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(54) **Title:** FC-FREE ANTIBODIES COMPRISING TWO FAB FRAGMENTS AND METHODS OF USE

(57) **Abstract:** The present invention relates to bispecific antibodies comprising at least two fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein either the variable regions or the constant regions of the second Fab heavy and light chain are exchanged; and wherein the bispecific antibody is devoid of a Fc domain; methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.



FC-FREE ANTIBODIES COMPRISING TWO FAB FRAGMENTS AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to bispecific antibodies comprising at least two fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein either the variable regions or the constant regions of the second Fab heavy and light chain are exchanged; and wherein the bispecific antibody is devoid of a Fc domain; methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

BACKGROUND

Monoclonal antibodies (mAbs) are an increasingly important class of therapeutic agents. Apart from mAb products composed of the full-size form of IgG, a wide variety of multispecific recombinant antibody formats have been developed, e.g. tetravalent bispecific antibodies by fusion of, e.g., an IgG antibody format and single chain domains (see e.g. Coloma, M.J., et al., Nature Biotech 15 (1997) 159-163; WO 2001/077342; and Morrison, S.L., Nature Biotech 25 (2007) 1233-1234).

Several other new formats wherein the antibody core structure (IgA, IgD, IgE, IgG or IgM) is no longer retained such as dia-, tria- or tetrabodies, minibodies, several single chain formats (scFv, Bis-scFv), which are capable of binding two or more antigens, have been developed (Holliger, P., et al., Nature Biotech 23 (2005) 1126-1136; Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14; Shen, J., et al., Journal of Immunological Methods 318 (2007) 65-74; Wu, C., et al., Nature Biotech. 25 (2007) 1290-1297).

All such formats use linkers either to fuse the antibody core (IgA, IgD, IgE, IgG or IgM) to a further binding protein (e.g. scFv) or to fuse e.g. two Fab fragments or scFvs (Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14). Tandem scFVs are two scFv fragments linked by an extra peptide linker and are also referred to as (scFv)₂. By reducing the length of the peptide linker between the variable domains, Diabodies are generated. Adding an extra peptide linker between the two polypeptides yields so called single chain diabodies. US 2007/0274985 relates to antibodies comprising single chain Fab (scFab) fragments. Antibody fragments have both pros and cons as therapeutics compared with full-size monoclonal antibodies: One advantage is that

they are smaller and penetrate tissues and tumors more rapidly. In addition, the small size of fragments has been suggested to permit binding to epitopes not accessible to full-sized monoclonal antibodies. On the downside, fragments demonstrate short circulating half-lives in humans, likely due to kidney clearance. The shorter half-life may prevent sufficient
5 accumulation of therapy at the targeted site. Production of antibody fragments is not trivial, as fragments are likely to form aggregates and can be less stable than full-size monoclonal antibodies. In addition, unwanted pairing of noncongnate heavy and light chains results in formation of inactive antigen-binding sites and/or other non-functional undesired side-products, which is a major problem in clinical-scale production and therapeutic application of antibody
10 fragments. In particular Tandem-Fab constructs where two or more Fabs are fused with each other via one connector are not feasible due to the random association of the two light chains resulting in inactive, undesired side products. These drawbacks have now been overcome with the new antibody format of the invention. Provided therein is a new bispecific antibody format that can be easily produced with an increased yield due to a decreased amount of mispaired side-
15 products, which show less aggregation than bispecific antibody fragments known in the art. . Using the crossover approach correct LC association can be enforced without the need for the generation of a common light chain. Common light chain approach is not possible for existing antibodies. In addition, the new bispecific antibody format has a higher molecular weight compared to many conventional bispecific antibody fragments, thus preventing excessive kidney
20 clearance and leading to an improved half-life in vivo. The new bispecific antibody format is fully functional and has comparable or improved binding and activity as corresponding conventional bispecific antibodies.

SUMMARY

25 The present invention relates to bispecific antibodies comprising at least two Fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen wherein either the variable regions or the constant regions of the second Fab heavy and light chain are exchanged; and wherein the bispecific antibody is devoid of a Fc
30 domain. In one embodiment the first and second Fab fragments are connected via a peptide linker. Preferably said peptide linker is a (G4S)₂ linker.

In one embodiment said antibody additionally comprises a third Fab fragment. In another embodiment said third Fab fragment comprises at least one antigen binding site specific for the first or second antigen, preferably for the first antigen.

In one embodiment the third Fab fragment is connected to the light chain or the heavy chain of the first Fab fragment. In another embodiment the third Fab fragment is connected to N or C-terminus of the light chain or the heavy chain of the second Fab fragment. In one embodiment the third Fab fragment is connected to the first or second Fab fragment via a peptide linker. Preferably said peptide linker is a (G4S)₂ linker.

The bispecific antibodies according to the invention are at least bivalent and can be trivalent or multivalent e.g. tetravalent. In one embodiment said bispecific antibodies are bivalent (1+1 format) with one binding site each targeting a first antigen and a second antigen, respectively. In another embodiment said bispecific antibodies are trivalent (2+1 format) with two binding sites each targeting a first antigen and one binding site targeting a second antigen, as detailed in the following section.

In a second object the present invention relates to a pharmaceutical composition comprising a bispecific antibody of the present invention.

In a third object the present invention relates to a bispecific antibody of the present invention for the treatment of cancer. In another embodiment, use of the bispecific antibody as a medicament is provided. Preferably said use is for the treatment of cancer.

In further objects the present invention relates to a nucleic acid sequence comprising a sequence encoding a heavy chain of a bispecific antibody of the present invention, a nucleic acid sequence comprising a sequence encoding a light chain of a bispecific antibody of the present invention, an expression vector comprising a nucleic acid sequence of the present invention and to a prokaryotic or eukaryotic host cell comprising a vector of the present invention. In addition a method of producing an antibody comprising culturing the host cell so that the antibody is produced is provided.

In a further embodiment an immunoconjugate comprising the bispecific antibody of the invention and a cytotoxic agent is provided.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Schematic illustration of exemplary bispecific antibody formats of the invention. a) Fab-Crossfab molecule C-terminal, b) Fab-Crossfab molecule N-terminal c) (Fab)₂-Crossfab molecule C-terminal d) (Fab)₂-Crossfab molecule N-terminal e) Fab-Crossfab-Fab molecule.

Figure 2: Analysis of hu Fab(MCSP)-Crossfab(CD3) production and purification: SDS-Page: 4-12 % Bis/Tris (NuPage [invitrogen]; coomassie stained): a) 1 – Mark 12 (invitrogen),

2 – hu Fab(MCSP)-Crossfab(CD3) non reduced; b) 1 – Mark 12 (invitrogen), 2 – hu Fab(MCSP)-Crossfab(CD3) reduced.

Figure 3: Analysis Fab(MCSP)-Crossfab(CD3) production and purification. Analytical size exclusion chromatography, Chromatogram A280 (Superdex 200 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 µg sample were injected).

Figure 4: Analysis of hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) production and purification: SDS-Page: 4-12 % Bis/Tris (NuPage [invitrogen]; coomassie stained): a) 1 – Mark 12 (invitrogen), 2 – hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) non reduced; b) 1 – Mark 12 (invitrogen), 2 – hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) reduced.

Figure 5: Analysis of hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) production and purification. Analytical size exclusion chromatography, Chromatogram A280 (Superdex 200 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 µg sample were injected).

Figure 6: Analysis of hu Fab(MCSP)- Crossfab(CD3) -Fab(MCSP) production and purification. SDS-Page: 4-12 % Bis/Tris (NuPage [invitrogen]; coomassie stained): a) 1 – Mark 12 (invitrogen), 2 – hu Fab(MCSP)- Crossfab(CD3) -Fab(MCSP) non reduced; b) 1 – Mark 12 (invitrogen), 2 – hu Fab(MCSP)- Crossfab(CD3) -Fab(MCSP) reduced.

Figure 7: Analysis of hu Fab(MCSP)- Crossfab(CD3) -Fab(MCSP) production and purification. Analytical size exclusion chromatography, Chromatogram A280 (Superdex 200 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 µg sample were injected).

Figure 8: Analysis of murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) production and purification. SDS-Page: 4-12 % Bis/Tris (NuPage [invitrogen]; coomassie stained): a) 1 – Mark 12 (invitrogen), 2 – murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) non reduced; b) 1 – Mark 12 (invitrogen), 2 – murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) reduced.

Figure 9: Analysis of murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) production and purification. Analytical size exclusion chromatography, Chromatogram A280 (Superdex 200 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 µg sample were injected).

Figure 10: Killing (as measured by LDH release) of MDA-MB-435 tumor cells upon co-culture with human pan T cells (E:T ratio = 5:1) and activation for 20 hours by different concentrations of the hu Fab(MCSP)-Crossfab(CD3) (=“Fab-Crossfab”), hu Fab(MCSP)-

Crossfab(CD3) -Fab(MCSP) (= "Fab-Crossfab-Fab"), hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) (= "(Fab)2-Crossfab"), as well as the (scFv)₂ (antiMCSP/anti huCD3e) (= "(scFv)₂") bispecific molecules. The constructs with bivalent MCSP-targeting show comparable cytotoxic activity compared to the "(scFv)₂" construct, whereas the "Fab-Crossfab" construct with monovalent MCSP binding is clearly less potent.

Figure 11: Comparison of the hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) (= "(Fab)2-Crossfab") and the (scFv)₂ (antiMCSP/anti huCD3e) (= "(scFv)₂") construct, Depicted is the LDH release from MDA-MB-435 tumor cells upon co-culture with human pan T cells (E/T ratio = 5:1), and activation for 21 hours by different concentrations of the bispecific constructs and corresponding IgGs. The "(Fab)2-Crossfab" induces apoptosis in target cells at least comparably good as the (scFv)₂ molecule.

Figure 12: Comparison of the hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) (= "(Fab)2-Crossfab") and the (scFv)₂ (antiMCSP/anti huCD3e) (= "(scFv)₂") construct. Depicted is the LDH release from MV-3 human melanoma tumor cells upon co-culture with human PBMCs (E/T ratio = 10:1), and activation for 26 hours by different concentrations of the bispecific constructs and corresponding IgGs. The "(Fab)2-Crossfab" induces apoptosis in target cells at least comparably good as the (scFv)₂ molecule.

Figure 13: LDH release from B16/F10-huMCSP Fluc2, clone 48 tumor cells, induced by primary murine T cell activation with the murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) construct (= (Fab)2-CrossFab), targeting human MCSP, as well as the murine CD3. The effector to target cell ratio was 5:1. The assay was analyzed after incubation for 23.5 hours at 37°C, 5 % CO₂. The construct induces concentration-dependent, T cell-mediated apoptosis of human MCSP-expressing target cells.

Figure 14: LDH release from B16/F10-huMCSP Fluc2, clone 48 tumor cells, induced by primary murine T cell activation with 50 nM of the murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) construct (= (Fab)2-CrossFab), targeting human MCSP, as well as the murine CD3. The effector to target cell ratio was 5:1. The assay was analyzed after incubation for 23.5 hours at 37°C, 5 % CO₂. The construct induces T cell-mediated apoptosis of human MCSP-expressing target cells. There is only weak hyperactivation of T cells at this concentration of the construct.

Figure 15: Different cytokine levels measured in the supernatant of whole blood after treatment with 1 nM of different CD3-MCSP bispecific constructs (hu Fab(MCSP)-Fab(MCSP)-Crossfab(CD3) (= "(Fab)2-Crossfab") and (scFv)₂ (antiMCSP/anti huCD3e) (= "(scFv)₂") in the presence (A, B) or absence (C, D) of Colo-38 tumor cells for 24 hours. 280 µl whole blood

were plated per well of a 96-well plate and 30 000 Colo-38 cells added, as indicated. The main cytokine that was secreted upon activation of T cells in the presence of Colo-38 tumor cells, is IL-6, followed by IFN γ . In addition, also the levels of granzyme B increased enormously upon activation of T cells in the presence of target cells. In general, the "(scFv)²" construct elevated the levels of TNF and IFN γ , as well as granzyme B in the presence of target cells (A and B) a bit more compared to the other bispecific construct.

There was no significant secretion of Th2 cytokines (IL-10 and IL-4) upon activation of T cells by the bispecific constructs in the presence (or absence) of target cells. In this assay there was also a weak secretion of IFN γ , induced by the "(Fab)₂-Crossfab" construct in the absence of target cells.

Figure 16: Surface expression level of the late activation marker CD25 on murine pan T cells, isolated from splenocytes. Murine pan T cells were incubated with 50 nM of the murine Crossfab(CD3) -Fab(MCSP)-Fab(MCSP) construct (= (Fab)₂-CrossFab) bispecific construct (targeting murine CD3, as well as human MCSP), in the presence or absence of B16/F10-huMCSP Fluc2 clone 48 tumor target cells, as indicated (E:T ratio is 10:1). Depicted is the expression level of the late activation marker CD25 on CD8⁺ T cells after 70 hours. Up-regulation of CD25 on CD8⁺ T cells with the (Fab)₂-CrossFab construct occurs only in the presence of target cells. The reference IgGs, used adjusted to the same molarity, were not able to up-regulate CD25.

Figure 17: Analysis of Fab(CD33)-CrossFab (CD3) production and purification. SDS-Page: a) 3-8% Tris/Acetate (NuPage [invitrogen]; coomassie stained): a) 1 – HiMark (invitrogen), 2 – Fab(CD33)-CrossFab (CD3) non reduced; b) 4-12 % Bis/Tris (NuPage [invitrogen]: 1 – Mark 12 (invitrogen), 2 – Fab(CD33)-CrossFab (CD3) reduced.

Figure 18: Analysis of Fab(CD33)-CrossFab (CD3) production and purification.

Analytical size exclusion chromatography, Chromatogram A280 (Superdex 200 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 μ g sample were injected).

Figure 19: Killing (as measured by LDH release) of MV-3 tumor cells upon co-culture with human PBMCs (E:T ratio = 10:1) and activation for 24 hours by different concentrations of CD3-MCSP bispecific constructs (hu Fab(MCSP)-Crossfab(CD3); designated as "1+1 non-Fc", and the (scFv)₂ (antiMCSP/anti huCD3e) (= "(scFv)²") reference molecule). The "1+1 non-Fc" construct induces apoptosis in MV-3 target cells with a calculated EC₅₀ of 25.4 pM, whereas the

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calculated EC50 for the “(scFv)2” reference molecule is 57 pM, showing a slight better potency of the “1+1 non-Fc” molecule in terms of EC50.

Figure 20: Activation of CD4+ or CD8+ T cells, as measured by up-regulation of CD69 (A), respective increase of CD69-positive cells (B) in the presence of huMCSP-positive MV-3 tumor cells upon co-culture with human PBMCs (E:T ratio = 10:1), treated with the CD3-MCSP bispecific constructs (hu Fab(MCSP)-Crossfab(CD3); designated as “1+1 non-Fc”, and the (scFv)2 (antiMCSP/anti huCD3e) (“(scFv)2”) reference molecule, respectively) for ~24 hours. In general, the CD69 median values are higher on CD8+ T cells compared to CD4+ T cells. There is a clear concentration-dependent increase in both, CD69 median values, as well percentage of CD69 positive cells for both constructs.

