described in detail in Example 3.

[0114] Besides, these fragment-based anti-PDL1xCD137xhSA antibodies have advantageous biophysical properties, in particular excellent stability.

[0115] In another aspect, the present invention relates to an antibody variable domain as defined herein, wherein said antibody variable domain specifically binds to human serum albumin.

[0116] The term "serum albumin" refers in particular to *human* serum albumin with UniProt ID number P02768 or a variant thereof. Human serum albumin (herein abbreviated as hSA) is a 66.4 kDa abundant protein in human serum (50 % of total protein) comprised of 585 amino acids (Sugio, Protein Eng, Vol. 12, 1999, 439-446). The structure of multifunctional hSA protein allows to bind and transport a number of metabolites such as fatty acids, metal ions, bilirubin and some drugs (Fanali, Molecular Aspects of Medicine, Vol. 33, 2012, 209-290). HSA concentration in serum is around 3.5-5 g/dl. Said hSA binding antibody variable domains of the present invention may thus be used, for example, to extend the *in vivo* serum half-life of drugs or proteins conjugated thereto.

[0117] In preferred embodiments, said antibody variable domain that specifically binds to human serum albumin comprises

- a) a VH domain selected from any one of the SEQ ID NOs: 35, 36 and 37, and from variants of SEQ ID NOs: 35, 36 and 37, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions (the non-italicized residues in Table 2) at positions different from 12, 103 and 144 (Aho numbering) have been mutated, provided that the VH domains selected from these variant sequences exhibit the functional features defined above in any one of the items 29 to 31; and
- b) a VL domain selected from any one of the SEQ ID NOs: 38, 39 and 40, and from variants of SEQ ID NOs: 38, 39 and 40, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions (the non-italicized residues in Table 2) have been mutated, provided that the VL domains selected from these variant sequences exhibit the functional features defined above in any one of the items 29 to 31.

[0118] In other preferred embodiments, said antibody variable domain that specifically binds to human serum albumin comprises

- a) a VH domain selected from any one of the SEQ ID NOs: 35, 36 and 37, and from variants of SEQ ID NOs: 35, 36 and 37, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions (the non-italicized residues in Table 2) at positions different from 12, 103 and 144 (Aho numbering) have been mutated, provided that the VH domains selected from these variant sequences exhibit the functional features defined above in any one of the items 29 to 31; and
- b) a VL domain selected from any one of the SEQ ID NOs: 38, 39 and 40, and from variants of SEQ ID NOs: 38, 39 and 40, wherein no more than 5 amino acids, particularly no more than 4 amino acids, particularly no more than 3 amino acids, particularly no more than 2 amino acids, particularly no more than 1 amino acid within the framework regions (the non-italicized residues in Table 2) have been mutated, provided that the VL domains selected from these variant sequences exhibit the functional features defined above in any one of the items 29 to 31.

[0119] In this connection, the term "mutation" means, as various non-limiting examples, an addition, substitution or deletion. The VH and VL regions further include VH and VL domains comprising at least positions 5 to 140 (Aho numbering), particularly at least positions 3 to 145, more particularly at least positions 2 to 147 of one of the sequences shown in the SEQ ID NOs: 35, 36 and 37, provided that such VL domains exhibit the functional features defined above in items 9 and 10.

[0120] In particular embodiments, said antibody variable domain that specifically binds to human serum albumin comprises

- a) a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
- b) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 39;
- c) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
- d) a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 40;
- e) a VH sequence of SEQ ID NO: 42 and a VL sequence of SEQ ID NO: 45;
- f) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 46;
- g) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 48; or
- h) a VH sequence of SEQ ID NO: 44 and a VL sequence of SEQ ID NO: 47.

[0121] Suitably, this hSA-binding antibody variable domain of the present invention is cross-reactive to other species. Particularly, the antibody variable domains of the invention are cross-reactive to Cynomolgus (*Macaca fascicularis*) serum albumin (herein abbreviated as cSA) and mouse (*Mus musculus*) serum albumin (herein abbreviated as mSA).

[0122] The hSA-binding antibody variable domain of the present invention, when being in scFv format, is further characterized by the following parameters:

- a. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by surface plasmon resonance (SPR);
- b. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR; and
- c. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR.

[0123] Specifically, the hSA-binding antibody variable domain of the present invention, when being in scFv format, is further characterized by the following parameters:

- a. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by surface plasmon resonance (SPR);
- b. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR; and
- c. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR;

and is additionally characterized by one or more of the following features:

- d. has a melting temperature (T_m), determined by differential scanning fluorimetry (DSF), of at least 70°C, preferably at least 75°C, wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- e. has a loss in monomer content, after storage for 28 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- f. has a loss in protein content, after storage for 28 days, at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- g. has a loss in protein content, after storage for 28 days, at 40°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or

- h. has a loss in monomer content, after storage for 14 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 50 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

[0124] Particularly, the hSA-binding antibody variable domain of the present invention, when being in scFv format, is further characterized by the following parameters:

- a. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by surface plasmon resonance (SPR);
- b. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR; and
- c. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR;

and is additionally characterized by one or more of the following parameters:

- d. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by surface plasmon resonance (SPR);
- e. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by SPR; and/or
- f. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 20 nM, particularly with a KD of 0.1 to 20 nM, particularly of 0.1 to 15 nM, at a pH value of 7.4, as measured by SPR.

[0125] More particularly, the hSA-binding antibody variable domain of the present invention, is further characterized by the following parameters:

- a. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by surface plasmon resonance (SPR);
- b. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR;
- c. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 5.5, as measured by SPR;

- d. binds to human serum albumin (hSA) with a monovalent dissociation constant (KD) of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by surface plasmon resonance (SPR);
- e. binds to *Macaca fascicularis* (Cynomolgus) serum albumin (cSA) with a monovalent KD of less than 10 nM, particularly with a KD of 0.05 to 10 nM, particularly of 0.05 to 5 nM, at a pH value of 7.4, as measured by SPR; and
- f. binds to *Mus musculus* (Mouse) serum albumin (mSA) with a monovalent KD of less than 20 nM, particularly with a KD of 0.1 to 20 nM, particularly of 0.1 to 15 nM, at a pH value of 7.4, as measured by SPR;

and is additionally characterized by one or more of the following features:

- g. has a melting temperature (T_m), determined by differential scanning fluorimetry (DSF), of at least 70°C, preferably at least 75°C, wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- h. has a loss in monomer content, after storage for 28 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- i. has a loss in protein content, after storage for 28 days, at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4;
- j. has a loss in protein content, after storage for 28 days, at 40°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 10 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or
- k. has a loss in monomer content, after storage for 14 days at 4°C, of less than 2 %, preferably less than 1 %, when said antigen-binding fragment is at a starting concentration of 50 mg/ml, and wherein said hSA-BD is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

[0126] As used herein, the term “affinity” refers to the strength of interaction between the antibody or the antibody variable domain and the antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody variable domain or the antibody “arm” interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

[0127] “Binding affinity” generally refers to the strength of the total sum of non-covalent interactions between a single binding site of a molecule (e. g., of an antibody or an antibody

variable domain) and its binding partner (e. g., an antigen or, more specifically, an epitope on an antigen). Unless indicated otherwise, as used herein, "binding affinity", "bind to", "binds to" or "binding to" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (e. g., an antibody variable domain and an antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies and antibody variable domains generally bind antigens slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigens faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity, *i. e.* binding strength are described in the following.

[0128] The term " K_{assoc} ", " K_a " or " K_{on} ", as used herein, are intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term " K_{dis} ", " K_d " or " K_{off} ", as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. In one embodiment, the term " K_D ", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i. e.* K_d/K_a) and is expressed as a molar concentration (M). The " K_D " or " K_D value" or "KD" or "KD value" according to this invention is in one embodiment measured by using surface plasmon resonance assays.

[0129] Suitably, the hSA-binding antibody variable domains of the present invention are selected from the group consisting of: a Fab, an Fv, a dsFv and an scFv.

[0130] The hSA-binding antibody variable domains of the present invention, when being in scFv format, exhibit a significantly reduced immunogenicity, *i. e.* exhibit a significantly reduced binding to pre-existing ADAs present in human serum samples, when compared to the corresponding hSA-binding scFvs that do not comprise the framework substitutions as defined above in item 1. The assay used for determining the binding of ADAs in said serum samples to said hSA-binding scFvs is described in detail in Example 3.

[0131] The antibody variable domains and antibodies of the invention can be produced using any convenient antibody-manufacturing method known in the art (see, e. g., Fischer, N. & Leger, O., Pathobiology 74 (2007) 3-14 with regard to the production of bispecific constructs; Hornig, N. & Färber-Schwarz, A., Methods Mol. Biol. 907 (2012) 713-727, and WO 99/57150 with regard to bispecific diabodies and tandem scFvs). Specific examples of suitable methods for the preparation of the multispecific constructs further include, *inter alia*, the Genmab (see Labrijn et al., Proc. Natl. Acad. Sci. USA 110 (2013) 5145-5150) and Merus (see de Kruif et al., Biotechnol. Bioeng. 106 (2010) 741-750) technologies.

[0132] These methods typically involve the generation of monoclonal antibodies or monoclonal antibody variable domains, for example by means of fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen using the hybridoma technology (see, e. g., Yokoyama et al., Curr. Protoc. Immunol. Chapter 2, Unit 2.5, 2006) or by means of recombinant antibody engineering (repertoire cloning or phage display/yeast display) (see, e. g., Chames & Baty, FEMS Microbiol. Letters 189 (2000) 1-8), and the combination of the antigen-binding domains or fragments or parts thereof of two or more different monoclonal antibodies to give a bispecific or multispecific construct using known molecular cloning techniques.

[0133] The antibodies of the invention that are multispecific, e.g. bispecific, trispecific, tetraspecific or pentaspecific, and/or multivalent, can be prepared by conjugating the constituent binding specificities, using methods known in the art. For example, each binding specificity of these antibodies can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-5-acetyl-thioacetate (SATA), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (sulfo-SMCC) (see e. g., Karpovsky et al., 1984 J. Exp. Med. 160: 1686; Liu, M A et al., 1985 Proc. Natl. Acad. Sci. USA 82:8648). Other methods include those described in Paulus, 1985 Behring Ins. Mitt. No. 78, 118-132; Brennan et al., 1985 Science 229:81-83, and Glennie et al., 1987 J. Immunol. 139: 2367-2375. Conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, Ill).

[0134] Alternatively, two or more binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x Fab, a mAb x scFv, a mAb x dsFv or a mAb x Fv fusion protein. Methods for preparing multispecific and/or multivalent antibodies and molecules are described for example in US 5,260,203; US 5,455,030; US 4,881,175; US 5,132,405; US 5,091,513; US 5,476,786; US 5,013,653; US 5,258,498; and US 5,482,858.

[0135] Binding of the antibody variable domains and multispecific antibodies to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (REA), FACS analysis, bioassay (e. g., growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e. g., an antibody) specific for the complex of interest.

[0136] In a further aspect, the invention provides a nucleic acid or two nucleic acids encoding the antibody variable domain or the antibody of the invention. Such nucleic acids can be optimized for expression in mammalian cells.

[0137] The term “nucleic acid” is used herein interchangeably with the term “polynucleotide(s)” and refers to one or more deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphorates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e. g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081, 1991; Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608, 1985; and Rossolini et al., *Mol. Cell. Probes* 8:91-98, 1994).

[0138] The invention provides substantially purified nucleic acid molecules which encode polypeptides comprising segments or domains of the antibody variable domains or the antibodies described above. When expressed from appropriate expression vectors, polypeptides encoded by these nucleic acid molecules are capable of exhibiting antigen-binding capacities of the antibody variable domains or the antibodies of the present invention.

[0139] The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (*e. g.*, sequences as described in the Examples below) encoding the antibody variable domain or the antibody of the invention. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., 1979, *Meth. Enzymol.* 68:90; the phosphodiester method of Brown et al., *Meth. Enzymol.* 68: 109, 1979; the diethylphosphoramidite method of Beaucage et al., *Tetrahedron Lett.*, 22: 1859, 1981; and the solid support method of US 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, *e. g.*, *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Erlich (Ed.), Freeman Press, NY, N.Y., 1992; *PCR Protocols: A Guide to Methods and Applications*, Innis et al. (Ed.), Academic Press, San

Diego, Calif, 1990; Mattila et al., Nucleic Acids Res. 19:967, 1991; and Eckert et al., PCR Methods and Applications 1:17, 1991.

[0140] Also provided in the invention are expression vectors and host cells for producing the antibody variable domain or the antibody of the invention.

[0141] The term “vector” is intended to refer to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a “plasmid”, which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e. g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e. g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

[0142] Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply, “expression vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e. g., replication defective retroviruses, adenoviruses and adeno- associated viruses), which serve equivalent functions. In this particular context, the term “operably linked” refers to a functional relationship between two or more polynucleotide (e. g., DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, *i. e.*, they are *cis*-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0143] Various expression vectors can be employed to express the polynucleotides encoding the antibody variable domain or the antibody chain(s). Both viral-based and non-viral expression vectors can be used to produce the antibodies or antibody variable domains in a mammalian host cell. Non-viral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial

chromosomes (see, e. g., Harrington et al., Nat Genet. 15:345, 1997). For example, non-viral vectors useful for expression of the hSA-binding polypeptides, or of polynucleotides encoding such polypeptides, in mammalian (e. g., human) cells include pThioHis A, B and C, pcDNA3.1/His, pEBVHis A, B and C, (Invitrogen, San Diego, Calif.), MPS V vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, Vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent et al., supra; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld et al., Cell 68: 143, 1992.