Figure 21: Illustration of (scFv)2 reference molecule.

Figure 22: Analysis of (scFv)2 (antiMCSP/anti huCD3e) production and purification. SDS-Page: 4-12 % Bis/Tris (NuPage [Invitrogen]; coomassie stained): 1 – Mark 12 (Invitrogen), 2 – (scFv)2 (antiMCSP/anti huCD3e) reduced; 3 – (scFv)2 (antiMCSP/anti huCD3e) non reduced

Figure 23: Analysis of (scFv)2 (antiMCSP/anti huCD3e) production and purification. Analytical size exclusion chromatography, Chromatogram A280 (Superdex 75 10/300 GL [GE Healthcare]; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02 % (w/v) NaCl; 50 µg sample ((scFv)2 (antiMCSP/anti huCD3e)) were injected).

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some

embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

5 A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication
10 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally
15 defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1),
20 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) The
25 terms hypervariable regions (HVRs) and complementarity determining regions (CDRs), are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), where the definitions include overlapping or subsets of
30 amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a

comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions¹

CDR	Kabat	Chothia	AbM ²
V _H CDR1	31-35	26-32	26-35
V _H CDR2	50-65	52-58	50-58
V _H CDR3	95-102	95-102	95-102
V _L CDR1	24-34	26-32	24-34
V _L CDR2	50-56	50-52	50-56
V _L CDR3	89-97	91-96	89-97

¹ Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

² "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat *et al.* also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

With the exception of CDR1 in V_H, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they

exhibit the desired antigen-binding activity. In particular the term "antibody" also includes the bispecific antibodies of the invention comprising at least two fab fragments but no Fc domain.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as a NS0 or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant human antibodies have variable and constant regions in a rearranged form. The recombinant human antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germ line VH and VL sequences, may not naturally exist within the human antibody germ line repertoire in vivo.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Other forms of "humanized antibodies" encompassed by the present invention are those in which the constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a murine variable region and a human

constant region are preferred. Other preferred forms of "chimeric antibodies" encompassed by the present invention are those in which the constant region has been modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding. Such chimeric antibodies are also referred to as "class-switched antibodies.". Chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding immunoglobulin variable regions and DNA segments encoding immunoglobulin constant regions. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art. See e.g. Morrison, S.L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855; US Patent Nos. 5,202,238 and 5,204,244.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g. described in Houston, J.S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together

with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

As used herein, "Fab fragment" refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL), and a VH domain and a first constant domain (CH1) of a heavy chain. The bispecific antibodies of the invention comprise at least two Fab fragments, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged. Due to the exchange of either the variable regions or the constant regions, said second Fab fragment is also referred to as "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment". Two different chain compositions of a crossover Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the variable regions of the Fab heavy and light chain are exchanged, i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab_(VLVH). On the other hand, when the constant regions of the Fab heavy and light chain are exchanged, the crossover Fab molecule comprises a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). This crossover Fab molecule is also referred to as CrossFab_(CLCH1).

In one embodiment said Fab fragments are connected via a peptide linker. By "connected" is meant that the Fab fragments are linked by peptide bonds, either directly or via one or more peptide linker.

The term "peptide linker" as used within the invention denotes a peptide with amino acid sequences, which is preferably of synthetic origin. These peptide linkers according to invention are used to connect one of the Fab fragments to the C-or N-terminus of the other Fab fragment to form a multispecific antibody according to the invention. Preferably said peptide linkers are peptides with an amino acid sequence with a length of at least 5 amino acids, preferably with a length of 5 to 100, more preferably of 10 to 50 amino acids. In one embodiment said peptide linker is (GxS)_n or (GxS)_nG_m with G = glycine, S = serine, and (x = 3, n= 3, 4, 5 or 6, and m= 0, 1, 2 or 3) or (x = 4, n= 2, 3, 4 or 5 and m= 0, 1, 2 or 3), preferably x = 4 and n= 2 or 3, more preferably with x = 4, n= 2. Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. In one embodiment said peptide linker is (G₄S)₂ (SEQ ID: NO 28). Other peptide linkers suitable for connecting the Fab

fragments, for example, (G₄S)₆-GG (SEQ ID NO: 147) or (SG₃)₂-(SEG₃)₄-(SG₃)-SG (SEQ ID NO: 148), or EPKSC(D)-(G₄S)₂ (SEQ ID NOs 145 and 146).

The term "antigen binding domain" refers to the part of an antigen binding molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "antigen-binding site of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding portion of an antibody comprises amino acid residues from the "complementary determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chain variable domains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding and defines the antibody's properties. CDR and FR regions are determined according to the standard definition of Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and/or those residues from a "hypervariable loop".

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in

certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

The term “Fc domain” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. For example in natural
5 antibodies, the Fc domain is composed of two identical protein fragments, derived from the second and third constant domains of the antibody's two heavy chains in IgG, IgA and IgD isotypes; IgM and IgE Fc domains contain three heavy chain constant domains (C_H domains 2–4) in each polypeptide chain. The bispecific antibodies of the invention are devoid of the Fc domain. “Devoid of the Fc domain” as used herein means that the bispecific antibodies of the
10 invention do not comprise a CH_2 , CH_3 or CH_4 domain; i.e. the constant heavy chain consists solely of one or more CH_1 domains.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity
15 which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and 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. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

20 As used herein, the term “binding” or “specifically binding” means that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed on a
25 BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain embodiments, an antigen binding moiety that binds to the antigen, or an antigen binding molecule comprising that
30 antigen binding moiety, has a dissociation constant (K_D) of $\leq 1 \mu M$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

An “affinity matured” antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

5 In one embodiment, the extent of binding of a bispecific antibody that specifically binds a first antigen and a second antigen to an unrelated, protein is less than about 10% of the binding of the antibody to the first or second antigen as measured, e.g., by a radioimmunoassay (RIA) or flow cytometry (FACS). In certain embodiments, a bispecific antibody that specifically binds a first antigen and a second antigen has a dissociation constant (KD) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, ≤ 10
10 nM , $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, a bispecific antibody that specifically binds a first antigen and a second antigen binds to an epitope of the first antigen or the second antigen that is conserved among the first or second antigen from different species.

Antibody specificity refers to selective recognition of the antibody for a particular
15 epitope of an antigen. Natural antibodies, for example, are monospecific. “Bispecific antibodies” according to the invention are antibodies which have two different antigen-binding specificities. Antibodies of the present invention are specific for two different antigens, i.e. for a first antigen and a second antigen.

The term “monospecific” antibody as used herein denotes an antibody that has one or
20 more binding sites each of which bind to the same epitope of the same antigen.

The term “bispecific” antibody as used herein denotes an antibody that has at least two binding sites each of which bind to different epitopes of the same antigen or a different antigen.

The antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two
25 different sites. Provided herein is a bispecific antibody, with binding specificities for a first antigen and a second antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of a first antigen or a second antigen.

Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a first antigen or a second antigen.

30

The term “valent” as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. As such, the terms “bivalent”, “tetravalent”, and “hexavalent” denote the presence of two binding sites, four binding sites, and

six binding sites, respectively, in an antibody molecule. The bispecific antibodies according to the invention are at least “bivalent” and may be “trivalent” or “multivalent” (e.g. “tetravalent” or “hexavalent”).

Antibodies of the present invention have two or more binding sites and are bispecific.

- 5 That is, the antibodies may be bispecific even in cases where there are more than two binding sites (i.e. that the antibody is trivalent or multivalent).

An “antibody that binds to the same epitope” as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its
10 antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

“No substantial cross-reactivity” means that a molecule (e.g., an antibody) does not recognize or specifically bind an antigen different from the actual target antigen of the molecule (e.g. an antigen closely related to the target antigen), particularly when compared to that target
15 antigen. For example, an antibody may bind less than about 10% to less than about 5% to an antigen different from the actual target antigen, or may bind said antigen different from the actual target antigen at an amount selected from the group consisting of less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1%, preferably less than about 2%, 1%, or 0.5%, and most preferably less than about 0.2% or 0.1% antigen different from the actual
20 target antigen.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity,
25 and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to
30 achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user

documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

“Isolated nucleic acid encoding a bispecific antibody of the invention” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

5 The term “amino acid” as used within this application denotes the group of naturally occurring carboxy α -amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S),
10 threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

 The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of
15 nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

 As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transfectants” and “transfected cells” include the primary subject cell and cultures derived there from without
20 regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

 The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably
25 and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as
30 screened or selected for in the originally transformed cell are included herein.

 A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

The term “N-terminus” denotes the last amino acid of the N-terminus, the term “C-terminus” denotes the last amino acid of the C-terminus.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "cancer" as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

II. COMPOSITIONS AND METHODS

A. Exemplary bispecific antibodies

The present invention relates to bispecific antibodies comprising at least two Fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged; and wherein the bispecific antibody is devoid of a Fc domain. In one embodiment the first and second Fab fragments are connected via a peptide linker. Preferably said peptide linker is a peptide with an amino acid sequence with a length of at least 5 amino acids, preferably with a length of 5 to 100, more preferably of 10 to 50

amino acids. In one embodiment said peptide linker is $(GxS)_n$ or $(GxS)_nGm$ with G = glycine, S = serine, and $(x = 3, n = 3, 4, 5 \text{ or } 6, \text{ and } m = 0, 1, 2 \text{ or } 3)$ or $(x = 4, n = 2, 3, 4 \text{ or } 5 \text{ and } m = 0, 1, 2 \text{ or } 3)$, preferably $x = 4$ and $n = 2$ or 3 , more preferably with $x = 4, n = 2$. In one embodiment said peptide linker is $(G_4S)_2$. The peptide linker is used to connect the first and the second Fab
 5 fragment. In one embodiment the first Fab fragment is connected to the C- or N- terminus of the second Fab fragment.

In one embodiment the first Fab fragment is connected to the N-terminus of the second Fab fragment. Depending on whether the variable or the constant domains of the heavy and the light chains of the second Fab fragment are exchanged, different bispecific antibody molecules
 10 are possible when the first Fab fragment is connected to the N-terminus of the second Fab fragment.

In one embodiment the variable domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab $_{(VHVL)}$), and the C-terminus of the heavy or light chain of the first Fab fragment is connected to the N-terminus of the VLCH1 chain of the second Fab
 15 fragment. Preferably, the C-terminus heavy chain of the first Fab fragment is connected to the N-terminus of the VLCH1 chain of the second Fab fragment. Thus, in one embodiment the bispecific antibody comprises three chains: a light chain (VLCL) of the first Fab fragment, the heavy chain of the first Fab fragment connected to the VLCH1 chain of the second Fab fragment via a peptide linker (VHCH1-linker-VLCH1) and a VHCL chain of the second Fab fragment.

In another embodiment the constant domains of the second Fab fragment are exchanged (i.e the second Fab fragment is a CrossFab $_{(CLCH1)}$) and the C-terminus of the heavy or light chain of the first Fab fragment is connected to the N-terminus of the VHCL chain of the second Fab
 20 fragment. Preferably, the C-terminus of the heavy chain of the first Fab fragment is connected to the N-terminus of the VHCL chain of the second Fab fragment. Thus, in one embodiment the bispecific antibody comprises three chains: a light chain (VLCL) of the first Fab fragment, the heavy chain of the first Fab fragment connected to the VHCL chain of the second Fab fragment
 25 via a peptide linker (VHCH1-linker-VHCL) and a VLCH1 chain of the second Fab fragment.

In one embodiment the first Fab fragment is connected to the C-terminus of the second Fab fragment. Depending on whether the variable or the constant domains of the heavy and the
 30 light chains of the second Fab fragment are exchanged different bispecific antibody molecules are possible when the first Fab fragment is connected to the C-terminus of the second Fab fragment.

In one embodiment the variable domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab $_{(VHVL)}$), and the CH1 domain of the second Fab fragment
 35 is connected to the N-terminus of the heavy or light chain of the first Fab fragment. Preferably,

the CH1 domain of the second Fab fragment is connected to the N-terminus of the heavy chain of the first Fab fragment. Thus, in one embodiment the bispecific antibody comprises three chains: a light chain (VLCL) of the first Fab fragment, the VLCH1 chain of the second Fab fragment connected to the heavy chain of the first Fab fragment via a peptide linker (VLCH1-linker-VHCH1) and a VHCL chain of the second Fab fragment.

In another embodiment the constant domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(CLCH1)), and the CL domain of the second Fab fragment is connected to the N-terminus of the heavy of light chain of the first Fab fragment. Preferably, the CL domain of the second Fab fragment is connected to the N-terminus of the heavy chain of the first Fab fragment. Thus, in one embodiment the bispecific antibody comprises three chains: a light chain (VLCL) of the first Fab fragment, the VHCL chain of the second Fab fragment connected to the heavy chain of the first Fab fragment via a peptide linker (VLCH1-linker-VHCH1) and a VLCH1 chain of the second Fab fragment.

The bispecific antibodies according to the invention are at least bivalent and can be trivalent or multivalent e.g. tetravalent. In one embodiment said bispecific antibodies are bivalent (1+1 format) with one binding site each targeting a first antigen and a second antigen, respectively. In another embodiment said bispecific antibodies are trivalent (2+1 format) with two binding sites each targeting a first antigen and one binding site targeting a second antigen, as detailed in the following section.

In one embodiment said antibody additionally comprises a third Fab fragment. In another embodiment said third Fab fragment comprises at least one antigen binding site specific for the first or second antigen, preferably for the first antigen.

In one embodiment the third Fab fragment is connected to the N or C-terminus of the first Fab fragment. In one embodiment the third Fab fragment is connected to the first Fab fragment via a peptide linker. Preferably said peptide linker is a (G4S)₂ linker.

In one embodiment the third Fab fragment is connected to the N or C-terminus of the light chain or the heavy chain of the first Fab fragment. Depending on which terminus of the first Fab fragment is connected to the second Fab fragment (as detailed above), the third Fab fragment is connected on the opposite (free) terminus of the first fragment.

In one embodiment, the bispecific antibody of the invention comprises three Fab fragments wherein said Fab fragments and said linker are connected in the following order from N-terminal to C-terminal direction: Fab fragment 3- linker- Fab fragment 1- linker- Fab fragment 2, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged. In this embodiment the C-terminus of the third Fab

fragment is connected to the N-terminus of the first Fab fragment. As detailed above, the Fab fragments can be connected to each other via the heavy or the light chains. In one embodiment the C-terminus of the heavy chain of the third Fab fragment is connected to the N-terminus of the heavy chain of the first Fab fragment via a peptide linker; and the C-terminus of the first Fab
5 fragment is connected to the N-terminus of the second Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged. Depending on whether the variable or the constant domains of the heavy and the light chains of the second Fab fragment are exchanged different bispecific antibody molecules are possible.