[0144] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (e. g., enhancers) that are operably linked to the polynucleotides encoding a multispecific antibody chain or a variable domain. In one embodiment, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, e. g., arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under non-inducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of a multispecific antibody chain or a variable domain. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e. g., Scharf et al., Results Probl. Cell Differ. 20: 125, 1994; and Bittner et al., Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[0145] Vectors to be used typically encode the antibody variable domain or the antibody light and heavy chain including constant regions or parts thereof, if present. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies and antibody variable domains thereof. Typically, such constant regions are human.

[0146] The term "recombinant host cell" (or simply "host cell") refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0147] The host cells for harboring and expressing the antibody variable domain or the antibody of the invention can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing the polynucleotides of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (e. g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express the antibody variable domain or multispecific antibodies of the invention. Insect cells in combination with baculovirus vectors can also be used.

[0148] In one embodiment, mammalian host cells are used to express and produce the antibody variable domain or the antibody of the invention. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes or a mammalian cell line harboring an exogenous expression vector. These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed including the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, e. g., Winnacker, FROM GENES TO CLONES, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, e. g., Queen, et al., Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPS V promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[0149] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See generally Green, M. R., and Sambrook, J., *Molecular Cloning: A Laboratory Manual* (Fourth Edition), Cold Spring Harbor Laboratory Press (2012)). Other methods include, e. g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation-nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, *Cell* 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express the antibody variable domain or the antibody of the invention can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1 to 2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type. The present invention thus provides a method of producing the antibody variable domain or the antibody of the invention, wherein said method comprises the step of culturing a host cell comprising a nucleic acid or a vector encoding the antibody variable domain or the antibody of the invention, whereby said antibody variable domain or said antibody of the disclosure is expressed.

[0150] In one aspect, the present invention relates to a method of producing the antibody variable domain or the antibody of the invention, the method comprising the step of culturing a host cell expressing a nucleic acid or two nucleic acids encoding the antibody variable domain or the antibody of the invention. In particular, the present invention relates to a method of producing the antibody variable domain or the antibody of the invention, the method comprising (i) providing a nucleic acid or two nucleic acids encoding the antibody variable domain or the antibody of the invention or one or two vectors encoding the antibody variable domain or the antibody of the invention, expressing said nucleic acid or nucleic acids, or said vector or vectors, and collecting said antibody variable domain or said antibody from the expression system, or (ii) providing a host cell or host cells expressing a nucleic acid or two nucleic acids encoding the antibody variable domain or the antibody of

the invention, culturing said host cell or said host cells; and collecting said antibody variable domain or said multispecific antibody from the cell culture.

[0151] In a further aspect, the present invention relates to a pharmaceutical composition comprising the antibody of the invention, and a pharmaceutically acceptable carrier.

"Pharmaceutically acceptable carrier" means a medium or diluent that does not interfere with the structure of the antibodies. Pharmaceutically acceptable carriers enhance or stabilize the composition, or facilitate preparation of the composition. Pharmaceutically acceptable carriers include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

[0152] Certain of such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject. Certain of such carriers enable pharmaceutical compositions to be formulated for injection, infusion or topical administration. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution.

[0153] Pharmaceutical compositions in accordance with the present disclosure may further routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents. The composition may also include antioxidants and/or preservatives. As antioxidants may be mentioned thiol derivatives (*e. g.* thioglycerol, cysteine, acetylcysteine, cystine, dithioerythritol, dithiothreitol, glutathione), tocopherols, butylated hydroxyanisole, butylated hydroxytoluene, sulfurous acid salts (*e. g.* sodium sulfate, sodium bisulfite, acetone sodium bisulfite, sodium metabisulfite, sodium sulfite, sodium formaldehyde sulfoxylate, sodium thiosulfate) and nordihydroguaiaretic acid. Suitable preservatives may for instance be phenol, chlorobutanol, benzylalcohol, methyl paraben, propyl paraben, benzalkonium chloride and cetylpyridinium chloride.

[0154] The pharmaceutical composition of the invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. Administration can be intravenous, intramuscular, intraperitoneal, or subcutaneous, or administered proximal to the site of the target. The pharmaceutically acceptable carrier should be suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e. g.*, by injection or infusion). Depending on the route of administration, the active compound, *i. e.*, the antibody of the invention, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[0155] The pharmaceutical compositions of the invention can be prepared in accordance with methods well known and routinely practiced in the art. See, *e. g.*, Remington: The

Science and Practice of Pharmacy, Mack Publishing Co., 20th ed., 2000; and Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically, a therapeutically effective dose or efficacious dose of the antibody of the invention is employed in the pharmaceutical compositions of the invention. The antibodies of the invention are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (*e. g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0156] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors.

[0157] The antibody of the invention is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the multispecific antibody of the invention in the patient. Alternatively, the antibody of the invention can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, humanized antibodies show longer half-life than that of chimeric antibodies and non-human antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage

is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0158] In one aspect, the present invention relates to the antibody of the invention or the pharmaceutical composition of the invention for use as a medicament. In a suitable embodiment, the present invention provides the multispecific antibody or the pharmaceutical composition for use in the treatment of a proliferative disease, such as cancer, or a disease selected from allergic, inflammatory and autoimmune diseases.

[0159] In another aspect, the present invention provides the pharmaceutical composition of the invention for use in the manufacture of a medicament for the treatment of a proliferative disease, such as cancer, or a disease selected from allergic, inflammatory and autoimmune diseases.

[0160] In another aspect, the present invention relates to the use of the antibody or the pharmaceutical composition of the present invention for treating a proliferative disease, such as cancer, or a disease selected from allergic, inflammatory and autoimmune diseases, in a subject in need thereof.

[0161] In another aspect, the present invention relates to a method of treating a subject comprising administering to the subject a therapeutically effective amount of the antibody of the present invention. In a suitable embodiment, the present invention relates to a method for the treatment of a proliferative disease, such as cancer, or a disease selected from allergic, inflammatory and autoimmune diseases, in a subject comprising administering to the subject a therapeutically effective amount of the antibody of the present invention.

[0162] The term "subject" includes human and non-human animals.

[0163] The term "animals" include all vertebrates, *e. g.*, non-human mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[0164] The terms "treatment", "treating", "treat", "treated", and the like, as used herein, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease or delaying the disease progression. "Treatment", as used herein, covers any treatment of a disease in a mammal, *e. g.*, in a human, and includes: (a)

inhibiting the disease, *i. e.*, arresting its development; and (b) relieving the disease, *i. e.*, causing regression of the disease.

[0165] The term “therapeutically effective amount” or “efficacious amount” refers to the amount of an agent that, when administered to a mammal or other subject for treating a disease, is sufficient to affect such treatment for the disease. The “therapeutically effective amount” will vary depending on the agent, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0166] In a final aspect, the present invention relates to a method for modifying an antibody, where the antibody is fragment-based or is an antibody comprising one or more scFv fragments, the method comprises the step of introducing the following substitutions (AHo numbering) in the VH sequence(s) of said fragment-based antibody or in the VH sequence(s) of the scFv fragment(s) of said antibody:

- an arginine (R) at amino acid position 12;
- a glutamine (Q) at amino acid position 144;
- an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
- an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144;
- a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
- an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144

to obtain a modified antibody;

wherein the modified antibody exhibits a decreased binding to pre-existing anti-drug-antibodies (ADAs) present in human sera from healthy donors when compared to its unmodified version, and wherein the decrease in binding is determined by an ELISA-based pre-existing anti-drug-antibody binding assay.

[0167] In particular embodiments of said final aspect, said modified antibody comprises an antibody variable domain in accordance with the present invention, *i. e.* as defined in the claims, in items 1 to 32 or in the detailed description of the invention.

Sequence listing (residues designated according to AHo numbering scheme; the CDRs defined according to Numab CDR definition, unless specified otherwise)

Table 1. Examples of VH/VL sequences of NM21-1480 variants according to the present invention and reference VH/VL sequences of unmodified NM21-1480 variants (modifications relative to NM21-1480 references are shown in bold; CDR residues are shown in bold and italic letters).

CD137 binding domain			Sequence:
SEQ ID NO:	Description:		
1	VH 38-27-A11-sc02		EVQLVESGGGLVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A N W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A V Y C A R S A W Y S G W G G D L W G Q G T L V T V S
2	VH 38-27-A11-sc02_ (CDR2: CIYGGSSDITYDAQWTK)		EVQLVESGGGLVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A Q W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A V Y C A R S A W Y S G W G G D L W G Q G T L V T V S
3	VH 38-27-A11-sc02_ (L12R, V103T, L144Q)		EVQLVESGGGRVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A N W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A T Y C A R S A W Y S G W G G D L W G Q G T Q V T V S
4	VH 38-27-A11-sc02_ (CDR2: CIYGGSSDITY DAQWTK)_ (L12R, V103T, L144Q)		EVQLVESGGGRVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A Q W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A T Y C A R S A W Y S G W G G D L W G Q G T Q V T V S
5	VL 38-27-A11-sc02		IQMTQSPSSLSASVGDRTITTC Q A S Q S I S N R L A W Y Q Q K P K A P K L L I Y S A S T L A S G V P S R F S G S G T D F T L T I S S L Q P E D F A T Y C Q S T Y Y G N D G N A F G T G T K V T V L G
6	VH 38-27-A11-sc02_ (V89L)		EVQLVESGGGLVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A N W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A V Y C A R S A W Y S G W G G D L W G Q G T L V T V S
7	VH 38-27-A11-sc02_ (CDR2: CIYGGSSDITYDAQWTK)_ (V89L)		EVQLVESGGGLVQPGGSLRLSCAAS GF S F S A N Y P C W R Q A P G K G L E W I G C I Y G G S S D I T D A Q W T K G R F T I S R D N S K N T V L Q M N S L R A E D T A V Y C A R S A W Y S G W G G D L W G Q G T L V T V S

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8	VH 38-27-A11-sc02_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRSLSCAASGFSFSAHYPCWVRQAPGKGLEWIGCI YGGSSDITYDANWTKGRFTISRDN SKNTLYLQMNSLRAEDTATYYCARSAWY SGWGGDLWGQGTQVTVSS
9	VH 38-27-A11-sc02_(CDR2: CIYGGSSDITY DAQWTK)_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRSLSCAASGFSFSAHYPCWVRQAPGKGLEWIGCI YGGSSDITYDAQWTKGRFTISRDN SKNTLYLQMNSLRAEDTATYYCARSAWY SGWGGDLWGQGTQVTVSS
10	VL 38-27-A11-sc02_(Q24R)	IQMTQSPSSLSASVGDRTITCRASQSIISNRLAWYQQKPGKAPKLLIYSASTLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCCQSTYYGNDGNALFGTGTKVT LG
PDL1 binding domain		
SEQ ID NO:	Description:	Sequence:
11	VH 37-20-B03-sc09.1	EVQLVESGGGLVQPGGSLRSLSCAASGFSFNDSYWIYVWRQAPGKGLEWIASI YGGSSGNTQYASWAQGRFTISRDN SKNTVYLQMNSLRAEDTAVYFCARGYV DYGGATDLWGQGTQVTVSS
12	VH 37-20-B03-sc09.1_(G51C)	EVQLVESGGGLVQPGGSLRSLSCAASGFSFNDSYWIYVWRQAPGKCLEWIASI YGGSSGNTQYASWAQGRFTISRDN SKNTVYLQMNSLRAEDTAVYFCARGYV DYGGATDLWGQGTQVTVSS
13	VH 37-20-B03-sc09.1_(L12R, V103T, L144Q)	EVQLVESGGGRVQPGGSLRSLSCAASGFSFNDSYWIYVWRQAPGKGLEWIASI YGGSSGNTQYASWAQGRFTISRDN SKNTVYLQMNSLRAEDTATYFCARGYV DYGGATDLWGQGTQVTVSS
14	VH 37-20-B03-sc09.1_(L12R, G51C, V103T, L144Q)	EVQLVESGGGRVQPGGSLRSLSCAASGFSFNDSYWIYVWRQAPGKCLEWIASI YGGSSGNTQYASWAQGRFTISRDN SKNTVYLQMNSLRAEDTATYFCARGYV DYGGATDLWGQGTQVTVSS
15	VL 37-20-B03-sc09.1	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFIL ASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGTG TKVTVLG
16	VL 37-20-B03-sc09.1_(T141C)	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFIL ASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGCG TKVTVLG
17	VH 37-20-B03-sc09.1_(V89L)	EVQLVESGGGLVQPGGSLRSLSCAASGFSFNDSYWIYVWRQAPGKGLEWIASI YGGSSGNTQYASWAQGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCARGYVD YGGATDLWGQGTQVTVSS

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18	VH 37-20-B03-sc09.1_(G51C, V89L)	EVQLVESGGGLVQPGGSLRLSCAASGFSFNSDYMIVWVRQAPGKCLEWIASI YGSSSGNTQYASWAQGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCARGYVD YGGATDLWGQGLTVTVSS
19	VH 37-20-B03-sc09.1_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFNSDYWIYVWVRQAPGKGLEWIASI YGSSSGNTQYASWAQGRFTISRDNSKNTLYLQMNSLRAEDTATYFCARGYVD YGGATDLWGQGTQVTVSS
20	VH 37-20-B03-sc09.1_(L12R, G51C, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFNSDYWIYVWVRQAPGKCLEWIASI YGSSSGNTQYASWAQGRFTISRDNSKNTLYLQMNSLRAEDTATYFCARGYVD YGGATDLWGQGTQVTVSS
21	VL 37-20-B03-sc09.1_(Q24R)	DIQMTQSPASLSASVGDRTVITTCRASQSIGTYLAWYQQKPGKPPKLLIYRAFIL ASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSNFYSDSTTIGPNAFGTG TKVTVLG
22	VL 37-20-B03-sc09.1_(Q24R, T141C)	DIQMTQSPASLSASVGDRTVITTCRASQSIGTYLAWYQQKPGKPPKLLIYRAFIL ASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSNFYSDSTTIGPNAFGCG TKVTVLG
22a	scFv 37-20-B03-sc09.1 PRO2230	DIQMTQSPASLSASVGDRTVITTCRASQSIGTYLAWYQQKPGKPPKLLIYRAFIL ASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSNFYSDSTTIGPNAFGTG TKVTVLGGGGGGGGGGGGGGVQLVESGGGLVQPGGSLRLS CAASGFSFNSDYWIYVWVRQAPGKGLEWIASIYGGSSGNTQYASWAQGRFTISRD NSKNTVYLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQGLTVTVSS
hSA BINDING DOMAIN 19-01-H04		
SEQ ID NO:	Description:	SEQUENCE:
23	VH 19-01-H04-sc03	EVQLVESGGGLVQPGGSLRLSCAASGFSLSNMGVWVRQAPGKGLYIGISV GGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYFCARDRHHGGDSS GAFYLVGQGLTVTVSS
24	VH 19-01-H04-sc03_(G51C)	EVQLVESGGGLVQPGGSLRLSCAASGFSLSNMGVWVRQAPGKGLYIGISV GGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYFCARDRHHGGDSS GAFYLVGQGLTVTVSS
25	VH 19-01-H04-sc03_(L12R, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSNMGVWVRQAPGKGLYIGIIS VGGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYFCARDRHHGGDS SGAFYLVGQGTQVTVSS