10 In one embodiment the variable domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(VHVL)), and the chains of the three Fab fragments are connected in the following order from N-terminal to C-terminal direction: VHCH1-linker-VHCH1-linker-VLCH1. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the
15 heavy chain of the third fragment connected to the heavy chain of the first Fab fragment which itself is connected to the VLCH1 chain of the second Fab fragment via a peptide linker (VHCH1-linker-VHCH1-linker-VLCH1) and a VHCL chain of the second Fab fragment.

In one embodiment the constant domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(CLCH1)), and the chains of the three Fab fragments are
20 connected in the following order from N-terminal to C-terminal direction: VHCH1-linker-VHCH1-linker-VHCL. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the heavy chain of the third fragment connected to the heavy chain of the first Fab fragment which itself is connected to the VHCL chain of the second Fab fragment via a peptide linker (VHCH1-
25 linker-VHCH1-linker-VHCL) and a VLCH1 chain of the second Fab fragment.

In one embodiment the bispecific antibody of the invention comprises three Fab fragments wherein said Fab fragments and said linker are connected in the following order from N-terminal to C-terminal direction: Fab fragment 2- linker- Fab fragment 1- linker- Fab fragment 3, wherein either the variable regions or the constant regions of the heavy and light chain of the
30 second Fab fragment are exchanged. In this embodiment the N-terminus of the third Fab fragment is connected to the C-terminus of the first Fab fragment. As detailed above, the Fab fragments can be connected to each other via the heavy or the light chains. In one embodiment the N-terminus of the heavy chain of the third Fab fragment is connected to the C-terminus of the heavy chain of the first Fab fragment via a peptide linker; and the N-terminus of the first Fab
35 fragment is connected to the C-terminus of the second Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are

exchanged. Depending on whether the variable or the constant domains of the heavy and the light chains of the second Fab fragment are exchanged different bispecific antibody molecules are possible.

In one embodiment the variable domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(VHVL)), and the chains of the three Fab fragments are connected in the following order from N-terminal to C-terminal direction: VLCH1-linker-VHCH1-linker-VHCH1. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the VLCH1 chain of the second Fab fragment connected to the heavy chain of the first fragment which itself is connected to the heavy chain of the first Fab fragment via a peptide linker (VLCH1-linker-VHCH1-linker-VHCH1) and a VHCL chain of the second Fab fragment.

In one embodiment the constant domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(CLCH1)), and the chains of the three Fab fragments are connected in the following order from N-terminal to C-terminal direction: VHCL-linker-VHCH1-linker-VHCH1. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the VHCL chain of the second Fab fragment connected to the heavy chain of the first fragment which itself is connected to the heavy chain of the first Fab fragment via a peptide linker (VHCL-linker-VHCH1-linker-VHCH1) and a VLCH1 chain of the second Fab fragment.

In another embodiment the third Fab fragment is connected to N or C-terminus of the light chain or the heavy chain of the second Fab fragment. In one embodiment the third Fab fragment is connected to the second Fab fragment via a peptide linker. Preferably said peptide linker is a (G4S)₂ linker. As detailed above, the Fab fragments can be connected to each other via the heavy or the light chains.

In one embodiment the bispecific antibody of the invention comprises three Fab fragments wherein said Fab fragments and said linker are connected in the following order from N-terminal to C-terminal direction: Fab fragment 1- linker- Fab fragment 2- linker- Fab fragment 3, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged. In one embodiment the N-terminus of the third Fab fragment is connected to the C-terminus of the second Fab fragment.

In another embodiment the C-terminus of the heavy chain of the third Fab fragment is connected to the N-terminus of the second Fab fragment via a peptide linker; and the N-terminus of the first Fab fragment is connected to the C-terminus of the second Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain of the second Fab fragment are exchanged.

Depending on whether the variable or the constant domains of the heavy and the light chains of the second Fab fragment are exchanged different bispecific antibody molecules are possible.

In one embodiment the variable domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(VHVL)), and the chains of the three Fab fragments are connected in the following order from N-terminal to C-terminal direction: VHCH1-linker-VLCH1-linker-VHCH1. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the heavy chain of the third fragment connected to to the N-terminus of the VLCH1 chain of the second Fab fragment, and the C-terminus of said VLCH1 chain connected to the N-terminus of the heavy chain of the first Fab fragment via a peptide linker (VHCH1-linker-VLCH1-linker-VHCH1) and a VHCL chain of the second Fab fragment.

In one embodiment the constant domains of the second Fab fragment are exchanged (i.e. the second Fab fragment is a CrossFab_(CLCH1)), and the chains of the three Fab fragments are connected in the following order from N-terminal to C-terminal direction: VHCH1-linker-VHCL-linker-VHCH1. In one embodiment the bispecific antibody comprises four chains: a light chain (VLCL) of the third Fab fragment, a light chain (VLCL) of the first Fab fragment, the heavy chain of the third fragment connected to to the N-terminus of the VHCL chain of the second Fab fragment, and the C-terminus of said VHCL chain connected to the N-terminus of the heavy chain of the first Fab fragment via a peptide linker (VHCH1-linker-VHCL-linker-VHCH1) and a VLCH1 chain of the second Fab fragment.

In one embodiment of the invention the bispecific antibody is a humanized antibody, as detailed below.

In another embodiment of the invention the bispecific antibody is a human antibody, as detailed below.

In a second object the present invention relates to a pharmaceutical composition comprising a bispecific antibody of the present invention.

In a third object the present invention relates to a bispecific antibody of the present invention for the treatment of cancer. In another embodiment, use of the bispecific antibody as a medicament is provided. Preferably said use is for the treatment of cancer.

In further objects the present invention relates to a nucleic acid sequence comprising a sequence encoding a heavy chain of a bispecific antibody of the present invention, a nucleic acid sequence comprising a sequence encoding a light chain of a bispecific antibody of the present invention, an expression vector comprising a nucleic acid sequence of the present invention and

to a prokaryotic or eukaryotic host cell comprising a vector of the present invention. In addition a method of producing an antibody comprising culturing the host cell so that the antibody is produced is provided.

In a further embodiment an immunoconjugate comprising the bispecific antibody of the invention and a cytotoxic agent is provided.

In a further aspect, a bispecific antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-5 below:

1. Antibody Affinity

The affinity of the bispecific antibody provided herein for a target antigen can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of bispecific antibodies for different receptors or target antigens may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS).

In certain embodiments, a bispecific antibody provided herein has a dissociation constant (KD) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

According to one embodiment, KD is measured using surface plasmon resonance assays using a BIAcore®-2000 or a BIAcore®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore, Inc.) are activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_a or k_{on}) and dissociation rates (k_d or k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (KD) is calculated as the ratio

$k_{\text{off}}/k_{\text{on}}$. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCOTM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Chimeric and Humanized Antibodies

In certain embodiments, a bispecific antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a “class switched” antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing “resurfacing”); Dall’Acqua et al., *Methods* 36:43-60 (2005) (describing

“FR shuffling”); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the “guided selection” approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

3. Human Antibodies

In certain embodiments, a bispecific antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSETM technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York,

1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

4. Library-Derived Antibodies

Bispecific antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas.

Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be

made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

5. *Antibody Variants*

In certain embodiments, amino acid sequence variants of the bispecific antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the bispecific antibody. Amino acid sequence variants of a bispecific antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the bispecific antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding or decreased immunogenicity.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys

Original Residue	Exemplary Substitutions	Preferred Substitutions
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes

for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent

antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant
5 antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury,
10 *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by
15 any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using
20 alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided
25 herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be
30 targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the

interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions.

Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring

residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered bispecific antibodies, e.g., "thioMAbs," in which one or more residues of a bispecific antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the bispecific antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain and A118 (EU numbering) of the heavy chain. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

c) Antibody Derivatives

In certain embodiments, a bispecific antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available.

The moieties suitable for derivatization of the bispecific antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol,

carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene

glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of a bispecific antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Bispecific antibodies of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the bispecific antibody (fragment), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of a bispecific antibody (fragment) along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the bispecific antibody (fragment) (i.e. the coding region) is cloned in operable

association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the bispecific antibody (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and

retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit α -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoter inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the bispecific antigen binding molecule is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding a bispecific antibody of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, e.g. an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the bispecific antibody may be included within or at the ends of the bispecific antibody (fragment) encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) a bispecific antibody of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the bispecific antibodies of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of bispecific antibodies are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the bispecific antibody for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, *Nat Biotech* 22, 1409-1414 (2004), and Li et al., *Nat Biotech* 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J Gen Virol* 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol Reprod* 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76),

human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., *Annals N.Y. Acad Sci* 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr⁻ CHO cells (Urlaub et al., *Proc Natl Acad Sci USA* 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., YO, NS0, Sp20 cell). Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain. In one embodiment, a method of producing a bispecific antibody according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the bispecific antibody, as provided herein, under conditions suitable for expression of the bispecific antigen binding molecule, and recovering the bispecific antibody from the host cell (or host cell culture medium).

The components of the bispecific antibody are genetically fused to each other. bispecific antibody can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different components of bispecific antibodies are found in the sequences provided herein. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

In certain embodiments the one or more antigen binding moieties of the bispecific antibodies comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can form part of and be derived from naturally or non-naturally

occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as
5 described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

Any animal species of antibody, antibody fragment, antigen binding domain or variable region can be used in the bispecific antibodies of the invention. Non-limiting antibodies,
10 antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the antibody is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be
15 achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen
20 interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front Biosci 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 332, 323-329 (1988); Queen et al., Proc Natl Acad Sci USA 86, 10029-
25 10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., Nature 321, 522-525 (1986); Morrison et al., Proc Natl Acad Sci 81, 6851-6855 (1984); Morrison and Oi, Adv Immunol 44, 65-92 (1988); Verhoeven et al., Science 239, 1534-1536 (1988); Padlan, Molec Immun 31(3), 169-217 (1994); Kashmiri et al., Methods 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, Mol Immunol 28, 489-498 (1991) (describing
30 "resurfacing"); Dall'Acqua et al., Methods 36, 43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36, 61-68 (2005) and Klimka et al., Br J Cancer 83, 252-260 (2000) (describing the "guided selection" approach to FR shuffling). Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies

are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, *Nat Biotech* 23, 1117-1125 (2005). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in *Methods in Molecular Biology* 178, 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); and McCafferty et al., *Nature* 348, 552-554; Clackson et al., *Nature* 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments.

In certain embodiments, the bispecific antibodies of the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the bispecific antibody of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljeblad, et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). Competition assays may be used to identify an antibody, antibody fragment, antigen binding domain or variable domain that competes with a reference antibody for binding to a particular antigen, e.g. an antibody that competes with the V9 antibody for binding to CD3. In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay, immobilized antigen (e.g. CD3) is incubated in a solution comprising a first labeled antibody that binds to the antigen (e.g. V9 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first

labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to the antigen. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Bispecific antibodies prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the bispecific antibody binds. For example, for affinity chromatography purification of bispecific antibody of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate a bispecific antibody essentially as described in the Examples. The purity of the bispecific antibodies can be determined by any of a variety of well known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like.

C. Assays

Bispecific antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. *Binding assays and other assays*

In one aspect, a bispecific antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with a specific antibody for binding to the first or second antigen. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by a specific antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

2. Activity assays

In one aspect, assays are provided for identifying bispecific antibodies thereof having biological activity. Biological activity may include, e.g., lysis of targeted cells, or induction of apoptosis. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, a bispecific antibody of the invention is tested for such biological activity. Assays for detecting cell lysis (e.g. by measurement of LDH release) or apoptosis (e.g. using the TUNEL assay) are well known in the art.

D. Immunoconjugates

The invention also provides immunoconjugates comprising a bispecific antibody of the invention conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises a bispecific antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-

sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises a bispecific antibody as
 5 described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} , Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123 , or a spin label for nuclear magnetic resonance (NMR) imaging (also
 10 known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of a bispecific antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC),
 15 iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a
 20 ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker,
 25 dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-
 30 EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the bispecific antibodies provided herein is useful for detecting the presence of a first and / or a second antigen in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain
5 embodiments, a biological sample comprises a cell or tissue.

In one embodiment, a bispecific antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of a first and / or a second antigen in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with a bispecific antibody as described herein under conditions
10 permissive for binding of the bispecific antibody to a first and / or a second antigen, and detecting whether a complex is formed between the bispecific antibody a first and / or a second antigen. Such method may be an *in vitro* or *in vivo* method. In one embodiment, a bispecific antibody is used to select subjects eligible for therapy with a bispecific antibody, e.g. where a first and / or a second antigen is a biomarker for selection of patients.

15 Exemplary disorders that may be diagnosed using an antibody of the invention include cancer.

In certain embodiments, labeled bispecific antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties,
20 such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ³²P, ¹⁴C, ¹²⁵I, ³H, and ¹³¹I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones,
25 horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free
30 radicals, and the like.

F. Pharmaceutical Formulations

Pharmaceutical formulations of a bispecific antibody as described herein are prepared by mixing such bispecific antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose

or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

5 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

10 **G. Therapeutic Methods and Compositions**

Any of the bispecific antibodies provided herein may be used in therapeutic methods.

In one aspect, a bispecific antibody for use as a medicament is provided. In further aspects, a bispecific antibody for use in treating cancer is provided. In certain embodiments, a bispecific antibody for use in a method of treatment is provided. In certain embodiments, the
15 invention provides a bispecific antibody for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the bispecific antibody . In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. An “individual” according to any of the above embodiments is preferably a human.

20 In a further aspect, the invention provides for the use of a bispecific antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer. In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the
25 individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating cancer. In one embodiment, the method comprises administering to an individual having cancer an effective amount of a bispecific antibody . In one such embodiment, the method further comprises
30 administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the bispecific antibodies provided herein, e.g., for use in any of the above therapeutic methods.

In one embodiment, a pharmaceutical formulation comprises any of the bispecific antibody provided herein and a pharmaceutically acceptable carrier. In another embodiment, a

5 pharmaceutical formulation comprises any of the bispecific antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

The bispecific antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, a bispecific antibody of the invention may be co-administered with at least one additional therapeutic agent.

10 Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Bispecific antibodies of the invention can also be used in combination with radiation
15 therapy.

A bispecific antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing
20 can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Bispecific antibodies of the invention would be formulated, dosed, and administered in a
25 fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The bispecific antibody need not be, but is optionally formulated with one or more
30 agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages

described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of a bispecific antibody of the invention (when used alone or in combination with one or more other additional
5 therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the bispecific antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the bispecific antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and
10 severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of bispecific antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the
15 condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the bispecific antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that
20 the patient receives from about two to about twenty, or e.g. about six doses of the bispecific antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried
25 out using an immunoconjugate of the invention in place of or in addition to a bispecific antibody of the invention.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The
30 article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective

for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a bispecific antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a bispecific antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to a bispecific antibody of the invention .