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26	VH 19-01-H04-sc03_(L12R, G51C, L144Q)	EVQLVESGGGRVQPGGSLRLSCAAS GFSLSSNAM GWWRQAPGKCLEYIGIIS VGGFTYYASWAK GRFTISRDNKNTVYLQMNSLRAEDTATYFCAR DRRHGGDS SGAFYLWGQGTQTVSS
27	VL 19-01-H04-sc03	IQMTQSPSSLSASVGDRVTITC QSSES VYNNQLSWYQQKPGQPPKLLIY DAS DLASGVPSR FRFGSGSGTDFTLTISLQPEDFATY YCAGGFSSSDTA FGGGTK LTVL
28	VL 19-01-H04-sc03_(G141C)	IQMTQSPSSLSASVGDRVTITC QSSES VYNNQLSWYQQKPGQPPKLLIY DAS DLASGVPSR FRFGSGSGTDFTLTISLQPEDFATY YCAGGFSSSDTA FGGGTK LTVLG
29	VH 19-01-H04-sc03_(V89L)	EVQLVESGGGLVQPGGSLRLSCAAS GFSLSSNAM GWWRQAPGKCLEYIGIISV GGFTYYASWAK GRFTISRDNKNTLYLQMNSLRAEDTATYFCAR DRRHGGDSS GAFYLWGQGTQTVSS
30	VH 19-01-H04-sc03_(G51C, V89L))	EVQLVESGGGLVQPGGSLRLSCAAS GFSLSSNAM GWWRQAPGKCLEYIGIISV GGFTYYASWAK GRFTISRDNKNTLYLQMNSLRAEDTATYFCAR DRRHGGDSS GAFYLWGQGTQTVSS
31	VH 19-01-H04-sc03_(L12R, L144Q)	EVQLVESGGGRVQPGGSLRLSCAAS GFSLSSNAM GWWRQAPGKCLEYIGIIS VGGFTYYASWAK GRFTISRDNKNTLYLQMNSLRAEDTATYFCAR DRRHGGDS SGAFYLWGQGTQTVSS
32	VH 19-01-H04-sc03_(L12R, G51C, L144Q)	EVQLVESGGGRVQPGGSLRLSCAAS GFSLSSNAM GWWRQAPGKCLEYIGIIS VGGFTYYASWAK GRFTISRDNKNTLYLQMNSLRAEDTATYFCAR DRRHGGDS SGAFYLWGQGTQTVSS
33	VL 19-01-H04-sc03_(Q24R)	IQMTQSPSSLSASVGDRVTITC RSSES VYNNQLSWYQQKPGQPPKLLIY DAS DLASGVPSR FRFGSGSGTDFTLTISLQPEDFATY YCAGGFSSSDTA FGGGTK LTVL
34	VL 19-01-H04-sc03_(Q24R_G141C)	IQMTQSPSSLSASVGDRVTITC RSSES VYNNQLSWYQQKPGQPPKLLIY DAS DLASGVPSR FRFGSGSGTDFTLTISLQPEDFATY YCAGGFSSSDTA FGGGTK LTVLG

Table 2. Examples of VH/VL sequences of hSA binding domain 19-04-A10 variants according to the present invention (modifications relative to original hSA binding domain 19-04-A10-sc01 and 19-04-A10-sc02 are shown in bold; CDR residues are shown in bold and italic letters).

hSA binding domain 19-04-A10

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SEQ ID NO:	Description:	Sequence:
35	VH 19-04-A10-sc01_(L12R, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYYCARAGAGGFSTGPFKLWGQ GTQVTVSS
36	VH 19-04-A10-sc02_(L12R, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARAGAGGFSTGPFKLWGQ GTQVTVSS
37	VH 19-04-A10-sc02_(L12R, G51C, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARAGAGGFSTGPFKLWGQ GTQVTVSS
38	VL 19-04-A10-sc01	MDIQMTQSPSSLSASVGDRTITTCQASEINSRLAWYQQKPKGAPKLLIYDASDLTSG VPSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG
39	VL 19-04-A10-sc02 (PRO2155)	AFELTQSPSSLSASVGDRTITTCQASEINSRLAWYQQKPKGAPKLLIYDASDLTSGV PSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG
40	VL 19-04-A10-sc06_(G141C) (PRO2317)	AFELTQSPSSLSASVGDRTITTCQASEINSRLAWYQQKPKGAPKLLIYDASDLTSGV PSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG
41	VL 19-04-A10-sc02_(G141T)	AFELTQSPSSLSASVGDRTITTCQASEINSRLAWYQQKPKGAPKLLIYDASDLTSGV PSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG
42	VH 19-04-A10-sc01_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTLYLQMNSLRAEDTATYYCARAGAGGFSTGPFKLWGQ GTQVTVSS
43	VH 19-04-A10-sc02_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTLYLQMNSLRAEDTATYFCARAGAGGFSTGPFKLWGQ GTQVTVSS
44	VH 19-04-A10-sc02_(L12R, G51C, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKGLGWIGHINAGDI AYYATWAKGRFTISRDNKNTLYLQMNSLRAEDTATYFCARAGAGGFSTGPFKLWGQ GTQVTVSS
45	VL 19-04-A10-sc01_(Q24R)	MDIQMTQSPSSLSASVGDRTITTCRASESINSRLAWYQQKPKGAPKLLIYDASDLTSG VPSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG
46	VL 19-04-A10-sc02 (PRO2155)_(Q24R)	AFELTQSPSSLSASVGDRTITTCRASESINSRLAWYQQKPKGAPKLLIYDASDLTSGV PSRFGSGSGTDFTLTISLQPEDFATYYCQGYGGSSTTTFGGGTKLTVLG

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47	VL 19-04-A10-sc06_(G141C) (PRO2317)_(Q24R)	AFELTQSPSSLSASVGDVRTTTCRASEINSRLAWYQQKPGQPPKLLIYDASDLTSGV PSRFSGSGGTDFLTLSLQLPEDFATYYCQGYGGSSTTTTFCGCTKLTVLG
48	VL 19-04-A10-sc02_(Q24R, G141T)	AFELTQSPSSLSASVGDVRTTTCRASEINSRLAWYQQKPGQPPKLLIYDASDLTSGV PSRFSGSGGTDFLTLSLQLPEDFATYYCQGYGGSSTTTTFCGCTKLTVLG

Table 3. NM21-1480 reference and examples of NM21-1480 variants according to the present invention (linker residues and modifications relative to unmodified NM21-1480 are shown in bold).

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		ASQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGSGDTFTLTISLQPEDF ATYYCQSTYYGNDGNAGFTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAA SGFSFNSDYWIYWRQAPGKGLEWIASYGGSSGNTQYASWAQGRFTISRDNKNTV YLQMNSLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGSIQMT QSPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGQPPKLLIYDASDLASGVPSR FSGSGGTDFLTISLQPEDFATYYCAGGFSSTDAFSGGKTLTVLGGGGGGGG GGGGGGGGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSNAMGWRQAPG KLEYIGIISVGGFTYYASWAKGRFTISRDNKNTVYLMNSLRAEDTATYFCARDRHG GDSSGAFYLVGGGTQVTVSS
51	scMATCH3 PRO2759	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGVP SRFSGSGGTDFLTISLQPEDFATYYCQSNFYSDSTTIGPNAFGTKVTVLGGGG GSEVQLVESGGGLVQPGGSLRLSCAASGFSFANYPCWWRQAPGKLEWIGCIYG GSSDITYDAQWTKGRFTISRDNKNTVYLMNSLRAEDTAVYCARSAWYSGWGGD LWGQGTQVTVSSGGGGGGGGGGGGSIQMTQSPSSLSASVGDRTITCQ ASQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGSGDTFTLTISLQPEDF ATYYCQSTYYGNDGNAGFTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAA SGFSFNSDYWIYWRQAPGKGLEWIASYGGSSGNTQYASWAQGRFTISRDNKNTV YLQMNSLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGSIQMT QSPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGQPPKLLIYDASDLASGVPSR FSGSGGTDFLTISLQPEDFATYYCAGGFSSTDAFSGGKTLTVLGGGGGGGG GGGGGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSLSNAMGWRQAPGK KLEYIGIISVGGFTYYASWAKGRFTISRDNKNTVYLMNSLRAEDTATYFCARDRHG GDSSGAFYLVGGGTQVTVSS
52	scMATCH3 PRO2760	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGVP SRFSGSGGTDFLTISLQPEDFATYYCQSNFYSDSTTIGPNAFGTKVTVLGGGG GSEVQLVESGGGRVQPGGSLRLSCAASGFSFANYPCWWRQAPGKLEWIGCIYG GSSDITYDAQWTKGRFTISRDNKNTVYLMNSLRAEDTATYCARSAWYSGWGGD LWGQGTQVTVSSGGGGGGGGGGGGSIQMTQSPSSLSASVGDRTITCQ ASQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGSGDTFTLTISLQPEDF ATYYCQSTYYGNDGNAGFTGKTVTLVGGGGSEVQLVESGGGLVQPGGSLRLSCAA SGFSFNSDYWIYWRQAPGKGLEWIASYGGSSGNTQYASWAQGRFTISRDNKNTV YLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGGGSIQMT QSPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGQPPKLLIYDASDLASGVPSR FSGSGGTDFLTISLQPEDFATYYCAGGFSSTDAFSGGKTLTVLGGGGGGGG GGGGGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSLSNAMGWRQAPGK

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		ASQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGGTFTLTISSLQPEDF ATYYCQSTYYGNDGNFAGTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAA SGFSFNSDYWIYWRQAPGKCLEWIASIYGGSSGNTQYASWAQGRFTISRDNKNTV YLQMNSLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGSIQMT QSPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGKPPKLLIYDASDLASGVPSR FSGSGGTFTLTISSLQPEDFATYYCAGGFSSTDAFGCGTKLTVLGGGGGGGG GGGGGGGGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSNAMGWWRQAPG KCLEYIGISVGGFTYYASWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARDRHG GDSSGAFYLVWGQGTQVTVSS
56	scMATCH3 PRO2764	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGVP SRFSGSGGTFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGCGTKVTVLGGGG GSEVQLVESGGGRVQPGGSLRLSCAASGFSFANYPCWWRQAPGKLEWIGCIYG GSSDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTATYCARSAWYSGWGGDL WGQGTQVTVSSGGGGGGGGGGGGGGSIQMTQSPSSLSASVGDRTITCQA SQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGGTFTLTISSLQPEDFA TYCQSTYYGNDGNFAGTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAAS GFSFNSDYWIYWRQAPGKCLEWIASIYGGSSGNTQYASWAQGRFTISRDNKNTVY LQMNSLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGSIQMTQ SPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGKPPKLLIYDASDLASGVPSRF SGSGGTFTLTISSLQPEDFATYYCAGGFSSTDAFGCGTKLTVLGGGGGGGGGG SGGGGGGGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSNAMGWWRQAPGK GLEIYIGISVGGFTYYASWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARDRHG GDSSGAFYLVWGQGTQVTVSS
57	scMATCH3 PRO2765	DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGVP SRFSGSGGTFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGCGTKVTVLGGGG GSEVQLVESGGGRVQPGGSLRLSCAASGFSFANYPCWWRQAPGKLEWIGCIYG GSSDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTATYCARSAWYSGWGGDL WGQGTQVTVSSGGGGGGGGGGGGGGSIQMTQSPSSLSASVGDRTITCQA SQISNRLAWYQQKPGKAPKLLIYASASTLASGVPSRFSGSGGTFTLTISSLQPEDFA TYCQSTYYGNDGNFAGTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAAS GFSFNSDYWIYWRQAPGKCLEWIASIYGGSSGNTQYASWAQGRFTISRDNKNTVY LQMNSLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGSIQMTQ SPSSLSASVGDRTITCQSSSESVYNNQLSWYQQKPGKPPKLLIYDASDLASGVPSRF SGSGGTFTLTISSLQPEDFATYYCAGGFSSTDAFGCGTKLTVLGGGGGGGGGG SGGGGGGGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSNAMGWWRQAPGK

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58	scMATCH3 PRO3351	CLEYIGIISVGGFTYYASWAKGRFTISRDN SKNTVYLQMN SLRAEDTATYFCARDRHG GDSSGAFYLGQGTQVTVSS DIQMTQSPASLSASVGDRTITCQASQSIGTYLAWYQQKPGKPKLLIYRAFILASGVP SRFSGSGGTDFTLTISSLQPEDFATYYCQSNFYSDTTIGPNAFGTGKTVTLVGGGG GSEVQLVESGGGRVQPGGSLRLSCAASGFSFANYPCWVRQAPGKGLEWIGCIYG GSSDITYDANWTKGRFTISRDN SKNTVYLQMN SLRAEDTATYYCARSAWYSGWGGDL WGQGTQVTVSSGGGGGGGGGGGGGSIQMTQSPSSLSASVGDRTITCQA SQSISNRLAWYQQKPGKAPKLLIYSASTLASGVPSRFSGSGGTDFTLTISSLQPEDFA TYYCQSTYYGNDGNAFGTGKTVTLVGGGGSEVQLVESGGGRVQPGGSLRLSCAAS GFSFNSDYWYWRQAPGKGLEWIASIYGGSSGNTQYASWAGRFTISRDN SKNTVY LQMN SLRAEDTATYFCARGYVDYGGATDLWGQGTQVTVSSGGGGGGGGGGSIQMTQ SPSSLSASVGDRTITCQSSSEVYNNQLSWYQQKPGQPKLLIYDASDLASGVPSRF SGSGGTDFTLTISSLQPEDFATYYCAGGFSSTDTAFGGGKTLTVLGGGGGGGGGG SGGGGGGGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSNMGWVRQAPGK GLEYIGIISVGGFTYYASWAKGRFTISRDN SKNTVYLQMN SLRAEDTATYFCARDRHG GDSSGAFYLGQGTQVTVSS
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Table 4. Examples of VH/VL sequences of NM26 variants according to the present invention and reference VH/VL sequences of unmodified NM26 variants (modifications relative to unmodified NM26 and linker residues, if present, are shown in bold; CDR residues in the VH/VL sequences are shown in bold and italic letters).

IL-31 binding domain		
SEQ ID NO:	Description:	Sequence:
59	VH 50-09-D07-sc03	QSQLVESGGGLVQPGGSLRLSCAASGFSFSANYWICWVRQAPGKGLEWIVCIYIGSR ADTYAPWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCARFSSGIDLDRFFFLW GQGTLVTVSS
60	VH 50-09-D07-sc03_(L12R, V103, L144Q)	QSQLVESGGGRVQPGGSLRLSCAASGFSFSANYWICWVRQAPGKGLEWIVCIYIGSR ADTYAPWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCARFSSGIDLDRFFFLW GQGTQVTVSS
61	VL 50-09-D07-sc03	AFQLTQSPSSLSASVGDRVTITCQASQSIDSWLAWYQQKPGKPKLLIYQASKLASGV PSRFSGSGSGTDYTLTISSLQPEDFATYYCQTYYGGGHIGWGFGTGKTVTLG
IL-4R binding domain		

SEQ ID NO:	Description:	Sequence:
62	VH 44-34-C10-sc09	EVQLVESGGGLVQPGGSLRLSCAASGFFSSSAHYMCWVRQAPGKGLEWIGCIYAGS RGSTYYASWAKGRFTISKASSTTVYLQMNSLRAEDTAVYYCARAAAFVNRGVSWIWPY YFSLWGQGTLVTVSS
63	VH 44-34-C10-sc09_(L12R, V103, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFFSSSAHYMCWVRQAPGKGLEWIGCIYAGS RGSTYYASWAKGRFTISKASSTTVYLQMNSLRAEDTATYYCARAAAFVNRGVSWIWPY YFSLWGQGTLVTVSS
64	VL 44-34-C10-sc09	DIQMTQSPSSLSASVGDRVTITCQASEDIESYLAWYQQKPKGAPKLLIYRASTLAYGV PSRFGSGSGTDFTLTISSLQPEDFATYYCLGSSYSTGSMIFGTGKTVTLG
Examples of NM26 multispecific antibody variants according to the present invention		
SEQ ID NO:	Ab Format:	Sequence:
PRO2198		
65	Morrison-H, LC	DIQMTQSPSSLSARVGDRTITCQASEDIESYLAWYQQKPKGAPKLLIYRASTLAYGVP SRFSGSGGRDFTLTISLQPEDFATYYCLGSSYSTGSIIFGQGTKEIKRTVAAPSVFI FPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS LSSTLTLSKADYEHKHYACEVTHQGLSSPVTKSFNRGEC
66	Morrison-H, HC	EVQLVESGGGLVQPGGSLRLSCAASGFFSSSAHYMCWVRQAPGKGLEWIGCIYAGS RGSTYYASWAKGRFTISRDNKNTVYLQMNSLRAEDTAVYYCARAAAFVNRGVSWWP YYFSLWGQGTLLVTVSSASTKGPSVFLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGKTKTYCNVDHKPSNTKVDKRV SKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSEQEDPEVQFN WYVDGVEVHNAKTPREEQFNSTYRVSVLTVHQDWLNGKEYCKKVSNGKGLPSSIE KTISKAKGQPREPVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVSFCSVMHEALHNHYTQKSLSLGG GGSGGGGSAFQLTQSPSSLSASVGDRTITCQASQSIDSWLAWYQQKPKGPPKLLI YQASKLASGVPSRFSGSGGTDTLTISLQPEDFATYYCQTYGGGHIGWGFGTGT KVTVLGGGGGGGGGGGGGGGSSQLVESGGGRVQPGGSLRLSCAASGFS FSANYWICWVRQAPGKGLEWIVCIYISRADTYAPWAKGRFTISKDNKNTVYLMQIN SLRAEDTATYFCARFSSGIYDLDRFFLWGQGTQVTVSS
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67	Morrison-H, LC	DIQMTQSPSSLSASVGRVTITCQASEDIESYLAWYQQKPKGKAPKLLIYRASTLAYGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCLGSSYSTGSNIFGQGTVEIKRTVAAPSVFI FPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC
68	Morrison-H, HC	EVQLVESGGGRVQPGGSLRLSCAASGFSSSSAHYMCWRQAPGKGLIEWIGCIYAGS RGSTYYASWAKGRFTISKASSTTVYQMNSLRAEDTATYYCARAAFNRGVSWWIPY YFSLWGQGTQVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTITTCNVDPKPSNTKVDKRVES KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSEQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVSVLTVLTQDNLNGKEYCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLGGG GGSGGGSAFQLTQSPSSLSASVGRVTITCQASQSIDSWLAWYQQKPKPKLLIY QASKLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQTYGGGHIGWGFGTGTK VTVLGGGGGGGGGGGGGSSQLVESGGGRVQPGGSLRLSCAASGFSF SANYMICWVRQAPGKGLIEWIVCIYIGSRADTYYPWAKGRFTISKDNSKNTVYLMQNS LRAEDTATYFCARFSSGIYDLDRFFLWGQGTQVTVSS

Table 6. Examples of VH/VL sequences according to the present invention and reference VH/VL sequences of unmodified NM28 references (modifications relative to NM28 references and linker residues, if present, are shown in bold; CDR residues in the VH/VL sequences are shown in bold and italic letters).

MSLN binding domain		
SEQ ID NO:	Description:	Sequence:
54-32-A07		
69	VH 54-32-A07-sc06	QSQLVESGGGLVQPGGSLRLSCAVSG FSLS SYAMG WVRQAPGKGL EYIGYISKIGTT YYASWAKGRFTISKDNSKNTVYLMNSLRAEDTAVYFCAR RGSSGGYLDGDFDPWG QGTLVTVSS
70	VH 54-32-A07-sc06_(G51C)	QSQLVESGGGLVQPGGSLRLSCAVSG FSLS SYAMG WVRQAPGKGL EYIGYISKIGTT YYASWAKGRFTISKDNSKNTVYLMNSLRAEDTAVYFCAR RGSSGGYLDGDFDPWG QGTLVTVSS

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71	VH 54-32-A07-sc06_(L12R, V103T, L144Q)	QQLVESGGGRVQPGGSLRLSCAIVSGFSLSSYAMGWWRQAPGKGLYIGYISKIGTT YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCAR ~~GS~~ SSGGYLD ~~DD~~ GFDPWG QGTQVTSS
72	VH 54-32-A07-sc06_(L12R, G51C, V103T, L144Q)	QQLVESGGGRVQPGGSLRLSCAIVSGFSLSSYAMGWWRQAPGKGLYIGYISKIGTT YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCAR ~~GS~~ SSGGYLD ~~DD~~ GFDPWG QGTQVTSS
73	VL 54-32-A07-sc06_(S14R, T22K, T87R)	ALQMTQSPSSLSARVGDRTIKCQASQISN ~~Y~~ LA ~~W~~ YQQKPGKPPKFLIY ~~DA~~ SDLASGV PSRFGSGSGRDTLTISLQPEDFATY ~~C~~ Q ~~Q~~ VYDS ~~NN~~ VEN ~~V~~ FGCGTKVTVLG
74	VL 54-32-A07-sc06_(S14R, T22K, T87R, T141C)	ALQMTQSPSSLSARVGDRTIKCQASQISN ~~Y~~ LA ~~W~~ YQQKPGKPPKFLIY ~~DA~~ SDLASGV PSRFGSGSGRDTLTISLQPEDFATY ~~C~~ Q ~~Q~~ VYDS ~~NN~~ VEN ~~V~~ FGCGTKVTVLG
54-21-H03		
75	VH 54-21-H03-sc01	EVQLVESGGGLVQPGGSLRLSCAASGFSFST ~~TY~~ MCWWRQAPGKGLWIGCTNTAS SVRTYYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAR ~~RD~~ MGFADYALNLW GGTQVTSS
76	VH 54-21-H03-sc01_(L12R, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFST ~~TY~~ MCWWRQAPGKGLWIGCTNTAS SVRTYYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYCAR ~~RD~~ MGFADYALNLW GGTQVTSS
77	VH 54-21-H03-sc01_(L12R, G51C, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFST ~~TY~~ MCWWRQAPGKGLWIGCTNTAS SVRTYYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYCAR ~~RD~~ MGFADYALNLW GGTQVTSS
78	VL 54-21-H03-sc01	DIQMTQSPSSLSASVGDRTITCQASESYSSLA ~~W~~ YQQKPGKAPKLLIY ~~LA~~ STLASGVP SRFSGSGGTDFLTISLQPEDFATY ~~C~~ Q ~~SD~~ YTT ~~ST~~ HRNSFGTKVTVLG
79	VL 54-21-H03-sc01_(G141C)	DIQMTQSPSSLSASVGDRTITCQASESYSSLA ~~W~~ YQQKPGKAPKLLIY ~~LA~~ STLASGVP SRFSGSGGTDFLTISLQPEDFATY ~~C~~ Q ~~SD~~ YTT ~~ST~~ HRNSFGCGTKVTVLG
79a	scFv 54-21-H03-sc01 (PRO1922)	DIQMTQSPSSLSASVGDRTITCQASESYSSLA ~~W~~ YQQKPGKAPKLLIY ~~LA~~ STLASGVP SRFSGSGGTDFLTISLQPEDFATY ~~C~~ Q ~~SD~~ YTT ~~ST~~ HRNSFGTKVTVLGGGGGS GGGGGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSFST ~~TY~~ MCWWR QAPGKGLWIGCTNTASSVRTYYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTAVY YCAR ~~RD~~ MGFADYALNLWGQGTQVTSS
CD3 binding domain		
SEQ ID NO:	Description:	Sequence:

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80	VH 28-21-D09 sc04	EVQLVESGGGLVQPGGSLRLSCAASGGFSLSSYDMSWVRQAPGKGLAWIGASYASGP TYASWAKGRFTISRDN SKNTVYLQMNSLRAEDTATYFCARGGWTGTS ~~SHSNMW~~ GGG TLVTVSS
81	VH 28-21-D09 sc04_ (L12R, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGGFSLSSYDMSWVRQAPGKGLAWIGASYASGP TYASWAKGRFTISRDN SKNTVYLQMNSLRAEDTATYFCARGGWTGTS ~~SHSNMW~~ GGG TQVTVSS
82	VL 28-21-D09 sc04	DIQMTQSPSSLSASVGDRTITCQSSQSVFSNNYLAWFQQKPGQSPKRLIYASSTLAS GVPSRFSGSGGTDFLTLSLQPEDFATYCLGSYACSSADCYVFGTGKTVLGL
NM28 multispecific antibodies references		
SEQ ID NO:	Ab Format:	Sequence:
PRO2660 reference		
83	MATCH4, CHAIN_1	DIQMTQSPSSLSASVGDRTITCQASESYSSLA WYQQKPGKAPKLLIYLASTLASGVP SRFSGSGGTDFLTLSLQPEDFATYQCSTDYTTSTHRNSFGTGKTVLGGGGGS GGGSGGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSFTYYMCWVR QAPGKLEWGCTNTASSVRYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVY YCARDMGFADYALNLWGQGLTVTVSSGGGGGGGGSEVQLVESGGGLVQPGGSLR LSCAASGFSLSYDMSWVRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDN SKN TVYLQMNSLRAEDTATYFCARGGWTGTS ~~SHSNMW~~ GGGLTVTVSSGGGGGGGGGS GEVQLVESGGGLVQPGGSLRLSCAASGFSLSYAMNWWVRQAPGKLEWIGHINAGDI AYYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVYFCARGAGGFSTGPFKLWGQ GTLTVTVSS
84	MATCH4, CHAIN_2	DIQMTQSPSSLSASVGDRTITCQASESYSSLA WYQQKPGKAPKLLIYLASTLASGVP SRFSGSGGTDFLTLSLQPEDFATYQCSTDYTTSTHRNSFGTGKTVLGGGGGS GGGSGGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSFTYYMCWVR QAPGKLEWGCTNTASSVRYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVY YCARDMGFADYALNLWGQGLTVTVSSGGGGGGGGSAFELTQSPSSLSASVGDRTV ITCQASESINRLAWYQQKPGQPPLIYDASDLTSGVPSRFSGSGGTDFLTLSLQ PEDFATYQCQYGGSTTTFCGCKLTVLGGGGGGSDIQMTQSPSSLSASVGDRTI TCQSSQSVFSNNYLAWFQQKPGQSPKRLIYASSTLASGVPSRFSGSGGTDFLTLSL LQPEDFATYCLGSYACSSADCYVFGTGKTVLGL
PRO2567 reference		