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Example 1: Preparation of Fab (MCSP)-CrossFab(CD3)

The resulting variable region of heavy and light chain DNA sequences have been subcloned in frame with either the constant heavy chain or the constant light chain pre-inserted into the respective recipient mammalian expression vector. The antibody expression is driven by

an MPSV promoter and carries a synthetic polyA signal sequence at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

The molecule is produced by co-transfecting HEK293-EBNA cells with the mammalian expression vectors using a calcium phosphate-transfection. Exponentially growing HEK293-EBNA cells are transfected by the calcium phosphate method. Alternatively, HEK293-EBNA cells growing in suspension are transfected by polyethylenimine. The cells are transfected with the corresponding expression vectors in a 1:1:1 ratio ("vector CH1-VH – CK-VH" : "vector light chain" : "vector light chain CH1-VL").

For transfection using calcium phosphate cells are grown as adherent monolayer cultures in T-flasks using DMEM culture medium supplemented with 10 % (v/v) FCS, and are transfected when they are between 50 and 80 % confluent. For the transfection of a T150 flask, 15 million cells are seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% v/v final), and cells are placed at 37 °C in an incubator with a 5 % CO₂ atmosphere overnight. For each T150 flask to be transfected, a solution of DNA, CaCl₂ and water is prepared by mixing 94 µg total plasmid vector DNA divided in the corresponding ratio, water to a final volume of 469 µl and 469 µl of a 1 M CaCl₂ solution. To this solution, 938 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄ solution at pH 7.05 are added, mixed immediately for 10 s and left to stand at room temperature for 20 s. The suspension is diluted with 10 ml of DMEM supplemented with 2 % (v/v) FCS, and added to the T150 in place of the existing medium. Then additional 13 ml of transfection medium are added. The cells are incubated at 37 °C, 5 % CO₂ for about 17 to 20 hours, then medium is replaced with 25 ml DMEM, 10 % FCS. The conditioned culture medium is harvested approx. 7 days post-media exchange by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % (w/v) is added, and kept at 4 °C.

For transfection using polyethylenimine HEK293 EBNA cells are cultivated in suspension serum free in CD CHO culture medium. For the production in 500 ml shake flask 400 million HEK293 EBNA cells are seeded 24 hours before transfection. For transfection cells are centrifuged for 5 min by 210 x g, supernatant is replaced by pre-warmed 20 ml CD CHO medium. Expression vectors are mixed in 20 ml CD CHO medium to a final amount of 200 µg DNA. After addition of 540 µl PEI solution is vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells are mixed with the DNA/PEI solution, transferred to a 500 ml shake flask and incubated for 3 hours by 37 °C in an incubator with a 5 % CO₂ atmosphere. After incubation time 160 ml F17 medium is added and cell are cultivated for

24 hours. One day after transfection 1 mM valporic acid and 7 % Feed 1 (Lonza) is added. After 7 days cultivation supernatant is collected for purification by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v is added, and kept at 4 °C.

5 The secreted protein is purified from cell culture supernatants by affinity chromatography using Protein A and Protein G affinity chromatography, followed by a size exclusion chromatographic step. For affinity chromatography supernatant is loaded on a HiTrap Protein A HP column (CV = 5 ml, GE Healthcare) coupled to a HiTrap Protein G HP column (CV = 5 ml, GE Healthcare) each column equilibrated with 30 ml 20 mM sodium phosphate, 20 mM sodium
10 citrate, pH 7.5. Unbound protein is removed by washing both columns with 6 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Subsequently an additional wash step is necessary to wash only the HiTrap Protein G HP column using at least 8 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. The target protein is eluted from HiTrap Protein G HP column using a step gradient with 7 column volume 8.8 mM formic acid,
15 pH 3.0. Protein solution is neutralized by adding 1/10 of 0.5 M sodium phosphate, pH 8.0. Target protein is concentrated and filtrated prior loading on a HiLoad Superdex 200 column (GE Healthcare) equilibrated with 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine solution of pH 6.7.

 The protein concentration of purified protein samples is determined by measuring the
20 optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of antibodies are analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiotreitol) and staining with Coomassie (SimpleBlue™ SafeStain from Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen, USA) is used according to the manufacturer's instruction (4-12 % Tris-Acetate gels
25 or 4-12 % Bis-Tris). The aggregate content of antibody samples is analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare, Sweden) in 2 mM MOPS, 150 mM NaCl, 0.02 % (w/v) NaN₃, pH 7.3 running buffer at 25°C.

Analysis of production and purification of an exemplary Fab-Crossfab molecule (consisting of
30 three chains: VHCH1(MCSP)-VLCH1(CD3_{v9}) = SEQ ID NO:25, VLCL(MCSP) = SEQ ID NO:17 and VHCL(CD3_{v9}) = SEQ ID NO:23; with an orientation as depicted in Figure 1 a)) is shown in Figures 2 and 3. This molecule is further referred to as Fab (MCSP)-Crossfab (CD3) or hu Fab (MCSP)-Crossfab (CD3).

Example 2: Preparation of Fab (MCSP)- Fab (MCSP)-CrossFab(CD3) and Fab (MCSP)- CrossFab(CD3)- Fab (MCSP)

The resulting variable region of heavy and light chain DNA sequences have been subcloned in frame with either the constant heavy chain or the constant light chain pre-inserted into the respective recipient mammalian expression vector. The antibody expression is driven by an MPSV promoter and carries a synthetic polyA signal sequence at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

The molecule is produced by co-transfecting HEK293-EBNA cells with the mammalian expression vectors using a calcium phosphate-transfection. Exponentially growing HEK293-EBNA cells are transfected by the calcium phosphate method. Alternatively, HEK293-EBNA cells growing in suspension are transfected by polyethylenimine. The cells are transfected with the corresponding expression vectors in a 1:2:1 ratio ("vector CH1-VH – CH1-VH – CK-VH" : "vector light chain" : "vector light chain CH1-VL").

For transfection using calcium phosphate cells are grown as adherent monolayer cultures in T-flasks using DMEM culture medium supplemented with 10 % (v/v) FCS, and are transfected when they are between 50 and 80 % confluent. For the transfection of a T150 flask, 15 million cells are seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% v/v final), and cells are placed at 37 °C in an incubator with a 5 % CO₂ atmosphere overnight. For each T150 flask to be transfected, a solution of DNA, CaCl₂ and water is prepared by mixing 94 µg total plasmid vector DNA divided in the corresponding ratio, water to a final volume of 469 µl and 469 µl of a 1 M CaCl₂ solution. To this solution, 938 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄ solution at pH 7.05 are added, mixed immediately for 10 s and left to stand at room temperature for 20 s. The suspension is diluted with 10 ml of DMEM supplemented with 2 % (v/v) FCS, and added to the T150 in place of the existing medium. Then additional 13 ml of transfection medium are added. The cells are incubated at 37 °C, 5 % CO₂ for about 17 to 20 hours, then medium is replaced with 25 ml DMEM, 10 % FCS. The conditioned culture medium is harvested approx. 7 days post-media exchange by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % (w/v) is added, and kept at 4 °C. For transfection using polyethylenimine HEK293 EBNA cells are cultivated in suspension serum free in CD CHO culture medium. For the production in 500 ml shake flask 400 million HEK293 EBNA cells are seeded 24 hours before transfection. For transfection cells are centrifuged for 5 min by 210 x g, supernatant is replaced by pre-warmed 20 ml CD CHO

medium. Expression vectors are mixed in 20 ml CD CHO medium to a final amount of 200 µg DNA. After addition of 540 µl PEI solution is vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells are mixed with the DNA/PEI solution, transferred to a 500 ml shake flask and incubated for 3 hours by 37 °C in an incubator with a 5 % CO₂ atmosphere. After incubation time 160 ml F17 medium is added and cell are cultivated for 24 hours. One day after transfection 1 mM valporic acid and 7 % Feed 1 (Lonza) is added. After 7 days cultivation supernatant is collected for purification by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v is added and kept at 4 °C.

The secreted protein is purified from cell culture supernatants by affinity chromatography using Protein A and Protein G affinity chromatography, followed by a size exclusion chromatographic step.

For affinity chromatography supernatant is loaded on a HiTrap Protein A HP column (CV = 5 ml, GE Healthcare) coupled to a HiTrap Protein G HP column (CV = 5 ml, GE Healthcare) each column equilibrated with 30 ml 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Unbound protein is removed by washing both columns with 6 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Subsequently an additional wash step is necessary to wash only the HiTrap Protein G HP column using at least 8 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. The target protein is eluted from HiTrap Protein G HP column using a step gradient with 7 column volume 8.8 mM formic acid, pH 3.0. Protein solution is neutralized by adding 1/10 of 0.5 M sodium phosphate, pH 8.0. Target protein is concentrated and filtrated prior loading on a HiLoad Superdex 200 column (GE Healthcare) equilibrated with 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine solution of pH 6.7.

The protein concentration of purified protein samples is determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of antibodies are analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiotreitol) and staining with Coomassie (SimpleBlue™ SafeStain from Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen, USA) is used according to the manufacturer's instruction (4-12 % Tris-Acetate gels or 4-12 % Bis-Tris). The aggregate content of antibody samples is analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare, Sweden) in 2 mM MOPS,

150 mM NaCl, 0.02 % (w/v) NaN₃, pH 7.3 running buffer at 25°C and compared with prior art antibody fragment (scFv)₂ (results see table below).

Construct	Yield [mg/l]	Aggregate after 1 st purification step [%]	HMW [%]	LMW [%]	Monomer [%]
(scFv) ₂	3.84	80	0	0	100
Fab-Crossfab	7.85	13.8	0	0	100
(Fab) ₂ -Crossfab	7.8	3.6	0	0	100
Fab-Crossfab-Fab	5.3	1.7	0.4	0	99.56

HMW = High Molecular Weight; LMW = Low Molecular Weight

- 5 Analysis of production and purification of an exemplary Fab-Fab-Crossfab molecule (consisting of four chains: VHCH1(MCSP)-VHCH1(MCSP)-VLCH1(CD3_{v9}) = SEQ ID NO:26, 2 VLCL(MCSP) chains = SEQ ID NO:17 and one VHCL(CD3_{v9}) chain = SEQ ID NO:23; with an orientation as depicted in Figure 1 c)) is shown in Figures 4 and 5. This molecule is further referred to as Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) or hu Fab (MCSP)- Fab (MCSP)-
- 10 Crossfab (CD3).

- Analysis of production and purification of an exemplary Fab-Crossfab-Fab molecule (consisting of four chains: VHCH1(MCSP)-VLCH1(CD3_{v9})- VHCH1(MCSP)= SEQ ID NO:27, 2 VLCL(MCSP) chains = SEQ ID NO:17 and one VHCL(CD3_{v9}) chain= SEQ ID NO:23; with an orientation as depicted in Figure 1 e)) is shown in Figures 6 and 7. This molecule is further
- 15 referred to as Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) or hu Fab (MCSP)- Fab (MCSP)-Crossfab (CD3).

- Analysis of production and purification of an exemplary Crossfab-Fab-Fab molecule (consisting of four chains: VLCH1(CD3_{2C11})- VHCH1(MCSP)- VHCH1(MCSP)= SEQ ID NO:42, 2 VLCL(MCSP) chains = SEQ ID NO:17 and one VHCL(CD3_{2C11}) chain = SEQ ID NO:43; with
- 20 an orientation as depicted in Figure 1 d)) is shown in Figures 8 and 9. This molecule is further referred to as murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP).

Example 3: Preparation of Fab(CD33)-CrossFab (CD3)

- The resulting variable region of heavy and light chain DNA sequences have been
- 25 subcloned in frame with either the constant heavy chain or the constant light chain pre-inserted into the respective recipient mammalian expression vector. The antibody expression is driven by an MPSV promoter and carries a synthetic polyA signal sequence at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

The molecule is produced by co-transfecting HEK293-EBNA cells with the mammalian expression vectors using a calcium phosphate-transfection. Exponentially growing HEK293-EBNA cells are transfected by the calcium phosphate method. Alternatively, HEK293-EBNA cells growing in suspension are transfected by polyethylenimine. The cells are transfected with the corresponding expression vectors in a 1:1:1 ratio ("vector CH1-VH – CK-VH" : "vector light chain" : "vector light chain CH1-VL").

For transfection using calcium phosphate cells are grown as adherent monolayer cultures in T-flasks using DMEM culture medium supplemented with 10 % (v/v) FCS, and are transfected when they are between 50 and 80 % confluent. For the transfection of a T150 flask, 15 million cells are seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% v/v final), and cells are placed at 37 °C in an incubator with a 5 % CO₂ atmosphere overnight. For each T150 flask to be transfected, a solution of DNA, CaCl₂ and water is prepared by mixing 94 µg total plasmid vector DNA divided in the corresponding ratio, water to a final volume of 469 µl and 469 µl of a 1 M CaCl₂ solution. To this solution, 938 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄ solution at pH 7.05 are added, mixed immediately for 10 s and left to stand at room temperature for 20 s. The suspension is diluted with 10 ml of DMEM supplemented with 2 % (v/v) FCS, and added to the T150 in place of the existing medium. Then additional 13 ml of transfection medium are added. The cells are incubated at 37 °C, 5 % CO₂ for about 17 to 20 hours, then medium is replaced with 25 ml DMEM, 10 % FCS. The conditioned culture medium is harvested approx. 7 days post-media exchange by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % (w/v) is added, and kept at 4 °C.

For transfection using polyethylenimine HEK293 EBNA cells are cultivated in suspension serum free in CD CHO culture medium. For the production in 500 ml shake flask 400 million HEK293 EBNA cells are seeded 24 hours before transfection. For transfection cells are centrifuged for 5 min by 210 x g, supernatant is replaced by pre-warmed 20 ml CD CHO medium. Expression vectors are mixed in 20 ml CD CHO medium to a final amount of 200 µg DNA. After addition of 540 µl PEI solution is vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells are mixed with the DNA/PEI solution, transferred to a 500 ml shake flask and incubated for 3 hours by 37 °C in an incubator with a 5 % CO₂ atmosphere. After incubation time 160 ml F17 medium is added and cell are cultivated for 24 hours. One day after transfection 1 mM valporic acid and 7 % Feed 1 (LONZA) is added.

After 7 days cultivation supernatant is collected for purification by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v is added, and kept at 4°C.

The secreted protein is purified from cell culture supernatants by affinity chromatography using Protein A and ProteinG affinity chromatography, followed by a size exclusion chromatographic step.

For affinity chromatography supernatant is loaded on a HiTrap ProteinA HP column (CV=5 mL, GE Healthcare) coupled to a HiTrap ProteinG HP column (CV=5 mL, GE Healthcare) each column equilibrated with 30 ml 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Unbound protein is removed by washing both columns with 6 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Subsequently an additional wash step is necessary to wash only the HiTrap ProteinG HP column using at least 8 column volume 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. The target protein is eluted from HiTrap ProteinG HP column using a step gradient with 7 column volume 8.8 mM formic acid, pH 3.0. Protein solution is neutralized by adding 1/10 of 0.5M sodium phosphate, pH 8.0. Target protein is concentrated and filtrated prior loading on a HiLoad Superdex 200 column (GE Healthcare) equilibrated with 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine solution of pH 6.7.