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85	MATCH4, CHAIN_1	AFELTQSPSSLSASVGDRTVITCQASEINSRLAWYQQKPGQPPKLLIYDASDLTSGVP SRFSGSGSGTDFTLTISLQPEDFATYYCQYGGSSITFTGTGKTLV GGGGSGG GGGGGGGGGG SEVQLVESGGGLVQPGGSLRLSCAASGFSLSYAMNWWVRQAPG KGLEWIGHINAGDIAYATWAKGRFTISRDNKNTVYLQMNSLRAEDTAVYFCARGAG GFSTGPFLWGQGLTVTVSS GGGGGGGG SEVQLVESGGGLVQPGGSLRLSCAAS GFSLSYDMSWVRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDNKNTVYLQ MNSLRAEDTATYFCARGGTGTSHNIWQGGTLTVSS GGGGGGGGGG SGGSSQL VESGGGLVQPGGSLRLSCAVSGFSLSYAMGWVRQAPGKCLEYIGYSKIGTITYAS WAKGRFTISKDNKNTVYLQMNSLRAEDTAVYFCARGSSGGYLDGDFDPWGQGLT VTVSS
86	MATCH4, CHAIN_2	ALQMTQSPSSLSARVGDRTVITKQASQISNLYAWYQQKPGKPPKFLYDASDLASGV PSRFSGSGSGRDTLTISLQPEDFATYYCQYVDSNNVENVFGCGTKTVL GGGG SGGGGGGGGG GSQSQLVESGGGLVQPGGSLRLSCAVSGFSLSSYAMGWVR QAPGKCLEYIGYSKIGTITYASWAKGRFTISKDNKNTVYLQMNSLRAEDTAVYFCAR GSSGGYLDGDFDPWGQGLTVTVSS GGGGGGGG GSALQMTQSPSSLSARVGDRTV IKQASQISNLYAWYQQKPGKPPKFLYDASDLASGVPSPRSGSGRDTLTISLQ PEDFATYYCQYVDSNNVENVFGCGTKTVL GGGGGG SDIQMTQSPSSLSASVGD VTITCQSSQSVFNLYLAWFQKPGQSPKRLYSASTLASGVPSPRSGSGSGTDFTLT SSLQPEDFATYYCLGSYACSSADCYVFGTGTKTVLG
Examples of NM28 multispecific antibody variants according to the present invention		
SEQ ID NO:	Ab Format:	Sequence:
PRO2741		
87	MATCH4, CHAIN_1	DIQMTQSPSSLSASVGDRTVITCQASEINSRLAWYQQKPGKAPKLLIYLASTLASGVP SRFSGSGSGTDFTLTISLQPEDFATYYCQSDTYTSTHRNSFGTGKTVL GGGGGS GGGGGGGGGG SEVQLVESGGGRVQPGGSLRLSCAASGFSSTTYMCWVR QAPGKLEWIGCTNTASSVRYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATY YCARDMGFADYALNLWGQGTQTVTVSS GGGGGGGG SEVQLVESGGGRVQPGGSL RLSCAASGFSLSYDMSWVRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDNK NTVYLQMNSLRAEDTATYFCARGGTGTSHNIWQGGTQTVTVSS GGGGGGGGGG SGEVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWWVRQAPGKCLEWIGHINAG DIAYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGAGGFGTGPFLKLVG QGTQTVTVSS

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88	MATCH4, CHAIN_2	DIQMTQSPSSLSASVGDRVITTCQASEISYSSSLAWYQQKPKAPKLLIYLASTLASGVP SRFSGSGSGTDFTLTISLQPEDFATYQCQSDYTTSTHRNSFGTKVTLVGGGGG GGGSGGGSGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSSTTYMCWVR QAPGKLEWIGCTNTASSVRTYYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATY YCARDMGFADYALNLWGQGTQTVSSGGGGGGGSAFELTQSPSSLSASVGDRV TITCQASEISYSSSLAWYQQKPKAPKLLIYDASDLTSGVPSRFSGSGTDFTLTISL QPEDFATYQCQYGGSTTTFCGKTLTVLGGGGSDIQTQSPSSLSASVGDRV ITCQSSQSVFSNNYLAWFQQKPGQSPKRLIYASATLASGVPSPRFSGSGSGTDFTLTIS LQPEDFATYCLGSYACSSADCYVFGTGKTVTLG
PRO2745		
89	MATCH4, CHAIN_1	AFELTQSPSSLSASVGDRVITTCQASEISYSSSLAWYQQKPKAPKLLIYDASDLTSGVP SRFSGSGSGTDFTLTISLQPEDFATYQCQYGGSTTTFTGTGKTLTVLGGGGGG GGSGGGSGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAP GKLEWIGHINAGDIAYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGA GGFSTGPFKLWGQGTQTVSSGGGGGGSEVQLVESGGGRVQPGGSLRLSCAA SGFSLSSYDMSWRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDNKNTVYLQ MNSLRAEDTATYFCARGGWTGTSNIWQGTQTVTVSSGGGGGGGGSGGSGGSQL VESGGGRVQPGGSLRLSCAVSGFSLSSYAMGWWRQAPGKLEYIGYISKIGTTYAS WAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGSSGGYLDGDFDPWGQGTQ VTSS
90	MATCH4, CHAIN_2	ALQMTQSPSSLSARVGDRVITKQASQISNLYAWYQQKPKPKFLIYDASDLASGV PSRFSGSGSGRDTLTISLQPEDFATYCCQVYDSNNVENVFGCGTKVTLVGGGG SGGGSGGGSGGGSQLVESGGGRVQPGGSLRLSCAVSGFSLSSYAMGWVR QAPGKLEYIGYISKIGTTYASWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCAR GSSGGYLDGDFDPWGQGTQTVTVSSGGGGGGGSAALQMTQSPSSLSARVGDRV IKQASQISNLYAWYQQKPKPKFLIYDASDLASGVPSPRFSGSGRDTLTISLQ PEDFATYCCQVYDSNNVENVFGCGTKVTLVGGGGGGGGSDIQTQSPSSL SASVGDRVITTCQSSQSVFSNNYLAWFQQKPGQSPKRLIYASATLASGVPSPRFSGSG SGTDFTLTISLQPEDFATYCLGSYACSSADCYVFGTGKTVTLG
PRO2746		
91	MATCH4, CHAIN_1	AFELTQSPSSLSASVGDRVITTCQASEISYSSSLAWYQQKPKAPKLLIYDASDLTSGVP SRFSGSGSGTDFTLTISLQPEDFATYQCQYGGSTTTFTGTGKTLTVLGGGGGG GGSGGGSGGGSEVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAP

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		GKLEWIGHINAGDIAYYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTATYFCARGA GGFSTGPFKLWGQGTQVTVSSGGGGGGSEVQLVESGGGRVQPGGSLRLSCAA SGFSLSSYDMSWVRQAPGKGLAWIGASYASGPTYYASWAKGRFTISRDN SKNTVYLQ MNSLRAEDTATYFCARGGWTGTSHSNIWGQGTQVTVSSGGGGGGGGSGGQSQQL VESGGGRVQPGGSLRLSCAVSGFSLSSYAMGW/RQAPGKCLEYIGYISKIGTTYAS WAKGRFTISKDN SKNTVYLQMNSLRAEDTATYFCARGSSGGYLDGDFDPWGQGTQ VTVSS
92	MATCH4, CHAIN_2	ALQMTQSPSSLSARVGDRTIKCQASQSSISNYLAWYQQKPGKPKFLIYDASDLASGV PSRFSGSGGRDFTLTISSLQPEDFATYYCQQVYDSNNVENVFGCGTKVTVLGGGGG SGGGGGGGGGGGSSQSLVESGGGRVQPGGSLRLSCAVSGFSLSSYAMGWVR QAPGKCLEYIGYISKIGTTYASWAKGRFTISRDN SKNTVYLQMNSLRAEDTATYFCAR GSSSGGYLDGDFDPWGQGTQVTVSSGGGGGGGGGALQMTQSPSSLSARVGDRT IKCQASQSSISNYLAWYQQKPGKPKFLIYDASDLASGVPSRFSGSGGRDFTLTISLQ PEDFATYYCQQVYDSNNVENVFGCGTKVTVLGGGGGGSDIQMTQSPSSLSASVGD VTITCQSSQSVFSNNYLAWFQQKPGQSPKRLIYASSTLASGVPSRFSGSGGTDFTLT SSLQPEDFATYYCLGSYACSSADCYVFGTGKVTVLG

Table 7. Examples of VH/VL sequences of NM32 variants according to the present invention and reference VH/VL sequences of unmodified NM32 references (modifications relative to NM32 references and linker residues, if present, are shown in bold; CDR residues in the VH/VL sequences are shown in bold and italic letters).

ROR1 binding domain		
SEQ ID NO:	Description:	Sequence:
55-39-G02		
93	VH 55-39-G02-sc02	QSQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWWRQAPGKGLEWIGIILTSGST YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCV RGIASSSLKSF WGQGTLV TVSS
94	VH 55-39-G02-sc02_(G51C)	QSQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWWRQAPGKGLEWIGIILTSGST YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCV RGIASSSLKSF WGQGTLV TVSS
95	VH 55-39-G02-sc02_(L12R, V103T, L144Q)	QSQLVESGGGRVQPGGSLRLSCAVSGLSLSRNAMSWWRQAPGKGLEWIGIILTSGST YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCV RGIASSSLKSF WGQGTV TVSS

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96	VH 55-39-G02-sc02_(L12R, G51C, V103T, L144Q)	QSQLVESGGGRVQPGGSLRLSCA VSGGLSLSRNAMMSWVRQAPGKCLEWIGILTSGST YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCV RGIASSSLKSF WGGGTQV TVSS
97	VL 55-39-G02-sc02	AQQLTQSPSSLSASVGDRVTITC QASQNVNNNN NYLSWFQKPGKPKLLIV TASTLA SGVSSRFSGSGGTDFLTLSLQPEDFATYYC AGGFSGEIRA FGTGKVTVLG
98	VL 55-39-G02-sc02_(T141C)	AQQLTQSPSSLSASVGDRVTITC QASQNVNNNN NYLSWFQKPGKPKLLIV TASTLA SGVSSRFSGSGGTDFLTLSLQPEDFATYYC AGGFSGEIRA FGCGTKVTVLG
55-38-D07		
99	VH 55-38-D07-sc02_(L12R, V103T, L144Q)	QSQVVESGGGRVQPGGSLRLSCA VSGGFDLSSYAVSVWVRQAPGKLEWIGI YIPRANT YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCAR DRYDSGAYL YTTYFNLW GGGTQVTVSS
100	VH 55-38-D07-sc02_(L12R, G51C, V103T, L144Q)	QSQVVESGGGRVQPGGSLRLSCA VSGGFDLSSYAVSVWVRQAPGKLEWIGI YIPRANT YYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCAR DRYDSGAYL YTTYFNLW GGGTQVTVSS
101	VL 55-38-D07-sc02	DVQMTQSPSSLSASVGDRVTITC RASENIY SLAWYQQKPGKPKLLI YRASTLASGV SSRFSGSGSGTDFTLTLSLQPEDFATYYC QGGYYSSSTYIA FGTGKVTVLG
102	VL 55-38-D07-sc02	DVQMTQSPSSLSASVGDRVTITC RASENIY SLAWYQQKPGKPKLLI YRASTLASGV SSRFSGSGSGTDFTLTLSLQPEDFATYYC QGGYYSSSTYIA FGCGTKVTVLG
NM32 multispecific antibodies references		
SEQ ID NO:	Ab Format:	Sequence:
PRO2510 reference		
103	scMATCH3	AFELTQSPSSLSASVGDRVTITCQASESINSRLAWYQQKPGQPPKLLIYDASDLTSGVP SRFSGSGSGTDFTLTLSLQPEDFATYYCQYGGSTTTFGCGTKLTVLGGGGGSEV QLVESGGGLVQPGGSLRLSCAASGFSLSYDMSWVRQAPGKGLAWIGASYSAGPTY YASWAKGRFTISRDNKNTVYLQMNSLRAEDATYFCARGGWTGTSHSNIWGGGTLV TVSSGGGGGGGGGGGGGGDIQMTQSPSSLSASVGDRVTITCQSSQSVFSN NYLAWFQQKPGQSPKRLIYASSTLASGVPSPRSFGSGSGTDFTLTLSLQPEDFATYYC LGSYACSSADCYVFGTGKVTVLGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF SLSSYAMNWVRQAPGKCLEWIGHINAGDIAYATWAKGRFTISRDNKNTVYLQMNSL RAEDTAVYFCARGAGGFTGPKLWGQGLTVTVSSGGGGGGGGGSAQQLTQSPSSL SASVGDRVTITCQASQNVNNNNYLSWFQKPGKPKLLIVTASTLASGVSSRFSGSG SGTDFTLTLSLQPEDFATYYCAGGESGEIRAFGCGIKTVI GGGGGGGGGGGGGGGG

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		SGGGGSQSQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWVRQAPGKCLEWIGII LTSGSTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCVRGIASSSLKSFWG QGTLVTVSS
PRO2658 reference		
104	MATCH4, chain_1	AQQLTQSPSSLSASVGDRTITCQASQNVWNNNYLSWFQQKPGKPPKLLIVTASTLAS GVSSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSGEIRAFGTGKTVTLV GGGGGS GGGGGGGGGGG SQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWVRQ APGKLEWIGIILTSGSTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCVRG IASSLKSFWGQGTLLTVSS GGGGGGGGG SAFELTQSPSSLSASVGDRTITCQASE SINSLAWYQQKPGQPPKLLIYDASDLTSGVPSRFSGSGTDFTLTISSLQPEDFATY YCGYGGSTTTFGCGTKTLV GGGGGGG DIQMTQSPSSLSASVGDRTITCQSSQS VFSNNYLAWFQQKPGQSPKRLIYASATLASGVPSRFSGSGTDFTLTISSLQPEDFA TYYCLGSYACSSADCYVFGTGKTVLG
105	MATCH4, chain_2	AQQLTQSPSSLSASVGDRTITCQASQNVWNNNYLSWFQQKPGKPPKLLIVTASTLAS GVSSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSGEIRAFGTGKTVTLV GGGGGS GGGGGGGGGGG SQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWVRQ APGKLEWIGIILTSGSTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCVRG IASSLKSFWGQGTLLTVSS GGGGGGGGG SEVQLVESGGGLVQPGGSLRLSCAASG FSLSSYDMSWVRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDNKNTVYLQM NSLRAEDTATYFCARGGTGTSHNIWGGQTLTVSS GGGGGGGGG SGGEVQLV ESGGGLVQPGGSLRLSCAASGFSLSYAMNWNWRQAPGKCLEWIGHINAGDIAYYATW AKGRFTISRDNKNTVYLQMNSLRAEDTAVYFCARGAGGFSTGPFLWGGQTLVTVS S
PRO2659 reference		
106	MATCH4, chain_1	AQQLTQSPSSLSASVGDRTITCQASQNVWNNNYLSWFQQKPGKPPKLLIVTASTLAS GVSSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSGEIRAFGCGTKTVTLV GGGGGS GGGGGGGGGGG SQLVESGGGLVQPGGSLRLSCAVSGLSLSRNAMSWVRQ APGKLEWIGIILTSGSTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCVRG IASSLKSFWGQGTLLTVSS GGGGGGGGG SAFELTQSPSSLSASVGDRTITCQASE SINSLAWYQQKPGQPPKLLIYDASDLTSGVPSRFSGSGTDFTLTISSLQPEDFATY YCGYGGSTTTFGCGTKTLV GGGGGGG DIQMTQSPSSLSASVGDRTITCQSSQS VFSNNYLAWFQQKPGQSPKRLIYASATLASGVPSRFSGSGTDFTLTISSLQPEDFA TYYCLGSYACSSADCYVFGTGKTVLG

107	MATCH4, chain_2	AQQLTQSPSSLSASVGDRTVITCQASQNVNNYLSWFQKPKGPPKLLIVTASTLAS GVSSRFSGSGGTDFTLTISSLQPEDFATYYCAGGFSGEIRAFGCGTKVTLVGGGGGS GGGSGGGSGGGGSQLVESGGLVQPGGSLRLSCAVSGLSLSRNAMSWSRQ APGKCLEWIGIILTSGSTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTAVYFCVRG IASSSLKSFWGQGLTVTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASG FSLSSYDMSWVRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDNKNTVYLQM NSLRAEDTATYFCARGGWGTGSHSNWGGTLTVTVSSGGGGGGGGSGGEVQLV ESGGLVQPGGSLRLSCAASGFSLSYAMNWRQAPGKCLEWIGHINAGDIAYATW AKGRFTISRDNKNTVYLQMNSLRAEDTAVYFCARGAGGFSTGPFKLWGQGLTVTVS S
Examples of NM32 multispecific antibody variants according to the present invention		
SEQ ID NO:	Ab Format:	Sequence:
PRO2667		
108	scMATCH3	AFELTQSPSSLSASVGDRTVITCQASEINSRLAWYQQKPGPPKLLIYDASDLTSGVP SRFSGSGGTDFTLTISSLQPEDFATYYCQYGGSSITTFGCGTKLTVLGGGGSEV QLVESGGGRVQPGGSLRLSCAASGFSLSYDMSWVRQAPGKGLAWIGASYASGPTY YASWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGGWGTGSHSNWGGGTQ VTSSGGGGGGGGGGGGGGGGGGDIQMTQSPSSLSASVGDRTVITCQSSQSVFS NNYLAWFQQKPGQSPKRLIYSASTLASGVPSRFSGSGGTDFTLTISLQPEDFATYY CLGSYACSSADCYVFGTKVTVLGGGGSEVQLVESGGGRVQPGGSLRLSCAASG FSLSSYAMNWRQAPGKCLEWIGHINAGDIAYATWAKGRFTISRDNKNTVYLQMN SLRAEDTATYFCARGAGGFSTGPFKLWGQGLTVTVSSGGGGGGGGSDVQMTQSP SSLSASVGDRTVITCRASENIYSGLAWYQQKPGPPKLLIYRASTLASGVSSRFSGSGS GTDFTLTISSLQPEDFATYYCQGGYSSSSTYAFGTGKTVTVLGGGGGGGGGGGG GGGGGGGSQLVESGGGRVQPGGSLRLSCAVSGFDLSSYAVSWVRQAPGKGLE WIGIYPRANTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCARDRYDSGA YLYTTYFNLWGQGTQVTVSS
PRO2668		
109	scMATCH3	AFELTQSPSSLSASVGDRTVITCQASEINSRLAWYQQKPGPPKLLIYDASDLTSGVP SRFSGSGGTDFTLTISSLQPEDFATYYCQYGGSSITTFGCGTKLTVLGGGGSEV QLVESGGGRVQPGGSLRLSCAASGFSLSYDMSWVRQAPGKGLAWIGASYASGPTY YASWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGGWGTGSHSNWGGGTQ

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[illegible]

		QAPGKLEWIGIYPRANTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCA RDRYDSGAYLYTTYFNLWGQGTQVTVSSGGGGGGGSAFELTQSPSSLSASVGD VTITCQASEINSRLAWYQKPGQPPKLLIYDASDLTSGVPSRFSGSGDTFTLTSS LQPEDFATYCYQGYGGSSTTTFCGCTKLTVLGGGGGSDIQTQSPSSLSASVGD TITCQSSQSVFSNNYLAWFQKPGQSPKRLIYASSTLASGVPSRFSGSGDTFTLTIS SLQPEDFATYCYCLGSACSSADCYVFGTGKTVTLG
113	MATCH4, chain_2	DVQMTQSPSSLSASVGDRTITCRASENIYSGLAWYQKPGKPPKLLIYRASTLASGV SSRFSGSGSGDTFTLTSSLPEDFATYCYQGGYSSSTYAFGCCGKTVTLGGGG SGGGGGGGGGGSSQSVESGGGRVQPGGSLRLSCAVSGFDLSSYAVSWR QAPGKLEWIGIYPRANTYYASWAKGRFTISKDNSKNTVYLQMNSLRAEDTATYFCA RDRYDSGAYLYTTYFNLWGQGTQVTVSSGGGGGGSEVQLVESGGGRVQPGGS LRLSCAASGFSLSYDMSWWRQAPGKGLAWIGASYASGPTYASWAKGRFTISRDN KNTVYLQMNSLRAEDTATYFCARGGWTGTSNHWGQGTQVTVSSGGGGGGGG GSSEVQLVESGGGRVQPGGSLRLSCAASGFSLSYAMNWRQAPGKCLEWIGHINA GDIAYYATWAKGRFTISRDNKNTVYLQMNSLRAEDTATYFCARGAGGFSTGPFKLW GQGTQVTVSS

Table 8. Other sequences related to the present invention.

SEQ ID NO:	Description	Sequence
114	VH3	EVQLVESGGGLVQPGGSLRLSCAASGFSFSAHYPCWWRQAPGKLEWIGCIYGGG SDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTAVYCARSAWYSGWGGDLW GQGTQVTVSS
115	VH3_(V89L)	EVQLVESGGGLVQPGGSLRLSCAASGFSFSAHYPCWWRQAPGKLEWIGCIYGGG SDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTAVYCARSAWYSGWGGDLW GQGTQVTVSS
116	VH3_(L12R, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFSAHYPCWWRQAPGKLEWIGCIYGGG SDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTATYCARSAWYSGWGGDLW GQGTQVTVSS
117	VH3_(L12R, V89L, V103T, L144Q)	EVQLVESGGGRVQPGGSLRLSCAASGFSFSAHYPCWWRQAPGKLEWIGCIYGGG SDITYDANWTKGRFTISRDNKNTVYLQMNSLRAEDTATYCARSAWYSGWGGDLW GQGTQVTVSS
118	Vkappa1	DIQMTQSPSSLSASVGDRTITCQASQSNINVLAWYQKPGKAPKLLIYRASTLASGV PSRFSGSGSGDTFTLTSSLPEDFATYCYQSSYGNYGDFGTGKTVTLG

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119	Vkappa1_(Q24R)	DIQMTQSPSSLSASVGDRTITTCRASQSNINVLAWYQQKPGKAPKLLIYRASTLASGV PSRFGSGSGTDFTLTISSLQPEDFATYYCQSSYGNYGDFGTGKVTVLG
120	VH1a	QVQLVQSGAEVKKPGSSVKVCKASGIDFNSNYIMCWWRQAPGQGLEWMGCIVVGS HVNITYYANWAKGRVTITADESTSTAYMELSSLRSEDTAVYYCATSGSSVL YFKFWGQ GTLTVSS
121	VH1b	QVQLVQSGAEVKKPGASVKVCKASGIDFNSNYIMCWWRQAPGQGLEWMGCIVVGS HVNITYYANWAKGRVTMTTRDTSISTAYMELSSLRSEDTAVYYCATSGSSVL YFKFWGQ GTLTVSS
122	VH4	QVQLQESGPGLVKPSSETLSLTCTVSGIDFNSNYIMCWIRQPPGKGLEWIGCIYVGS HVNITYYANWAKGRVTISVDTSKNQFSLKLSSTAAADTAVYYCATSGSSVL YFKFWGQGT LTVSS
123	VA germline-based FR4 (Sk17)	FGTGTKVTVLG
124	VA germline-based FR4 (Sk12)	FGGGTKLTVLG
125	VA germline-based FR4	FGGGTQLIILG
126	VA germline-based FR4	FGEGETLTVLG
127	VA germline-based FR4	FGSGTKVTVLG
128	VA germline-based FR4	FGGGTQLTVLG
129	VA germline-based FR4	FGGGTQLTALG
130	VA germline-based FR4 G141C	FGCGTKVTVLG
131	VA germline-based FR4 G141T	FGTGTKLTVLG

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[0168] Throughout the text of this application, should there be a discrepancy between the text of the specification (e. g., Tables 1 to 8) and the sequence listing, the text of the specification shall prevail.

[0169] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0170] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[0171] To the extent possible under the respective patent law, all patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference.

[0172] The following Examples illustrates the invention described above, but is not, however, intended to limit the scope of the invention in any way. Other test models known as such to the person skilled in the pertinent art can also determine the beneficial effects of the claimed invention.

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Examples

Example 1: Manufacturing of scFv variants according to the present invention

1.1. Manufacturing of variants of PRO1922 (MSLN binding scFv) and variants of PRO2230 (PDL1 binding scFv):

[0173] The manufacturing as well as the functional and biophysical characterization of PRO1922 are disclosed in detail in the patent application PCT/EP2021/064427.

[0174] PRO2230 is a scFv of the PD-L1 binding domain of the multispecific anti-PDL1xCD137xhSA antibody PRO1480. The design, characterization and manufacturing of PRO1480 and its binding domains is disclosed in detail in the patent application WO 2019/072868.

[0175] The variants of PRO1922 and PRO2230 of the present invention have been produced according to the methods described in said patent applications.

[0176] Briefly, the expression of the variants of PRO1922 and PRO2230, as defined herein, was performed in CHO cells using the ExpiCHO Expression System (ThermoFisher). Expression was conducted according to manufacturing instructions. Proteins were purified from clarified harvest by affinity chromatography. If necessary, variant scFvs were polished by SE-chromatography to a final monomeric content > 95 %. For quality control of the manufactured material, standard analytical methods such as SE-HPLC, UV₂₈₀ and SDS-PAGE were applied.

[0177] The variants of PRO1922 and PRO2230 produced are summarized in Table 9. The manufacturing details of the variants scFv of PRO1922 and PRO2230 are summarized in Table 10.

Table 9: Variants of PRO1922 and PRO2230.

PRO Number	Parental scFv	Target	Description	Mutations
PRO2922	PRO1922	MSLN	PRO1922-L12R	L12R
PRO2907	PRO2230	PD-L1	PRO2230-L12R	L12R
PRO2920	PRO1922	MSLN	PRO1922-L12R-L144Q	L12R-L144Q
PRO2905	PRO2230	PD-L1	PRO2230-L12R-L144Q	L12R-L144Q
PRO2919	PRO1922	MSLN	PRO1922-L12R-V103T	L12R-V103T
PRO2904	PRO2230	PD-L1	PRO2230-L12R-V103T	L12R-V103T

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PRO2918	PRO1922	MSLN	PRO1922-L12R-V103T-L144Q	L12R-V103T-L144Q
PRO2903	PRO2230	PD-L1	PRO2230-L12R-V103T-L144Q	L12R-V103T-L144Q
PRO2990	PRO1922	MSLN	PRO1922-L12S-V103T-L144T	L12S-V103T-L144T
PRO2984	PRO2230	PD-L1	PRO2230-L12S-V103T-L144T	L12S-V103T-L144T
PRO2925	PRO1922	MSLN	PRO1922-L12S-V103T-L144Q	L12S-V103T-L144Q
PRO2926	PRO2230	PD-L1	PRO2230-L12S-V103T-L144Q	L12S-V103T-L144Q
PRO2924	PRO1922	MSLN	PRO1922-L144Q	L144Q
PRO2909	PRO2230	PD-L1	PRO2230-L144Q	L144Q
PRO2923	PRO1922	MSLN	PRO1922-V103T	V103T
PRO2908	PRO2230	PD-L1	PRO2230-V103T	V103T
PRO2921	PRO1922	MSLN	PRO1922-V103T-L144Q	V103T-L144Q
PRO2906	PRO2230	PD-L1	PRO2230-V103T-L144Q	V103T-L144Q

1.2. Manufacturing of variants of PRO2155 and PRO2317 (hSA binding scFvs):

[0178] PRO2155 and PRO2317 are the scFvs of the hSA binding domain present in the multispecific anti-MSLNxCD3xhSA antibodies PRO2576 and PRO2660, and the multispecific anti-ROR1xCD3xhSA antibodies PRO2510, PRO2589, PRO2658 and PRO2659. Likewise, the variants of PRO2155 and PRO2317 according to the present invention are the scFvs of the hSA binding domain present in the anti-MSLNxCD3xhSA and anti-ROR1xCD3xhSA multispecific antibody variants PRO2741, PRO2745, PRO2746, PRO2667, PRO2668, PRO2669 and PRO2670.