The protein concentration of purified protein samples is determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of antibodies are analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiotreitol) and staining with Coomassie (SimpleBlue™ SafeStain from Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen, USA) is used according to the manufacturer's instruction (4-12% Tris-Acetate gels or 4-12% Bis-Tris). The aggregate content of antibody samples is analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare, Sweden) in 2 mM MOPS, 150 mM NaCl, 0.02 % (w/v) NaN₃, pH 7.3 running buffer at 25°C.

Analysis of production and purification of an exemplary Fab-Crossfab molecule (consisting of three chains: VHCH1(CD33)- VLCH1(CD3_{v9}) = SEQ ID NO:102, VLCL(CD33) = SEQ ID NO:100 and VHCL(CD3_{v9}) = SEQ ID NO:23 or SEQ ID NO:101; with an orientation as depicted in Figure 1 a)) is shown in Figures 17 and 18. This molecule is further referred to as Fab(CD33)-CrossFab (CD3) or hu Fab(CD33)-CrossFab (CD3).

Example 4: Preparation of the reference molecule (scFv)₂Cloning and production

The resulting variable region of heavy and light chain DNA sequences have been subcloned in frame into the respective recipient mammalian expression vector. The antibody expression is
5 driven by an MPSV promoter and carries a synthetic polyA signal sequence at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

The molecule is produced by transfecting HEK293-EBNA cells with the mammalian expression vector using polyethylenimine. HEK293 EBNA cells are cultivated in suspension serum free in CD CHO culture medium. For the production in 500 ml shake flask 400 million HEK293 EBNA
10 cells are seeded 24 hours before transfection. For transfection cells are centrifuged for 5 min by 210 x g, supernatant is replaced by pre-warmed 20 ml CD CHO medium. Expression vectors are mixed in 20 ml CD CHO medium to a final amount of 200 µg DNA. After addition of 540 µl PEI solution is vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells are mixed with the DNA/PEI solution, transferred to a 500 ml shake flask and
15 incubated for 3 hours by 37 °C in an incubator with a 5 % CO₂ atmosphere. After incubation time 160 ml F17 medium is added and cell are cultivated for 24 hours. One day after transfection 1 mM valporic acid and 7 % Feed 1 (LONZA) are added. After 7 days cultivation supernatant is collected for purification by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v is added, and kept at
20 4°C.

Purification of (scFv)₂ (anti MCSP/anti huCD3)

The secreted protein is purified from cell culture supernatants by affinity chromatography using Immobilized Metal Ion Affinity Chromatography (IMAC), followed by a size exclusion chromatographic step.

25 Prior first purification step disturbing components from the supernatant are removed by diafiltration using the tangential flow filtration system Sarcojet (Sartorius) equipped with a 5.000 MWCO membrane (Sartocon Slice Cassette, Hydrosart; Sartorius). Supernatant is concentrated to 210 ml and subsequently diluted in 1 l 20 mM sodium phosphate, 500 mM sodium chloride, pH 6.5. The protein solution is concentrated again to 210 ml. This process is
30 repeated twice to ensure a complete buffer exchange.

For affinity chromatography retentate of the diafiltration process is loaded on a NiNTA Superflow Cartridge (CV=5 mL, Qiagen) equilibrated with 25 ml 20 mM sodium phosphate, 500 mM sodium chloride, 15 mM imidazole, pH 6.5. Unbound protein is removed by washing

with at least 2 column volume 20 mM sodium phosphate, 500 mM sodium chloride, 15 mM imidazole, pH 6.5 followed by an additional wash step using 3 column volume 20 mM sodium phosphate, 500 mM sodium chloride, 62.5 mM imidazole, pH 6.5. Target protein is eluted in 2 column volume 20 mM sodium phosphate, 500 mM sodium chloride, 125 mM imidazole, pH 6.5.

- 5 Column is washed subsequently with 20 mM sodium phosphate, 500 mM sodium chloride, 250 mM imidazole, pH 6.5.

Target protein is concentrated prior loading on a HiLoad Superdex 75 column (GE Healthcare) equilibrated with 25 mM KH_2PO_4 , 125 mM NaCl, 200 mM Arginine, pH 6.7.

- 10 Yields, aggregate content after the first purification step and final monomer content is shown in the table above. Comparison of the aggregate content after the first purification step indicates the superior stability of the Fab-Crossfab construct in contrast to the (scFv)₂.

Characterization of (scFv)₂

- The protein concentration of purified protein samples is determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of antibodies are analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiotreitol) and staining with Coomassie (SimpleBlue™ SafeStain from Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen, USA) is used according to the manufacturer's instruction (4-12% Tris-Acetate gels or 4-12% Bis-Tris). The aggregate content of antibody samples is analyzed using a Superdex 75 10/300GL analytical size-exclusion column (GE Healthcare, Sweden) in 2 mM MOPS, 150 mM NaCl, 0.02 % (w/v) NaN_3 , pH 7.3 running buffer at 25°C.
- 20

A schematic drawing of the (scFv)₂ molecule is shown in Figure 21.

- Analysis of production and purification of an exemplary (scFv)₂ molecule (antiMCSP/anti huCD3; consisting two single chain Fvs: VL-VH (MCSP) and VH-VL (CD3_{v9}) = SEQ ID NO:149; is shown in Figures 22 and 23. This molecule is further referred to as (scFv)₂ (antiMCSP/anti huCD3e).
- 25

Example 5: Isolation of primary human pan T cells from PBMCs

- Peripheral blood mononuclear cells (PBMCs) were prepared by Histopaque density centrifugation from enriched lymphocyte preparations (buffy coats) obtained from local blood banks or from fresh blood from healthy human donors.
- 30

T-cell enrichment from PBMCs was performed using the Pan T Cell Isolation Kit II (Miltenyi Biotec #130-091-156), according to the manufacturer's instructions. Briefly, the cell pellets were diluted in 40µl cold buffer per 10 Mio cells (PBS with 0.5 % BSA, 2 mM EDTA -

sterile filtered) and incubated with 10µl Biotin-Antibody Cocktail per 10 Mio cells for 10 min at 4 °C.

30µl cold buffer and 20µl Anti-Biotin magnetic beads per 10 Mio cells were added, and the mixture incubated for another 15 min at 4 °C.

5 Cells were washed by adding 10-20x of labeling volume and a subsequent centrifugation step at 300g for 10 min. Up to 100 Mio cells were resuspended in 500 µl buffer.

Magnetic separation of unlabeled human pan T cells was performed using LS columns (Miltenyi Biotec #130-042-401) according to the manufacturer's instructions. The resulting T cell population was counted automatically (ViCell) and stored in AIM-V medium at 37°C, 5 %
10 CO₂ in the incubator until assay start (not longer than 24 h).

Example 6: Isolation of murine pan T cells from splenocytes

Spleens were isolated from C57BL/6 mice, transferred into a GentleMACS C-tube (Miltenyi Biotech #130-093-237) containing MACS buffer (PBS + 0.5 % BSA + 2 mM EDTA) and dissociated with the GentleMACS Dissociator to obtain single-cell suspensions according to
15 the manufacturers' instructions.

The cell suspension was passed through a pre-separation filter to get rid-off remaining undissociated tissue particles. After centrifugation at 400 g for 4 minutes at 4°C, ACK Lysis Buffer was added to lyse red blood cells (incubation for 5 minutes at room temperature). The remaining cells were washed with MACS buffer twice, counted and used for the isolation of
20 murine pan T cells. The negative (magnetic) selection was performed using the Pan T Cell Isolation Kit from Miltenyi Biotec (#130-090-861), following the manufacturers' instructions. The resulting T cell population was counted automatically (ViCell) and used immediately for further assays.

Example 7: Re-directed T cell cytotoxicity mediated by cross-linked bispecific 25 constructs targeting CD3 on T cells and MCSP on tumor cells (LDH release assay)

Bispecific constructs targeting CD3 on human, or mouse T cells and human on tumor cells, are analyzed by a LDH release assay regarding their potential to induce T cell-mediated apoptosis of target cells.

Briefly, target cells (human Colo-38, human MDA-MB-435, human melanoma MV-3 or
30 murine B16/F10-huMCSP Fluc 2 clone 48 cells, all expressing human MCSP) are harvested with Cell Dissociation Buffer (MCSP is trypsin-sensitive) or trypsin (and then plated the day before), washed and resuspended in the appropriate cell culture medium (see detailed description of the

different figures). 20 000 - 30 000 cells per well are plated in a round-bottom 96-well-plate and the respective antibody dilution was added as indicated (triplicates). Effector cells were added to obtain a final E:T ratio of 5:1 (for human pan T cells), 10:1 (for human PBMCs).

In addition, 1-10 µg/ml PHA-M (Sigma #L8902), a mixture of isolectins, isolated from Phaseolus vulgaris, was used as a mitogenic stimulus to induce human or cynomolgus T cell activation. For murine T cells, a 5 % solution of "rat T-Stim with ConA" (BD #354115) was used as a positive control for T cell activation.

For normalization, maximal lysis of the target cells (= 100 %) is achieved by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %) refers to target cells co-incubated with effector cells, but without any construct or antibody.

After an overnight incubation of at least 18 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant is measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

LDH release assay with Fab (MCSP)-Crossfab (CD3) and Fab (MCSP)- Fab (MCSP)- Crossfab (CD3) bispecific constructs

Purified Fab (MCSP)-Crossfab (CD3), Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) and the (scFv)₂ (antiMCSP/anti huCD3e) reference molecule were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells upon crosslinkage of the construct via binding of both targeting moieties to the respective antigens on cells. Briefly, huMCSP-expressing MDA-MB-435 human melanoma target cells are harvested with Cell Dissociation Buffer, washed and resuspended in AIM-V medium (Invitrogen # 12055-091). 30 000 cells per well were plated in a round-bottom 96-well-plate and the respective antibody dilution was added at the indicated concentrations. All constructs and controls were adjusted to the same molarity.

Human pan T effector cells were added to obtain a final E:T ratio of 5:1. As a positive control for the activation of human pan T cells, 1 µg/ml PHA-M (Sigma #L8902) was used. For normalization, maximal lysis of the target cells (= 100 %) was determined by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %) refers to target cells co-incubated with effector cells, but without any construct or antibody.

After an overnight incubation of 20 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant was measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

As depicted in Figure 10, the constructs with bivalent MCSP-targeting show comparable cytotoxic activity compared to the (scFv)₂ (antiMCSP/anti huCD3e) construct, whereas the Fab (MCSP)-Crossfab (CD3) construct with monovalent MCSP binding is clearly less potent.

LDH release assay with Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) bispecific construct with MDA-MB-435 human melanoma target cells

Purified Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) and the (scFv)₂ (antiMCSP/anti huCD3e) reference molecule were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells upon crosslinkage of the construct via binding of both targeting moieties to the respective antigens on cells.

Briefly, huMCSP-expressing MDA-MB-435 human melanoma target cells are harvested with Cell Dissociation Buffer, washed and resuspended in AIM-V medium (Invitrogen # 12055-091). 30 000 cells per well were plated in a round-bottom 96-well-plate and the respective antibody dilution was added at the indicated concentrations. All constructs and controls were adjusted to the same molarity.

Human pan T effector cells were added to obtain a final E:T ratio of 5:1. As a positive control for the activation of human pan T cells, 5 µg/ml PHA-M (Sigma #L8902) was used. For normalization, maximal lysis of the target cells (= 100 %) was determined by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %) refers to target cells co-incubated with effector cells, but without any construct or antibody.

After an overnight incubation of 21 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant was measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

As depicted in Figure 11, the Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) induces apoptosis in target cells at least comparably good as the (scFv)₂ (antiMCSP/anti huCD3e) molecule.

LDH release assay with Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) bispecific construct with MV-3 human melanoma target cells

Purified Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) and the (scFv)₂ (antiMCSP/anti huCD3e) molecule were analyzed for their potential to induce T cell-mediated apoptosis in tumor target cells upon crosslinkage of the construct via binding of both targeting moieties to the respective antigens on cells.

Briefly, huMCSP-expressing MV-3 human melanoma target cells are harvested with trypsin on the day before the LDH release assay was started. Cells were washed and resuspended in the appropriate cell culture medium. 30 000 cells per well were plated in a round-bottom 96-well-plate. The next day, the supernatant was discarded and 100 µl/well AIM-V medium (Invitrogen # 12055-091), as well as the respective antibody dilution were added at the indicated concentrations. All constructs and controls were adjusted to the same molarity.

Human PBMC effector cells were added to obtain a final E:T ratio of 10:1. As a positive control for the activation of human pan T cells, 5 µg/ml PHA-M (Sigma #L8902) was used. For normalization, maximal lysis of the target cells (= 100 %) was determined by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %) refers to target cells co-incubated with effector cells, but without any construct or antibody.

After an overnight incubation of 26 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant was measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

As depicted in Figure 12, the Fab (MCSP)- Crossfab (CD3) induces apoptosis in target cells at least comparably good as the (scFv)₂ (antiMCSP/anti huCD3e) molecule.

LDH release assay with Fab (MCSP)- Crossfab (CD3) bispecific construct with MV-3 human melanoma target cells

An LDH release assay was performed as outlined above. Figure 19 shows killing of huMCSP-positive MV-3 tumor cells upon co-culture with human PBMCs (E:T ratio = 10:1), treated with Fab (MCSP)- Crossfab (CD3), respective the (scFv)₂ (antiMCSP/anti huCD3e) reference molecule for ~ 24 hours.

LDH release assay with murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP) bispecific construct

Purified with murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP), targeting murine CD3, as well as human MCSP, was analyzed for its potential to induce T cell-mediated apoptosis in tumor target cells upon crosslinkage of the construct via binding of both targeting moieties to the respective antigens on cells.

Briefly, huMCSP-expressing B16/F10-huMCSP Fluc2 clone 48 tumor target cells are harvested with Cell Dissociation Buffer, washed and resuspended in RPMI1640 medium, including 1x NEAA, 10 mM Hepes, 50 μ M 2-b-ME and 1 mM sodium pyruvate.

20 000 cells per well were plated in a round-bottom 96-well-plate and the respective
5 antibody dilution was added at the indicated concentrations. The bispecific construct and the different IgG controls were adjusted to the same molarity. As an additional control for the activation of murine T cells "T Cell Stim with ConA" (BD #354115) was used, diluted 1:160 with assay medium.

10 Murine pan T effector cells, isolated from splenocytes (C57BL/6 mice) were added to obtain a final E:T ratio of 10:1. For normalization, maximal lysis of the target cells (= 100 %) was determined by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %) refers to target cells co-incubated with effector cells, but without any construct or antibody.

15 After an incubation for 70 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant was measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

As depicted in Figure 13, the bispecific construct induces concentration-dependent LDH release from target cells, comparable to the positive control with "T Cell Stim with ConA".

20 LDH release assay with murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP) bispecific construct

Purified murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP), targeting murine CD3, as well as human MCSP, was analyzed for its potential to induce T cell-mediated apoptosis in tumor target cells upon crosslinkage of the construct via binding of both targeting moieties to the respective antigens on cells.

25 Briefly, huMCSP-expressing B16/F10-huMCSP Fluc2 clone 48 tumor target cells are harvested with Cell Dissociation Buffer, washed and resuspended in RPMI1640 medium, including 1x NEAA, 10 mM Hepes, 50 μ M 2-b-ME and 1 mM sodium pyruvate.

30 20 000 cells per well were plated in a round-bottom 96-well-plate and the respective antibody dilution was added to obtain a final concentration of 50 nM. The bispecific construct and the different IgG controls were adjusted to the same molarity.

Murine pan T effector cells, isolated from splenocytes (C57BL/6 mice) were added to obtain a final E:T ratio of 10:1. To assess the level of hyperactivation of murine T cells in the

absence of target cells, control wells with 50 nM bispecific construct and T cells were plated accordingly.

For normalization, maximal lysis of the target cells (= 100 %) was determined by incubation of the target cells with a final concentration of 1 % Triton-X-100. Minimal lysis (= 0 %)
5 % refers to target cells co-incubated with effector cells, but without any construct or antibody.

After an incubation for 70 h at 37°C, 5 % CO₂, LDH release of apoptotic/necrotic target cells into the supernatant was measured with the LDH detection kit (Roche Applied Science, # 11 644 793 001), according to the manufacturer's instructions.

As depicted in Figure 14, the bispecific construct induces strong LDH release from target
10 cells. In the absence of target cells, there is only a slight increase of LDH (reflecting hyperactivation of T cells) compared to untreated murine T cells, co-incubated with target cells. None of the control IgGs induces LDH release of target cells.

Example 8: Cytokine release assay (CBA analysis)

To assess the *de novo* secretion of different cytokines upon T cell activation with CD3-
15 bispecific constructs in the presence or absence of target cells, human PBMCs were isolated from Buffy Coats and 0.3 Mio cells per well were plated into a round-bottom 96-well plate. Alternatively, 280 µl whole blood from a healthy donor were plated per well of a deep-well 96-well plate.

Tumor target cells (e.g. MDA-MB-435 cells for CD3-MCSP-bispecific constructs) were
20 added to obtain a final E/T-ratio of 10:1. Bispecific constructs and controls were added as indicated. After an incubation of up to 24 h at 37°C, 5 % CO₂, the assay plate was centrifuged for 5 min at 350 g and the supernatant was transferred into a new deep-well 96-well-plate for the subsequent analysis.

The CBA analysis was performed according to manufacturers' instructions for FACS
25 CantoII, using the combination of the following CBA Flex Sets: human granzyme B (BD 560304), human IFN-γ Flex Set (BD 558269), human TNF Flex Set (BD 558273), human IL-10 Flex Set (BD 558274), human IL-6 Flex Set (BD 558276), human IL-4 Flex Set (BD 558272).

Cytokine Release Assay with MCSP-CD3 bispecific constructs

30 The following purified bispecific constructs targeting human MCSP and human CD3 were analyzed for their ability to induce T cell-mediated *de novo* secretion of cytokines in the

presence (A, B) versus absence (C, D) of tumor target cells: “Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) and the (scFv)₂ (antiMCSP/anti huCD3e) reference molecule.

Briefly, 280 µl whole blood from a healthy donor were plated per well of a deep-well 96-well plate. 30 000 Colo-38 tumor target cells, expressing human MCSP, as well as the different bispecific constructs and IgG controls were added were added at 1 nM final concentration. The cells were incubated for 24 h at 37°C, 5 % CO₂ and then centrifuged for 5 min at 350 x g. The supernatant was transferred into a new deep-well 96-well-plate for the subsequent analysis.

The CBA analysis was performed according to manufacturers’ instructions for FACS CantoII, using the combination of the following CBA Flex Sets: human granzyme B (BD 560304), human IFN-γ Flex Set (BD 558269), human TNF Flex Set (BD 558273), human IL-10 Flex Set (BD 558274), human IL-6 Flex Set (BD 558276), human IL-4 Flex Set (BD 558272).

FIGURE 15 depicts different cytokine levels, that were measured in the supernatant of whole blood after treatment with 1 nM of different CD3-MCSP bispecific constructs (Fab (MCSP)- Fab (MCSP)-Crossfab (CD3) and the (scFv)₂ (antiMCSP/anti huCD3e)) in the presence (A, B) or absence (C,D) of Colo-38 tumor cells for 24 hours. 280 µl whole blood were plated per well of a 96-well plate and 30 000 Colo-38 cells added, as indicated.

The main cytokine that was secreted upon activation of T cells in the presence of Colo-38 tumor cells, is IL-6, followed by IFNγ. In addition, also the levels of granzyme B increased enormously upon activation of T cells in the presence of target cells. In general, the (scFv)₂ (antiMCSP/anti huCD3e) construct elevated the levels of TNF and IFNγ, as well as granzyme B in the presence of target cells (A and B) a bit more compared to the other bispecific construct.

There was no significant secretion of Th2 cytokines (IL-10 and IL-4) upon activation of T cells by the bispecific constructs in the presence (or absence) of target cells.

In this assay, there was also a weak secretion of IFNγ, induced by the Fab (MCSP)-Fab (MCSP)-Crossfab (CD3) construct in the absence of target cells.

Cytokine Release Assay with MCSP-murineCD3 bispecific constructs

The purified huMCSP-muCD3-targeting bispecific molecule as murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP) was tested by flow cytometry for its potential to up-regulate the late activation marker CD25 on CD8⁺ T cells in the presence of human MCSP-expressing tumor cells.

Briefly, MCSP-positive B16/F10-huMCSP Fluc2 clone 48 tumor cells were harvested with Cell Dissociation buffer, counted and checked for viability. Cells were adjusted to 0.3×10^6

(viable) cells per ml in RPMI1640 medium (including 1x NEAA, 10 mM Hepes, 50 μ M 2-b-ME, 1 mM sodium pyruvate), 100 μ l of this cell suspension were pipetted per well into a round-bottom 96-well plate (as indicated). 50 μ l of the (diluted) bispecific construct was added to the cell-containing wells to obtain a final concentration of 50 nM. Human murine T effector cells were isolated from splenocytes (C57BL/6 mice) and adjusted to 3×10^6 (viable) cells per ml in AIM-V medium. 50 μ l of this cell suspension was added per well of the assay plate (see above) to obtain a final E:T ratio of 10:1. To analyze, if the bispecific construct is able to activate T cells only in the presence of target cells, expressing huMCSP, wells were included that contained 50 nM of the respective bispecific molecule, as well as T effector, but no target cells.

After incubation for 70 hours at 37°C, 5 % CO₂, cells were centrifuged (5 min, 350 x g) and washed twice with 150 μ l/well PBS, including 0.1 % BSA.

Surface staining for CD8a (rat IgG2a; clone 53-6.7; BioLegend #100712) and CD25 (rat IgG2b; clone 3C7; BD #553075) was performed according to the suppliers' suggestions. Cells were washed twice with 150 μ l/well PBS, including 0.1 % BSA and fixed for 15 min at 4°C, using 100 μ l/well fixation buffer (BD #554655).

After centrifugation, the samples were resuspended in 200 μ l/well PBS, 0.1 % BSA and analyzed using a FACS CantoII machine (Software FACS Diva).

Figure 16 shows that the as murine Crossfab (CD3)-Fab (MCSP)- Fab (MCSP) construct induces up-regulation of CD25 in the presence of target cells only.

Example 9: Expression of surface activation markers on primary human T cells upon engagement of bispecific constructs

To check for specific activation of T cells upon binding of CD3 bispecific constructs exclusively in the presence of tumor target cells, primary human PBMCs (isolated as described above) were incubated with the indicated concentrations of bispecific constructs for at least 24 h in the presence or absence of tumor antigen-positive target cells.

Briefly, 0.3 million primary human PBMCs were plated per well of a flat-bottom 96-well plate, containing the huMCSP-positive target cells (MV-3 tumor cells) or medium. The final effector to target cell (E:T) ratio was 10:1. The cells were incubated with the indicated concentration of the CD3-MCSP bispecific constructs (Fab (MCSP)-Crossfab (CD3); designated as "1+1 non-Fc", and the (scFv)₂ (antiMCSP/anti huCD3e) reference molecule (designated as "(scFv)₂") for the indicated incubation times at 37°C, 5% CO₂. The effector cells were stained

for CD8, and the early activation marker CD69 or the late activation marker CD25 and analyzed by FACS CantoII.

Figure 20 shows the result of this experiment.

While there are shown and described presently preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims.

Sequences

While there are shown and described presently preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims. Legend: GA201= EGFR binder, 3F2= FAP binder, CH1A1A=CEA binder.

Protein sequences

SEQ ID. NO.	Description	Sequence
1	CDR1 VL MCSP	SASQGIRNYLN
2	CDR2 VL MCSP	YTSSLHS
3	CDR3 VL MCSP	QQYSKLPWT
4	CDR1 VH MCSP	GYSITSGYYWN
5	CDR2 VH MCSP	YITYDGSNNYNPSLKN
6	CDR3 VH MCSP	FDY
7	CDR1 VL CD3 _(V9)	RASQDIRNYLN
8	CDR2 VL CD3 _(V9)	YTSRLES
9	CDR3 VL CD3 _(V9)	QQGNTLPWT
10	CDR1 VH CD3 _(V9)	GYTMN
11	CDR2 VH CD3 _(V9)	LINPYKGVSTYNQKFKD
12	CDR3 VH CD3 _(V9)	SGYYGDSDWYFDV
29	CDR1 VL CD3 _(H2C)	GSSTGAVTSGYYPN
30	CDR2 VL CD3 _(H2C)	GTKFLAP
31	CDR3 VL CD3 _(H2C)	ALWYSNRWV
32	CDR1 VH CD3 _(H2C)	GFTFNKYAMN
33	CDR2 VH CD3 _(H2C)	RIRSKYNNYATYYADSVKD

SEQ ID. NO.	Description	Sequence
34	CDR3 VH CD3 _(H2C)	HGNFGNSYISYWAY
13	VL MCSP	DIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWY QQRPDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYS LTISNLEPEDIATYYCQQYSKLPWTFGGGTKLEIK
14	VH MCSP	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWN WIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISITR DTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTL TVSS
15	CL MCSP	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
16	CH1 MCSP	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCD
17	LIGHT CHAIN MCSP	DIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWYQQR PDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLE PEDIATYYCQQYSKLPWTFGGGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
18	HEAVY CHAIN MCSP	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIR QFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKN QFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCD
19	VL CD3 _(v9)	QSPSSLSASVGDRTITCRASQDIRNYLNWYQQKPGKA PKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFA TYYCQQGNTLPWTFGQGTKVEIK
20	VH CD3 _(v9)	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWV RQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKS KNTAYLQMNSLRAEDTAVYYCARSGYYGDSWDYFDV WGQGTLVTVSS
21	CL CD3 _(v9)	VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
22	CH CD3 _(v9)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSC
23	LIGHT CHAIN CD3 _(v9) (VHCL)	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWV RQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKS KNTAYLQMNSLRAEDTAVYYCARSGYYGDSWDYFDV WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCL

SEQ ID. NO.	Description	Sequence
		LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
24	HEAVY CHAIN CD3 _(v9) (VLCH1)	SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSC
35	VL CD3 _(H2C)	QTVVTQEPSTLVSPGGTVTLTCGSSTGAVTSGYYPN WVQQKPGQAPRGLIGGTFKFLAPGTPARFSGSLLGGK AALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTV L
36	VH CD3 _(H2C)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNW VRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSY ISYWAYWGQGLTVTVSS
37	CL CD3 _(H2C)	VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
38	CH1 CD3 _(H2C)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSC
39	LIGHT CHAIN CD3 _(H2C) (VHCL)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWW RQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISR DSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYW AYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
40	HEAVY CHAIN CD3 _(H2C) (VLCH1)	SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSC
25	FAB (MCSP)- XFAB (CD3 _(v9)) (VH-CH1—VL- CH1)	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIR QFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKN QFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSC
26	FAB (MCSP)- FAB (MCSP)- XFAB (CD3 _(v9)) (VH-CH1—VH- CH1—VL-CH1)	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIR QFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKN QFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT

SEQ ID. NO.	Description	Sequence
		YICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQ LQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFP GNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKNQFFL KLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDGGGGSGGGGSSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
27	FAB (MCSP)- XFAB (CD3 _(V9))- FAB (MCSP) (VH-CH1—VL- CH1—VH-CH1)	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIR QFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKN QFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSEVQL QESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPG NKLEWMGYITYDGSNNYNPSLKNRISITRDTSKNQFFL LNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSC
28	LINKER 1	GGGGSGGGGS
41	FAB (MCSP)- XFAB (CD3 _(H2C))- FAB (MCSP) (VH-CH1—VH- CH1—VL-CH1)	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIR QFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKN QFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQ LQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFP GNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKNQFFL KLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDGGGGSGGGGSSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
42	Murine LIGHT CHAIN CD3 _(2C11) (VHCL)	EVQLVESGGGLVQPGKSLKLSCEASGFTFSGYGMHWV RQAPGRGLESVAYITSSSINIKYADAVKGRFTVSRDNAK NLLFLQMNILKSEDAMYYCARFDWDKNYWGQGTMV TVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLT SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID. NO.	Description	Sequence
43	Murine XFAB (CD3 _(2C11))-FAB (MCSP)-FAB (MCSP) (VL-CH1—VH-CH1—VH-CH1)	DIQMTQSPSSLPASLGDRV TINCQASQDISNYLNWYQQ KPGKAPKLLIYYTNKLADGVPSRFSGSGSGRDSSFTISL ESEDIGSYQCQYYNYPWTFGPGTKLEIKSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCGGGGSGGGGSEVQLQESGPG LVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEW MGYITYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVT TEDTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDGGGGSGGGGSEVQLQESGPGLVK PSQSLSLTCSVTGYSITSGY YWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISIT RDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTL TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCD
68	GA201 CDR1 VH	DYKIH
69	GA201 CDR2 VH	YFNPNSGYSTYAQKFQG
70	GA201 CDR3 VH	LSPGGYYVMDA
71	GA201 CDR1 VL	RASQGINNYLN
72	GA201 CDR2 VL	NTNNLQT
73	GA201 CDR3 VL	LQHNSFPT
74	GA201 VH	QVQLVQSGAEVKKPGSSVKV SCKASGFTFTDYKIHVV RQAPGQGLEWMGYFNPNSGYSTYAQKFQGRVTITADK STSTAYMELSSLRSED TAVYYCARLSPGGYYVMDAWG QGTTVTVSS
75	GA201 VL	DIQMTQSPSSLSASVGDRTITCRASQGINNYLNWYQQ KPGKAPKRLIYNTNNLQTGVPSRFSGSGSGTEFTLTISL QPEDFATYYCLQHNSFPTFGQG TKLEIK
76	3F2 CDR1 VH	SYAMS
77	3F2 CDR2 VH	AISGSGGSTYYADSVK
78	3F2 CDR3 VH	YCAKGWFG
79	3F2 CDR1 VL	RASQSVTSSYL
80	3F2 CDR2 VL	NVGSRRRA