[0179] The manufacturing as well as the functional and biophysical characterization of PRO2155 and PRO2317 are disclosed in detail in the patent application WO/2021/089609.

[0180] The variants of PRO2155 and PRO2317 of the present invention have been produced according to the methods described in said patent applications and above in section 1.1.

Example 2: Manufacturing of antibody variants according to the present invention

2.1. Manufacturing of variants of NM21-1480 (PRO1480)

[0181] As mentioned above, the design, characterization and manufacturing of NM21-1480 (PRO1480) and its binding domains is disclosed in detail in the patent application WO2019/072868. The NM21-1480 variants of the present invention, have been produced according to the methods described therein.

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[0182] Briefly, the expression of the NM21-1480 variants (scMATCH3 constructs) as listed in Table 11 has been performed at 0.5 L scale using CHOgro expression kit (Mirus) and mammalian CHO-S cells. After 7 days of expression, proteins were purified from clarified culture supernatants by Protein A (MabSelect Prisma, Cytiva) affinity chromatography either followed by size exclusion chromatography (SEC) in 50 mM phosphate-citrate buffer with 300 mM sucrose at pH 6.5 or, where applicable, capture fractions with >95% purity were directly pooled and buffer exchanged to 50 mM phosphate-citrate buffer with 300 mM sucrose at pH 6.5 buffer. Monomeric content of SEC fractions was assessed by SE-HPLC analysis and fractions with a monomeric content >95% were pooled. For quality control of the manufactured material, standard analytical methods such as SE-HPLC, UV₂₈₀ and SDS-PAGE were applied. Molecule composition and a manufacture summary of scMATCH3 molecules are shown in Table 2. Thermal stability of selected molecules has been assessed by nDSF using Prometheus NT.48 device (NanoTemper) as also summarized in Table 12.

2.2. Manufacturing of NM32 variants:

[0183] As mentioned above, the design, characterization and manufacturing of the NM32 references PRO2510, PRO2589, PRO2658 and PRO2659 and its binding domains is disclosed in detail in the priority document EP21154786.4. The NM32 variants of the present invention, have been produced according to the methods described therein.

[0184] Briefly, the expression of the NM32 variants (scMATCH3 and MATCH4 constructs) as listed in Table 13 has been performed at 1 L scale at Evitria AG (Schlieren, Switzerland) using their proprietary mammalian expression system. Proteins were purified from clarified culture supernatants by Protein L (CaptorL, Cytiva) affinity chromatography followed by SEC in 50 mM phosphate-citrate buffer with 300 mM sucrose at pH 6.5. Monomeric content of SEC fractions was assessed by SE-HPLC analysis and fractions with a monomeric content >95% were pooled. For quality control of the manufactured material, standard analytical methods such as SE-HPLC, UV₂₈₀ and SDS-PAGE were applied. A manufacture summary of scMATCH3 and MATCH4 molecules is shown in Table 13. Thermal stability of molecules has been assessed by nDSF using Prometheus NT.48 device (NanoTemper) as also summarized in Table 13.

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Table 10: Manufacture summary of PRO1922 and PRO2230 variants including thermal stability by nDSF

Protein ID	Capture titer [mg/l]	Purity by SE-HPLC [% monomer]	nDSF T _M [°C]
PRO2922	221	100	69.1
PRO2907	35	100	82.2
PRO2920	69	100	69.1
PRO2905	29	100	82.5
PRO2919	103	100	69.0
PRO2904	17	99	82.3
PRO2918	23	100	69.6
PRO2903	43	99	82.5
PRO2990	116	99	68.5
PRO2984	61	100	NA
PRO2925	65	98	69.4
PRO2926	39	100	81.7
PRO2924	120	100	68.5
PRO2909	18	100	81.3
PRO2923	68	100	68.7
PRO2908	143	100	81.3
PRO2921	63	100	68.9
PRO2906	42	100	82.0
PRO1922	158	100	69.0
PRO2230	330	100	80.7

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Table 11: NM21-1480 variants of the present invention.

Protein ID	Description of the NM21-1480 variant *
PRO2758	ND21_v1_PRO1480_L130R_N183Q_V214T_L236Q_L386R_V470T_L492Q_L649R_L754Q
PRO2759	ND21_v2_PRO1480_N183Q_L386R_V470T_L492Q
PRO2760	ND21_v3_PRO1480_L130R_N183Q_V214T_L236Q
PRO2761	ND21_v4_PRO1480_N183Q_L649R_L754Q
PRO2762	ND21_v5_PRO1480_T106C_L130R_N183Q_V214T_L236Q_L386R_G420C_V470T_L492Q_L649R_L754Q
PRO2763	ND21_v6_PRO1480_T106C_L130R_N183Q_V214T_L236Q_L386R_G420C_V470T_L492Q_G610C_L649R_G682C_L754Q
PRO2764	ND21_v7_PRO1480_T106C_L130R_V214T_L236Q_L386R_G420C_V470T_L492Q_L649R_L754Q
PRO2765	ND21_v8_PRO1480_T106C_L130R_V214T_L236Q_L386R_G420C_V470T_L492Q_G610C_L649R_G682C_L754Q

* The numbering is based on a continuous numbering (from N- to C-terminus) of the amino acids of the scMATH3 constructs, whose sequences are defined in Table 3.

Table 12: Manufacture summary table of NM21-1480 variants including thermal stability by nDSF

Protein ID	Capture titer [mg/l]	SEC polishing	Titer [mg/l]	Purity by SE-HPLC [% monomer]	nDSF T _{m1} [°C]	nDSF T _{m2} [°C]	nDSF T _{m3} [°C]	nDSF T _{onset} [°C]	nDSF scattering onset [°C]
PRO2758	30.0	No	2.6	96.0	63.7	79.2	NA	59.4	61.1
PRO2759	34.6	YES	13.2	99.0	NA	NA	NA	NA	NA
PRO2760	13.7	YES	2.8	99.7	NA	NA	NA	NA	NA
PRO2761	10.6	YES	2.8	99.8	NA	NA	NA	NA	NA
PRO2762	24.4	YES	9.2	99.0	NA	NA	NA	NA	NA

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PRO2763	13.0	YES	2.5	99.6	65.0	87.9	NA	59.6	62.8
PRO2764	21.2	No	2.4	95.0	NA	NA	NA		NA
PRO2765	10.7	YES	1.9	99.7	65.0	72.1	87.9	60.2	63.5

Table 13: Manufacture of ND032-RTQ molecules

Protein ID	Format	Capture titer [mg/l]	SEC polishing	Titer [mg/l]	Purity by SE- HPLC [% monomer]	nDSF T _{M1} [°C]	nDSF T _{M2} [°C]	nDSF T _{onset} [°C]	nDSF Scattering onset [°C]
PRO2667	scMATCH3	66.3	Yes	25.7	98.7	62.5	71.2	NA	69.0
PRO2668	scMATCH3	47.1	Yes	23.9	99.7	64.6	74.0	57.1	67.8
PRO2669	MATCH4	99.1	Yes	15.4	98.5	63.1	71.2	50.0	67.7
PRO2670	MATCH4	50.2	Yes	21.0	98.7	61.5	76.2	NA	69.6

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2.3. Manufacturing of NM26 variants:

[0185] As mentioned above, the design, characterization and manufacturing of the NM26 variants PRO2198 and PRO2199 and its binding domains is disclosed in detail in the priority document EP20216957.9.

2.4. Manufacturing of NM28 variants:

[0186] As mentioned above, the design, characterization and manufacturing of the NM28 references PRO2567 and PRO2660 and the NM28 variants PRO2741, PRO2745 and PRO2746 and its binding domains is disclosed in detail in the patent application PCT/EP2021/064427.

Example 3: pre-existing ADA binding assay

3.1. General assay procedure:

[0187] A method was developed at Numab to detect pre-existing anti-drug-antibodies in human serum, using a direct assay format.

[0188] 96 well half-area plates were coated with 100 ng/ml of the test molecule (MATCH3 or scFv format) for 2 hours at room temperature. The plates were blocked for 1 hour with PBS containing 0.2% Tween and 1% BSA. Individual human sera were then added at a dilution of 1:20 (5% serum) or 1:100 (1% serum), either unspiked (screening assay) or spiked (confirmatory assay) with the same molecule as coated in the corresponding well. The spiking concentration ranged from 60 to 115 nM and spiked samples were pre-incubated for 1 hour. Antibodies bound to the molecules coated on the plate were then detected with 100 ng/ml rabbit anti-human IgG-HRP for 1 hour. TMB substrate was added as substrate and after a short incubation, the enzymatic reaction was stopped with 1M HCl. The optical density of each well was read at 450nm.

[0189] All steps were performed at room temperature. Between each step, plates were washed three times with 450 µl wash buffer. Except for the blocking and washing steps, all assay components were added in a volume of 25 µl/well and duplicates were used. For the incubation steps, the ELISA plates were placed on a rotating mixer (40 rpm).

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[0190] Generally, a first round of measurement was performed with unspiked human sera (screening assay). Then a screening cut-point (SCP) was calculated for each plate. Unspiked samples with signal below the SCP were termed "screening negative" and were not taken into account in the confirmatory assay. Unspiked samples with signal above the SCP were termed "screening positive".

[0191] With most of these "screening positive" samples, a second round of measurement was performed with spiked human sera (confirmatory assay), to determine whether the initially detected binding of antibodies in the respective sera sample is specific to the test molecule. A decrease of the absorbance signal in the spiked wells indicates that the signal observed in the unspiked well of the initial screening assay is specific to the molecule coated on the plate. The resulting percent inhibition (% inhibition) required to confirm specificity was either set at 20 to 30% or a confirmatory cut-point was calculated in percent (%CCP). In the latter case, only samples where percent inhibition was above the CCP were confirmed positive. % inhibition was calculated as reduction of the initial signal obtained for unspiked serum (screening assay) as follows: $\%CCP = \text{initial signal} (1 - (\text{spiked serum}/\text{signal unspiked serum})) * 100$.

[0192] In an alternative procedure, the initial screening assay was not performed. Instead, the test samples were directly analysed using the confirmatory assay procedure. For data analysis however, the same calculations were performed, which at least involves the calculation of the SCP and the %CCP.

3.2. Determination/calculation of Screening Cut Point (SCP), Normalization Factor (NF) and Floating Cut Point (FCP)

[0193] For each test compound, 40 individual human serum samples of healthy untreated subjects were analyzed. In other cases 20 individual human serum samples of healthy untreated subjects were analyzed.

Screening Cut-Point (SCP):

[0194] The screening cut point (SCP) is the threshold at which a signal is considered positive (screening positive). It is calculated such that 5% false positive sera are included.

[0195] The screening cut point (SCP) is calculated as follows:

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$$\text{SCP} = \text{mean N} + 1.645 \times \text{SDN}$$

Wherein:

- "Mean N" corresponds to mean signal from all unspiked individual sera measured for a specific test compound;
- "SDN" corresponds to standard deviation calculated from all unspiked individual sera measured for a specific test compound.

Normalization factor (NF):

[0196] The normalization factor (NF) is calculated as follow:

$$\text{NF} = \text{SCP} - \text{Negative control mean}$$

Wherein:

- "SCP" is as defined above; and
- "negative control mean" corresponds to mean signal from the negative controls (pooled individual human sera, same on each plate) analyzed in duplicates (*i. e.* 2 wells) per plate.

[0197] At least two NC samples (*i. e.* 4 wells) must be taken into account for the calculation of the normalization factor.

Calculation of the Floating Cut Point (FCP):

[0198] After the determination of the SCP and the NF, the Floating Cut Point (FCP) for each plate was used as the reference cut point. The FCP takes into account the analytical variability of each analytical run, by normalizing the SCP with the negative controls of the plate. The FCP is calculated for each analytical run as follows:

$$\text{FCP} = \text{NF} + \text{Mean NC}$$

Wherein:

- "Mean NC" refers to the mean signal of the negative control samples;

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- "NF" refers to the normalization factor, as defined above.

3.3. Pre-existing ADA binding assay results for PRO1922 (MSLN binding scFv) and variants of PRO2230 (PDL1 binding scFv)

[0199] Seven PRO1922 variants and seven PRO2230 variants according to the present invention have been measured for their immunogenic properties using the pre-existing ADA binding assay described above. Two references, i. e. PRO1922-L12S-V103T-L144T (PRO2990) and PRO2230-L12S-V103T-L144T (PRO2984) have been analyzed as well. The measurements were directly performed in the confirmatory assay setup using 20 human serum samples.

[0200] The data were analyzed by calculating the SCP for each individual plate, as described above. The screening positives sera were then further analysed by taking into account a %CCP of 30%. The number of positive serum samples for the tested molecule are summarized in Table 14. Some exemplary graphs of absorption levels of pre-existing ADAs in human serum and of reduction of absorbance level of spiked human serum for PRO1922, PRO2230, PRO2922 and PRO2925 are shown in figure 1.