SEQ ID. NO.	Description	Sequence
81	3F2 CDR3 VL	CQQGIMLPP
82	3F2 VH	EVQLLES G GGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS
83	3F2 VL	EIVLTQSPGTL SLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRL LINVGSR RATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGIMLPPTFGQGTKVEIK
84	CH1A1A CDR1 VH	EFGMN
85	CH1A1A CDR2 VH	WINTKTGEATYVEEFKG
86	CH1A1A CDR3 VH	WDFAYYVEAMDY
87	CH1A1A CDR1 VL	KASAAVGTYVA
88	CH1A1A CDR2 VL	SASYRKR
89	CH1A1A CDR3 VL	HQYYTYPLFT
90	CH1A1A VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTVTVSS
91	CH1A1A VL	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK
92	Anti-CD33 CDR1 VH	GYTITDSNIH
93	Anti-CD33 CDR2 VH	YIYPYNGGTDYNQ
94	Anti-CD33 CDR3 VH	GNPWLAY
95	Anti-CD33 CDR1 VL	RASESLDNYGIRFLT
96	Anti-CD33 CDR1 VL	AASNQGS
97	Anti-CD33 CDR1 VL	QQTKEVPWS
98	Anti-CD33 VH	EVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVRQAPGQSLEWIGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDTAFYYCVNGNPWLAYWGQGTLTVTVSS
99	Anti-CD33 VL	DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQQKPGKAPKLLMYAASNQGSQGVPSRFSGSGSGTEFTLTI

SEQ ID. NO.	Description	Sequence
		SSLQPDDFATYYCQQTKEVPWSFGQGTKVEVK
100	Light Chain antiCD33	DIQMTQSPSSLSASVGDRVTITCRASESDNYGISFMNW FQQKPGKAPKLLIYAASNQGSVPSRFSGSGSGTDFTLT ISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
101	Light Chain CD3 _(v9) (VH-CL)	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYT MNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTI SVDKSKNTAYLQMNSLRAEDTAVYYCARGSGYYGDS WYFDVWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
102	Fab (CD33)- XFab (CD3 _(v9)) (VH-CH1—VL- CH1)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHW VRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADE STNTAYMELSSLRSED TAVYYCARGRPAMDYWGQGTLL TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGG GGSDIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWY QQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTIS SLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVL
145	Linker 2	EPKSCGGGGSGGGGS
146	Linker 3	EPKSCDGGGGSGGGGS
147	Linker 4	GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
148	Linker 5	SGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSG
149	(scFv) ₂ antiMCSP/anti huCD3e (MCSP(VL-VH)— CD3 _(v9) (VH-VL))	DIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWYQQR PDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLE PEDIATYYCQQYSKLPWTFGGGKLEIKGGGGSGGGGS GGGGSEVQLQESGPGLVKPSQSLTCSVTGYSITSGYY WNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISITR DTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTLLT VSSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFT GYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKD RFTISVDKSKNTAYLQMNSLRAEDTAVYYCARGSGYYG DSDWYFDVWGQGTLLTVSSVEGGSGGSGGSGGGV DDIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQ QKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISS LQPEDFATYYCQQGNTLPWTFGQGTKVEIKHHHHHH
151	Light Chain	DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWF QQKPGKAPKLLMYAASNQGSVPSRFSGSGSGTEFTLTI SSLQPDDFATYYCQQTKEVPWSFGQGTKVEVKRTVAA

SEQ ID. NO.	Description	Sequence
	antiCD33 _(Myelotarg)	PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
152	Light Chain CD3 _(V9) (VL-CH1)	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQ KPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSL QPEDFATYYCQQGNTLPWTFGQGQTKVEIKSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
153	Fab (CD33 _(Myelotarg))- XFab (CD3 _(V9)) (VH-CH1—VH- CL)	EVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVR QAPGQSLEWIGYIYPYNGGTDYNQKFKNRATLTVDNPT NTAYMELSSLRSEDTAFYYCVNGNPWLAYWGQGTLLV VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGG GSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMN WVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISV DKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWY FDVWGQGTLLVTVSSASVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
157	CD3 _(CH2527) VL	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPD HLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAI YFCALWYSNLWVFGGGTKLTVL
158	CD3 _(CH2527) VH	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWW RQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISR DSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWF AYWGQGTLLVTVSA
159	CEA _{(CH1A1A (98/99))} VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWV RQAPGQGLEWMGWINTKTGEATYVEEFKGRVTFITDT STSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY WGQGTTVTVSS
160	CEA _{(CH1A1A (98/99))} VL	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQ KPGKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTLTISL QPEDFATYYCHQYYTYPLFTFGQGQTKLEIK
161	MCSP _(M4-3 ML2) VH	QVQLQESGPGLVKPSQTLSTCTVSGGSITSGYYWNWI RQHPGKGLEWIGYITYDGSNNYNPSLKSRTISRDTSKN QFSLKLSSVTAADTAVYYCADFDYWGQGTLLVTVSS
162	MCSP _(M4-3 ML2) VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLNWYQQ KPGKAPKLLIYYTSSLHSGVPSRFSGSGSGTDFTLTISL QPEDFATYYCQQYSKLPWTFGQGQTKVEIK

DNA Sequences

SEQ ID. NO.	Description	Sequence
44	VL MCSP	GATATTGTGCTCACACAGTCTCCATCCTCCCTGTCTGCC TCTCTGGGAGACAGAGTCACCATCAGTTGCAGTGCAAG TCAGGGCATTAGAAATTATTTAAACTGGTATCAGCAGA GACCAGATGGAAGTGTAAACTCCTGATCTATTACACAT CAAGTTTACACTCAGGAGTCCCATCAAGGTTTCAGTGGC AGTGGGTCTGGGACAGATTATTCTCTCACCATCAGCAAC CTGGAACCTGAAGATATTGCCACTTACTATTGTCAGCAG TATAGTAAGCTTCCTTGGACGTTTCGGTGGAGGCACCAA GCTGGAAATCAAA
45	VH MCSP	GAGGTGCAGCTGCAGGAATCTGGCCCTGGCCTGGTCAA GCCAAGCCAGAGTCTGAGCCTGACCTGCAGCGTGACCG GCTACAGCATTACCAGCGGCTACTACTGGAAGTGGATT CGGCAGTTCCCCGGCAATAAGCTGGAATGGATGGGCTA CATCACCTACGACGGCAGCAACAACCTACAACCCAGCC TGAAGAACCGGATCAGCATCACCCGGGACACCAGCAAG AACCAGTTCTTCCTGAAGCTGAACAGCGTGACCACCGA GGACACCGCCACATACTATTGCGCCGACTTCGACTACTG GGGCCAGGGCACCAACCCTGACCGTGTCCAGC
46	CL MCSP	CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACT CCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG CACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTT CAACAGGGGAGAGTGTTAG
47	CH1 MCSP	GCCAGCACAAAGGGGCCCTAGCGTGTTCCCTCTGGCCCC CAGCAGCAAGAGCACAAAGCGGCGGAACAGCCGCCCTG GGCTGCCTCGTGAAGGACTACTTCCCCGAGCCCGTGAC AGTGTCTTGGAACAGCGGAGCCCTGACAAGCGGCGTG ACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACT CCCTGAGCAGCGTGGTCACCGTGCCCTAGCAGCAGCCTG GGCACCCAGACCTACATCTGCAACGTGAACCACAAGCC CAGCAACACCAAAGTGGACAAGAAGGTGGAGCCCAAG AGCTGTGAT
48	LIGHT CHAIN MCSP	ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGCCTCCTG CTGCTCTGGTTCACAGGTGCCAGGTGTGATATTGTGCTC ACACAGTCTCCATCCTCCCTGTCTGCCTCTCTGGGAGAC AGAGTCACCATCAGTTGCAGTGCAAGTCAGGGCATTAG AAATTATTTAAACTGGTATCAGCAGAGACCAGATGGAA CTGTAAACTCCTGATCTATTACACATCAAGTTTACACT CAGGAGTCCCATCAAGGTTTCAGTGGCAGTGGGTCTGGG

SEQ ID. NO.	Description	Sequence
		ACAGATTATTCTCTCACCATCAGCAACCTGGAACCTGAA GATATTGCCACTTACTATTGTCAGCAGTATAGTAAGCTT CCTTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAA ACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCC ATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGT GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAA GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT TCAACAGGGGAGAGTGTTAG
49	HEAVY CHAIN MCSP	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCGGAGGTGCAGCTGCAGGAATC TGGCCCTGGCCTGGTCAAGCCAAGCCAGAGTCTGAGCC TGACCTGCAGCGTGACCGGCTACAGCATTACCAGCGGC TACTACTGGAAGTGGATTTCGGCAGTTCCCGGCAATAA GCTGGAATGGATGGGCTACATCACCTACGACGGCAGCA ACAACCTACAACCCAGCCTGAAGAACCGGATCAGCATC ACCCGGGACACCAGCAAGAACCAGTTCTTCCTGAAGCT GAACAGCGTGACCACCGAGGACACCGCCACATACTATT GCGCCGACTTCGACTACTGGGGCCAGGGCACCACTG ACCGTGTCCAGCGCCAGCACAAAGGGCCCTAGCGTGTT CCCTCTGGCCCCCAGCAGCAAGAGCACAAAGCGGCGGAA CAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTCCCCG AGCCCGTGACAGTGTCTTGGAACAGCGGAGCCCTGACA AGCGGCGTGCACACCTTCCCTGCCGTGCTGCAGAGCAG CGGCCTGTACTCCCTGAGCAGCGTGGTCACCGTGCCTAG CAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGT GGAGCCCAAGAGCTGTGAT
50	VL CD3 (v9)	GACATCCAGATGACCCAGAGCCCCTCTAGCCTGAGCGC CAGCGTGGGCGACAGAGTGACCATCACCTGTCGGGCCA GCCAGGACATCAGAACTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACTACAC CTCTAGACTGGAAAGCGGCGTGCCAGCCGGTTTAGCG GCAGCGGCTCCGGCACCGACTACACCCTGACCATCAGC AGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCA GCAGGGCAACACACTCCCCTGGACCTTCGGCCAGGGCA CCAAGGTGGAGATCAAGTCCAGC
51	VH CD3 (v9)	GAGGTGCAGCTGGTCGAGAGCGGAGGCGGCCTGGTGCA GCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG GCTACAGCTTCACCGGCTACACCATGAACTGGGTCCGG CAGGCACCTGGCAAGGGACTGGAATGGGTGGCCCTGAT

SEQ ID. NO.	Description	Sequence
		CAACCCCTACAAGGGCGTGAGCACCTACAACCAGAAGT TCAAGGACCGGTTACCATCAGCGTGGACAAGAGCAAG AACACCGCCTATCTGCAGATGAACAGCCTGCGGGCCGA GGACACCGCCGTGTACTACTGCGCCAGAAGCGGCTACT ACGGCGACAGCGACTGGTACTTCGACGTGTGGGGCCAG GGCACCCCTCGTGACCGTGTCTAGC
52	CL CD3 _(v9)	GTGGCTGCACCATCTGTCTTCATCTTCCCGCCATC TGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTG CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC AGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCAC CCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTGA
53	CH CD3 _(v9)	ACCAAGGGCCCCCTCCGTGTTCCCCCTGGCCCCCA GCAGCAAGAGCACCAAGCGGCGGCACAGCCGCCCTCGGC TGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGACCGTG TCCTGGAACAGCGGAGCCCTGACCTCCGGCGTGACAC CTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCT GTCCAGCGTGGTACCGTGCCCTCCAGCAGCCTGGGCA CCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGC AATACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCT GCTGA
54	LIGHT CHAIN CD3 _(v9) (VHCL)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGAGGTGCAGCTGGTCGAGAG CGGAGGCGGCCTGGTGCAGCCTGGCGGCAGCCTGAGAC TGAGCTGCGCCGCCAGCGGCTACAGCTTCACCGGCTAC ACCATGAACTGGGTCCGGCAGGCACCTGGCAAGGGACT GGAATGGGTGGCCCTGATCAACCCCTACAAGGGCGTGA GCACCTACAACCAGAAGTTCAAGGACCGGTTACCATC AGCGTGGACAAGAGCAAGAACACCGCCTATCTGCAGAT GAACAGCCTGCGGGCCGAGGACACCGCGTGTACTACT GCGCCAGAAGCGGCTACTACGGCGACAGCGACTGGTAC TTCGACGTGTGGGGCCAGGGCACCCCTCGTGACCGTGTCT AGC GCTAGC GTGGCTGCACCATCTGTCTTCATCTTCCCG CCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGA CAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAA GTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGTTGA
55	HEAVY CHAIN CD3 _(v9) (VLCH1)	GACATCCAGATGACCCAGAGCCCCCTCTA GCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACC TGTCGGGCCAGCCAGGACATCAGAAACTACCTGAACTG

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		GTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGA TCTACTACACCTCTAGACTGGAAAGCGGCGTGCCACAGC CGGTTTAGCGGCAGCGGCTCCGGCACCGACTACACCCT GACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCT ACTACTGCCAGCAGGGCAACACACTCCCCTGGACCTTC GGCCAGGGCACCAAGGTGGAGATCAAGTCCAGC <u>GCTA</u> <u>GC</u> ACCAAGGGCCCCTCCGTGTTCCCCCTGGCCCCCAGC AGCAAGAGCACCAGCGGCGGCACAGCCGCCCTCGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTC CTGGAACAGCGGAGCCCTGACCTCCGGCGTGACACACCT TCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTG TCCAGCGTGGTCACCGTGCCCTCCAGCAGCCTGGGCAC CCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCA ATACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTG CTGA
56	VL CD3 _(H2C)	CAGACCGTGGTGACACAGGAACCCAGCCTGACCGTCTC CCCTGGCGGCACCGTGACCCCTGACCTGTGGAAGCAGCA CAGGCGCCGTGACCAGCGGCTACTACCCCAACTGGGTG CAGCAGAAGCCCGGCCAGGCCCTAGAGGACTGATCGG CGGCACCAAGTTTCTGGCCCCTGGCACCCCCGCCAGATT CTCTGGCTCTCTGCTGGGCGGCAAGGCCGCCCTGACACT GTCTGGCGTGACGCCTGAGGACGAGGCCGAGTACTACT GCGCCCTGTGGTACAGCAACAGATGGGTGTTTCGGCGGA GGCACCAAGCTGACCGTGCTGAGCAGC
57	VH CD3 _(H2C)	GAGGTGCAGCTGGTGGAAAGCGGCGGAGGACTGGTGC AGCCTGGCGGAAGCCTGAAGCTGTCTTGCGCCGCCAGC GGCTTCACCTTCAACAAATACGCCATGAACTGGGTGCG CCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCCGGA TCAGAAGCAAGTACAACAACCTACGCCACCTACTACGCC GACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGA CAGCAAGAACACCGCCTACCTGCAGATGAACAACCTGA AAACCGAGGACACCGCCGTGTACTACTGCGTGCGGCAC GGCAACTTCGGCAACAGCTACATCAGCTACTGGGCCTA CTGGGGACAGGGCACCCCTGGTGACAGTGTCCAGC
58	CL CD3 _(H2C)	GTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT GAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTG CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTA CAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACT ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAG GGGAGAGTGTTGA
59	CH1 CD3 _(H2C)	ACCAAGGGCCCCTCCGTGTTCCCCCTGGCCCCCAGCAGC AAGAGCACCGAGCGGCGGCACAGCCGCCCTCGGCTGCCT GGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTG GAACAGCGGAGCCCTGACCTCCGGCGTGACACACCTTCC