Table 14: Number of positive serum samples above 30 % CCP of PRO1922 (MSLN binding scFv) variants and of PRO2230 (PDL1 binding scFv):

PRO Number	Parental scFv	Substitutions	No of positive serum samples above 30 % CCP	% positives of total tested serum samples
PRO1922	-	-	16	80
PRO1922	-	-	16	80
PRO2230	-	-	18	90
PRO2230	-	-	15	76
PRO2922	PRO1922	L12R	2	10
PRO2907	PRO2230	L12R	3	15
PRO2920	PRO1922	L12R-L144Q	2	10
PRO2905	PRO2230	L12R-L144Q	7	20
PRO2919	PRO1922	L12R-V103T	1	5
PRO2904	PRO2230	L12R-V103T	12	60
PRO2918	PRO1922	L12R-V103T-L144Q	5	25
PRO2903	PRO2230	L12R-V103T-L144Q	6	30
PRO2925	PRO1922	L12S-V103T-L144Q	5	25
PRO2926	PRO2230	L12S-V103T-L144Q	8	40
PRO2924	PRO1922	L144Q	15	75

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PRO2909	PRO2230	L144Q	10	50
PRO2921	PRO1922	V103T-L144Q	11	55
PRO2906	PRO2230	V103T-L144Q	19	95
PRO2990	PRO1922	L12S-V103T-L144T	5	25
PRO2984	PRO2230	L12S-V103T-L144T	5	25

3.4. Pre-existing ADA binding assay results for NM21-1480 (PRO1480) variants:

[0201] Eight NM21-1480 variants including NM21-1480 (PRO1480) as reference, have been measured for their immunogenic properties using the pre-existing ADA binding assay described above. The measurements were directly performed in the confirmatory assay setup using 40 human serum samples.

[0202] The data were analyzed by calculating the SCP, as described above. The screening positives sera were then further analysed by taking into account a %CCP of 31.56%. The %CCP was calculated as described above using the data from all measurements. The number of positive serum samples for each tested molecule are summarized in Table 15. Some exemplary graphs of absorption levels of pre-existing ADAs in human serum for PRO1480 and PRO2764 are shown in figure 2.

Table 15: Number of positive serum samples above 31,56 % CCP of PRO1922 (MSLN binding scFv) variants and of PRO2230 (PDL1 binding scFv):

PRO Number	Binding domains with 12R, 103T, 144Q	No of positive serum samples above 31.56 % CCP	% positives of total tested serum samples
PRO1480	-	9	22.5
PRO2758	PDL1, CD137, hSA	2	5.0
PRO2759	PDL1	6	15.0
PRO2760	CD137	10	25.0
PRO2761	hSA	3	7.5
PRO2762	PDL1, CD137, hSA	3	7.5
PRO2763	PDL1, CD137, hSA	1	2.5
PRO2764	PDL1, CD137, hSA	1	2.5
PRO2765	PDL1, CD137, hSA	1	2.5

3.5. Pre-existing ADA binding assay results for NM28 variants:

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[0203] The NM28 variant PRO2741 and, as comparison, the corresponding unmodified reference PRO2660, have been measured for their immunogenic properties using the pre-existing ADA binding assay described above. The measurements were directly performed in the confirmatory assay setup using 20 human serum samples.

[0204] The screening positives sera were then further analysed by taking into account a %CCP of 30%. The number of positive serum samples for each tested molecule are summarized in Table 16. Graphs of absorption levels of pre-existing ADAs in human serum as well as the reduction of absorbance level of spiked human serum (ADA binding inhibition) for PRO2741 and the reference PRO2660 are shown in figure 3.

Table 16: Number of positive serum samples above 30 % CCP of PRO2741 and the reference PRO2660:

PRO Number	No of positive serum samples above 30 % CCP	% positives of total tested serum samples
PRO2741	3	15
PRO2660	6	30

3.6. Pre-existing ADA binding assay results for NM32 variants:

[0205] The NM32 variants PRO2668 and PRO2669 and, as comparison, the corresponding unmodified references PRO2510 and PRO2589, have been measured for their immunogenic properties using the pre-existing ADA binding assay described above. The measurements were directly performed in the confirmatory assay setup using 20 human serum samples (19 in case of PRO2589).

[0206] The screening positives sera were then further analysed by taking into account a %CCP of 30%. The number of positive serum samples for each tested molecule are summarized in Table 17. Graphs of absorption levels of pre-existing ADAs in human serum as well as the reduction of absorbance level of spiked human serum (ADA binding inhibition) for PRO2741 and the reference PRO2660 are shown in figure 4.

Table 17: Number of positive serum samples above 30 % CCP of PRO2668 and PRO2669 and the references PRO2510 and PRO2589:

PRO Number	No of positive serum samples above 30 % CCP	% positives of total tested serum samples
PRO2668	0	0

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PRO2510	3	15
2669	1	5
2589	10	52.6

CLAIMS

1. An antibody variable domain, which specifically binds to a target antigen, comprising:
 - 5 (i) a variable heavy chain (VH) comprising from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from a VH framework subtype, and wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
 - an arginine (R) at amino acid position 12;
 - a glutamine (Q) at amino acid position 144;
 - an arginine (R) at amino acid position 12 and a threonine (T) at amino acid position 103;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144;
 - a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144; or
 - an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
 - (ii) a variable light chain (VL), wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein
 - 25 a. said variable light chain framework regions LFW1, LFW2, LFW3 and LFW4 are selected from a human antibody V_k framework subtype; or
 - b. said variable light chain framework regions LFW1, LFW2 and LFW3 are selected from a human antibody V_k framework subtype, and said variable light chain framework region LFW4 is selected from a V_λ framework subtype.
2. The antibody variable domain of claim 1, wherein said HFW1, HFW2, HFW3 and HFW4 have one of the following substitutions (AHo numbering):
 - an arginine (R) at amino acid position 12;
 - an arginine (R) at amino acid position 12 and a (Q) at amino acid position 144; or

- an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;
particularly wherein said HFW1, HFW2, HFW3 and HFW4 have the following substitutions (Aho numbering): an arginine (R) at amino acid position 12, a threonine (T) at amino acid position 103, and a glutamine (Q) at amino acid position 144.
3. The antibody variable domain of any one of claims 1 or 2, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from the VH framework subtypes VH1a, VH1b, VH3 or VH4, in particular from the VH framework subtype VH3; and wherein the human antibody Vk framework subtype is selected from the Vk1 framework subtype.
 4. The antibody variable domain of any one of the preceding claims, wherein said variable heavy chain framework regions HFW1, HFW2, HFW3 and HFW4 are selected from
 - a. the framework regions (*i. e.* the non-italicized residues in Tables 1, 2 and 4) of any one of the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117; and
 - b. the framework regions (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 3, 4, 8, 9, 13, 14, 19, 20, 25, 26, 31, 32, 35, 36, 37, 42, 43, 44, 60, 63, 71, 72, 76, 77, 81, 95, 96, 99, 100, 116 and 117 having 1, 2 or 3 mutations within the framework region at positions different from 12, 103 and 144 (Aho numbering);
 and wherein said variable light chain framework regions LFW1, LFW2 and LFW3, and also LFW4, if LFW4 is selected from a human antibody Vk framework subtype, are selected from
 - a. the framework regions (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119; and
 - b. the framework regions (*i. e.* the non-italicized residues in Table 1, 2 and 4) of any one of the SEQ ID NOs: 5, 10, 15, 16, 21, 22, 27, 28, 33, 34, 38, 39, 40, 41, 45, 46, 47, 48, 61, 64, 73, 74, 78, 79, 82, 97, 98, 101, 102, 118 and 119 having 1, 2 or 3 mutations within the framework region.
 5. The antibody variable domain of any one of the preceding claims, wherein said variable light chain framework region LFW4 is selected from a Vλ framework subtype, particularly

- has one of the sequences selected from the group consisting of SEQ ID NOs: 123, 124, 125, 126, 127, 128, 129, 130 and 131.
- 5 6. An antibody comprising one or more antibody variable domains as defined in any one of claims 1 to 5.
7. The antibody of claim 6, wherein the antibody is
- 10 (i) monospecific and monovalent, bivalent or trivalent for its target antigen;
- (ii) bispecific and, independently of each other, monovalent or bivalent for each target antigen;
- 15 (iii) trispecific and monovalent for each target antigen;
- (iv) trispecific and bivalent for one of the target antigens and monovalent for the other target antigens;
- (v) trispecific and bivalent for two of the target antigens and monovalent for the third target antigen;
- 20 (vi) tetraspecific and monovalent for each target antigen;
- (vii) tetraspecific and bivalent for one of the target antigens and monovalent for the other target antigens; or
- (viii) tetraspecific and bivalent for two of the target antigens and monovalent for the other target antigens.
8. The antibody of any one of claims 6 or 7, wherein the format of said antibody is selected from bivalent bispecific IgG formats, trivalent bispecific IgG formats and tetravalent bispecific IgG formats;
- 25 more particularly wherein the format of said antibody is selected from KiH-based IgGs; DVD-Ig; CODV-IgG and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)), even more particularly from DVD-Ig and Morrison (IgG CH₃-scFv fusion (Morrison-H) or IgG CL-scFv fusion (Morrison-L)).
- 30 9. The antibody of any one of claims 6 or 7, wherein said antibody does not comprise an immunoglobulin Fc region, and wherein said antibody does further not comprise CH1 and/or CL regions, particularly wherein said antibody is in a scDb-scFv, a triabody, a tetrabody or a MATCH format, more particularly wherein said antibody is in a MATCH or scDb-scFv format, more particularly wherein said antibody is in a MATCH format, more particularly a MATCH3 or a MATCH4 format.
- 35

10. The antibody of claim 9, wherein the antibody is trispecific and monovalent for each target antigen, and wherein the antibody comprises:
- 1) one binding domain, which specifically binds to CD137 (CD137-BD) comprising
 - a) a VH sequence of SEQ ID NO: 1 and a VL sequence of SEQ ID NO: 5;
 - b) a VH sequence of SEQ ID NO: 2 and a VL sequence of SEQ ID NO: 5;
 - c) a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5; or
 - d) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;
 - 2) one binding domain, which specifically binds to PDL1 (PDL1-BD) comprising
 - a) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 15;
 - b) a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 16;
 - c) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15; or
 - d) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;
 - 3) one human serum albumin binding domain (hSA-BD) comprising
 - a) a VH sequence of SEQ ID NO: 23 and a VL sequence of SEQ ID NO: 27;
 - b) a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 28;
 - c) a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27; or
 - d) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;
- with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d);
- or wherein the antibody comprises:
- 1) one binding domain, which specifically binds to CD137 (CD137-BD) comprising
 - a) a VH sequence of SEQ ID NO: 6 and a VL sequence of SEQ ID NO: 10;
 - b) a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 10;
 - c) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
 - d) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10;
 - 2) one binding domain, which specifically binds to PDL1 (PDL1-BD) comprising
 - a) a VH sequence of SEQ ID NO: 17 and a VL sequence of SEQ ID NO: 21;
 - b) a VH sequence of SEQ ID NO: 18 and a VL sequence of SEQ ID NO: 22;
 - c) a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
 - d) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22; and
 - 3) one human serum albumin binding domain (hSA-BD) comprising
 - a) a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 33;
 - b) a VH sequence of SEQ ID NO: 30 and a VL sequence of SEQ ID NO: 34;
 - c) a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
 - d) a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34;

with the proviso that at least one of the three binding domains comprises VH/VL sequence pairs selected from c) or d).

11. An antibody variable domain as defined in any one of the claims 1 to 5, wherein said antibody variable domain specifically binds to CD137, and comprises:
- a) a VH sequence of SEQ ID NO: 3 and a VL sequence of SEQ ID NO: 5;
 - b) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 5;
 - c) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 10; or
 - d) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10;
- or
- an antibody variable domain as defined in any one of the claims 1 to 5, wherein said antibody variable domain specifically binds to PDL1, and comprises:
- a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 15;
 - b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 16;
 - c) a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 21; or
 - d) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22;
- or
- an antibody variable domain as defined in any one of the claims 1 to 5, wherein said antibody variable domain specifically binds to human serum albumin, and comprises:
- a) a VH sequence of SEQ ID NO: 25 and a VL sequence of SEQ ID NO: 27;
 - b) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 28;
 - c) a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 33; or
 - d) a VH sequence of SEQ ID NO: 32 and a VL sequence of SEQ ID NO: 34;
- or
- an antibody variable domain as defined in any one of the claims 1 to 5, wherein said antibody variable domain specifically binds to human serum albumin, and comprises:
- a) a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
 - b) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 39;
 - c) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
 - d) a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 40;
 - e) a VH sequence of SEQ ID NO: 42 and a VL sequence of SEQ ID NO: 45;
 - f) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 46;
 - g) a VH sequence of SEQ ID NO: 43 and a VL sequence of SEQ ID NO: 48; or
 - h) a VH sequence of SEQ ID NO: 44 and a VL sequence of SEQ ID NO: 47.

12. A nucleic acid or two nucleic acids encoding the antibody variable domain of any one of claims 1 to 5 or 11, or the antibody of any one of claims 6 to 10.
13. A vector or two vectors comprising the nucleic acid or the two nucleic acids of claim 12.
- 5 14. A host cell or host cells comprising the vector or the two vectors of claim 13.
15. A method for producing the antibody variable domain of any one of claims 1 to 5 or 11, or the antibody of any one of claims 6 to 10, comprising (i) providing the nucleic acid or
10 the two nucleic acids of claim 12, or the vector or the two vectors of claim 13, expressing said nucleic acid sequence or nucleic acids, or said vector or vectors, and collecting said antibody variable domain or said antibody from the expression system, or (ii) providing a host cell or host cells of claim 14, culturing said host cell or said host cells; and collecting said antibody variable domain or said antibody from the cell culture.
- 15 16. A pharmaceutical composition comprising the antibody of any one of claims 6 to 10 and a pharmaceutically acceptable carrier.