SEQ ID. NO.	Description	Sequence
		CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCC AGCGTGGTCACCGTGCCCTCCAGCAGCCTGGGCACCCA GACCTACATCTGCAACGTGAACCACAAGCCCAGCAATA CCAAGGTGGACAAGAAGGTGGAGCCCCAAGAGCTGCTG A
60	LIGHT CHAIN CD3 _(H2C) (VHCL)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGAGGTGCAGCTGGTGGAAAG CGGCGGAGGACTGGTGCAGCCTGGCGGAAGCCTGAAGC TGTCTTGCGCCGCCAGCGGCTTCACCTTCAACAAATACG CCATGAACTGGGTGCGCCAGGCCCTGGCAAGGGACTG GAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATA CGCCACCTACTACGCCGACAGCGTGAAGGACCGGTTCA CCATCAGCCGGGACGACAGCAAGAACACCGCCTACCTG CAGATGAACAACCTGAAAACCGAGGACACCGCCGTGTA CTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTACA TCAGCTACTGGGCCTACTGGGGACAGGGCACCCCTGGTG ACAGTGTCCAGCGCTAGCGTGGCTGCACCATCTGTCTT CATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAG AGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGAC AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGAC GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACG CCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC ACAAAGAGCTTCAACAGGGGAGAGTGTTGA
61	HEAVY CHAIN CD3 _(H2C) (VLCH1)	CAGACCGTGGTGACACAGGAACCCAGCCTGACCGTCTC CCCTGGCGGCACCGTGACCCTGACCTGTGGAAGCAGCA CAGGCGCCGTGACCAGCGGCTACTACCCCAACTGGGTG CAGCAGAAGCCCGGCCAGGCCCTAGAGGACTGATCGG CGGCACCAAGTTTCTGGCCCCTGGCACCCCCGCCAGATT CTCTGGCTCTCTGCTGGGCGGCAAGGCCGCCCTGACACT GTCTGGCGTGCAGCCTGAGGACGAGGCCGAGTACTACT GCGCCCTGTGGTACAGCAACAGATGGGTGTTTCGGCGGA GGCACCAAGCTGACCGTGCTGAGCAGCGCTAGCACCA AGGGCCCCCTCCGTGTTCCCCCTGGCCCCCAGCAGCAAG AGCACCAGCGGCGGCACAGCCGCCCTCGGCTGCCTGGT CAAGGACTACTTCCCCGAGCCCGTGACCGTGTCTTGG ACAGCGGAGCCCTGACCTCCGGCGTGCACACCTTCCCC GCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCCAG CGTGGTCACCGTGCCCTCCAGCAGCCTGGGCACCCAGA CCTACATCTGCAACGTGAACCACAAGCCCAGCAATACC AAGGTGGACAAGAAGGTGGAGCCCCAAGAGCTGCTGA
62	FAB (MCSP)- XFAB (CD3) _(V9) (VH-CH1—VL- CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAG CAACAGCTACCGGTGTGCATTCCGAGGTGCAGCTGCAG GAAAGCGGCCCTGGCCTGGTGAACCCAGCCAGAGCCT GAGCCTGACCTGCAGCGTGACCGGCTACAGCATCACCA GCGGCTACTACTGGAAGTGGATCAGACAGTTCCCCGGC

SEQ ID. NO.	Description	Sequence
		AACAAGCTGGAATGGATGGGCTACATCACCTACGACGG CAGCAACAACCTACAACCCAGCCTGAAGAACAGAATCA GCATCACCCGGGACACCAGCAAGAACCAGTTCTTCCTG AAGCTGAACAGCGTGACCACCGAGGACACCGCCACCTA CTACTGCGCCGACTTCGACTACTGGGGCCAGGGCACCA CCCTGACCGTGTCTCC <u>GCTAGC</u> ACCAAGGGACCCAGC GTGTTCCCCCTGGCACCAGCAGCAAGAGCACATCTGG CGGAACAGCCGCTCTGGGCTGTCTGGTGAAAGACTACT TCCCCGAGCCCGTGACCGTGTCTTGGAACCTCTGGCGCCC TGACCAGCGGCGTGCACACCTTCCAGCCGTGCTGCAG AGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGACAGT GCCCAGCAGCAGCCTGGGAACCCAGACCTACATCTGCA ACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAA GAAGGTGGAACCCAAGAGCTGCGATGGCGGAGGAGGC TCCGGAGGCGGAGGCTCTGATATCCAGATGACCCAGAG CCCCAGCTCTCTGAGCGCCAGCGTGGGCGACAGAGTGA CCATCACCTGTTCGGGCCAGCCAGGACATCAGAACTAC CTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGATCTACTACACCAGCAGACTGGAAAGCGGCG TGCCCTCCAGATTTTCCGGCAGCGGCTCCGGCACCGACT ACACCCTGACCATCAGCAGCCTGCAGCCCGAGGATTTC GCCACATATTACTGCCAGCAGGGCAATAACCCTGCCCTG GACCTTCGGACAGGGCACAAAAGTGGAATCAAG
63	FAB (MCSP)- FAB (MCSP)- XFAB (CD3) _(V9) (VH-CH1—VH- CH1—VL-CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTTCGGAGGTGCAGCTGCAGGAATC TGGCCCTGGCCTGGTCAAGCCAAGCCAGAGTCTGAGCC TGACCTGCAGCGTGACCGGCTACAGCATTACCAGCGGC TACTACTGGAACCTGGATTTCGGCAGTTCCCCGGCAATAA GCTGGAATGGATGGGCTACATCACCTACGACGGCAGCA ACAACCTACAACCCAGCCTGAAGAACCGGATCAGCATC ACCCGGGACACCAGCAAGAACCAGTTCTTCCTGAAGCT GAACAGCGTGACCACCGAGGACACCGCCACATACTATT GCGCCGACTTCGACTACTGGGGCCAGGGCACCACTG ACCGTGTCCAGCGCCAGCACAAAGGGCCCTAGCGTGTT CCCTCTGGCCCCCAGCAGCAAGAGCACAAAGCGGCGGAA CAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTCCCCG AGCCCGTGACAGTGTCTTGGAACAGCGGAGCCCTGACA AGCGGCGTGCACACCTTCCCTGCCGTGCTGCAGAGCAG CGGCCTGTACTCCCTGAGCAGCGTGGTCACCGTGCCTAG CAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAAGTGGAACAAGAAGGT GGAGCCCAAGAGCTGTGATGGCGGAGGAGGGTCCGGA GGCGGTGGCTCCGAGGTGCAGCTGCAGGAATCTGGCCC TGGCCTGGTCAAGCCAAGCCAGAGTCTGAGCCTGACCT GCAGCGTGACCGGCTACAGCATTACCAGCGGCTACTAC TGGAACCTGGATTTCGGCAGTTCCCCGGCAATAAGCTGGA ATGGATGGGCTACATCACCTACGACGGCAGCAACAACCT

SEQ ID. NO.	Description	Sequence
		ACAACCCAGCCTGAAGAACCGGATCAGCATCACCCGG GACACCAGC AAGAACCAGTTCTTCTGAAGCTGAACAGCGTGACCAC CGAGGACACCGCCACATACTATTGCGCCGACTTCGACT ACTGGGGCCAGGGCACCACCCTGACCGTGTCCAGCGCC AGCACAAGGGGCCCTAGCGTGTTCCTCTGGCCCCCAG CAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCT GCCTCGTGAAGGACTACTTCCCCGAGCCCGTGACAGTG TCTTGGAACAGCGGAGCCCTGACAAGCGGCGTGACAC CTTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCT GAGCAGCGTGGTCACCGTGCCTAGCAGCAGCCTGGGCA CCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGC AACACCAAAGTGGACAAGAAGGTGGAGCCCAAGAGCT GTGATGGCGGAGGAGGGTCCGGCGGCGGTGGATCCGAC ATCCAGATGACCCAGAGCCCCTCTAGCCTGAGCGCCAG CGTGGGCGACAGAGTGACCATCACCTGTCGGGCCAGCC AGGACATCAGAACTACCTGAACTGGTATCAGCAGAAG CCCGGCAAGGCCCCCAAGCTGCTGATCTACTACACCTCT AGACTGGAAAGCGGCGTGCCAGCCGGTTTAGCGGCAG CGGCTCCGGCACCGACTACACCCTGACCATCAGCAGCC TGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAG GGCAACACACTCCCCTGGACCTTCGGCCAGGGCACCAA GGTGGAGATCAAGTCCAGC <u>GCTAGC</u> ACCAAGGGCCCCCT CCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGC GGCGGCACAGCCGCCCTCGGCTGCCTGGTCAAGGACTA CTTCCCCGAGCCCGTGACCGTGTCTGGAACAGCGGAG CCCTGACCTCCGGCGTGACACCTTCCCCGCCGTGCTGC AGAGCAGCGGCCTGTACAGCCTGTCCAGCGTGGTCACC GTGCCCTCCAGCAGCCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCAGCAATACCAAGGTGGACA AGAAGGTGGAGCCCAAGAGCTGCTGA
64	FAB (MCSP)- XFAB (CD3 _(V9))- FAB (MCSP) (VH-CH1—VL- CH1—VH- CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTTCGGAGGTGCAGCTGCAGGAAAG CGGCCCTGGCCTGGTGAAACCCAGCCAGAGCCTGAGCC TGACCTGCAGCGTGACCGGCTACAGCATCACCAGCGGC TACTACTGGAAGTGGATCAGACAGTTCCCCGGCAACAA GCTGGAATGGATGGGCTACATCACCTACGACGGCAGCA ACAACTACAACCCAGCCTGAAGAACAGAATCAGCATC ACCCGGGACACCAGCAAGAACCAGTTCTTCCTGAAGCT GAACAGCGTGACCACCGAGGACACCGCCACCTACTACT GCGCCGACTTCGACTACTGGGGCCAGGGCACCACCCTG ACCGTGTCCTCC <u>GCTAGC</u> ACCAAGGGACCCAGCGTGTT CCCCCTGGCACCCAGCAGCAAGAGCACATCTGGCGGAA CAGCCGCTCTGGGCTGTCTGGTGAAAGACTACTTCCCCG AGCCCGTGACCGTGTCTTGGAAGTCTGGCGCCCTGACCA GCGGCGTGACACCTTTCCAGCCGTGCTGCAGAGCAGC GGCCTGTACTCCCTGAGCAGCGTGGTGACAGTGCCAG

SEQ ID. NO.	Description	Sequence
		CAGCAGCCTGGGAACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGT GGAACCCAAGAGCTGCGATGGCGGAGGAGGCTCCGGA GGCGGAGGCTCTGATATCCAGATGACCCAGAGCCCCAG CTCTCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCA CCTGTTCGGGCCAGCCAGGACATCAGAACTACCTGAAC TGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCT GATCTACTACACCAGCAGACTGGAAAGCGGCGTGCCCT CCAGATTTTCCGGCAGCGGCTCCGGCACCGACTACACC CTGACCATCAGCAGCCTGCAGCCCGAGGATTTTCGCCAC ATATTACTGCCAGCAGGGCAATACCCTGCCCTGGACCTT CGGACAGGGCACAAAAGTGGAAATCAAGAGCAGCGCT TCCACCAAAGGCCCTTCCGTGTTTCCTCTGGCTCCTAGC TCCAAGTCCACCTCTGGAGGCACCGCTGCTCTCGGATGC CTCGTGAAGGATTATTTTCCTGAGCCTGTGACAGTGTCC TGGAATAGCGGAGCACTGACCTCTGGAGTGCATACTTT CCCCGCTGTGCTGCAGTCCTCTGGACTGTACAGCCTGAG CAGCGTGGTGACAGTGCCCAGCAGCAGCCTGGGCACCC AGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAACCCAAGTCTTGTGG CGGAGGCGGATCCGGCGGAGGGGGATCTGAGGTGCAG CTGCAGGAAAGCGGCCCTGGCCTGGTGAAACCCAGCCA GAGCCTGAGCCTGACCTGCAGCGTGACCGGCTACAGCA TCACCAGCGGCTACTACTGGAAGTGGATCAGACAGTTC CCCGGCAACAAGCTGGAATGGATGGGCTACATCACCTA CGACGGCAGCAACAATAACAACCCAGCCTGAAGAACA GAATCAGCATCACCCGGGACACCAGCAAGAACCAGTTC TTCCTGAAGCTGAACAGCGTGACCACCGAGGACACCGC CACCTACTACTGCGCCGACTTCGACTACTGGGGCCAGG GCACCACCCTGACCGTGTCTCCGCCTCTACCAAGGGCC CCAGCGTGTTCCTCCCTGGCACCCAGCAGCAAGAGCACA TCTGGCGGAACAGCCGCTCTGGGCTGTCTGGTGAAAGA CTACTTCCCCGAGCCCGTGACCGTGTCTTGGAACTCTGG CGCCCTGACCAGCGGCGTGACACACCTTTCAGCCGTGCT GCAGAGCAGCGGCCTGTACTCCCTGTCTCCGTGGTTCAC CGTGCCCTCTAGCTCCCTGGGAACACAGACATATATCTG TAATGTCAATCACAAGCCTTCCAACACCAAAGTCGATA AGAAAGTCGAGCCCAAGAGCTGCTGA
65	FAB (MCSP)- FAB (MCSP)- XFAB (CD ₃ (H ₂ C)) (VH-CH1—VH- CH1—VL-CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTTCGGAGGTGCAGCTGCAGGAATC TGGCCCTGGCCTGGTCAAGCCAAGCCAGAGTCTGAGCC TGACCTGCAGCGTGACCGGCTACAGCATTACCAGCGGC TACTACTGGAAGTGGATTTCGGCAGTTCCTCCGGCAATAA GCTGGAATGGATGGGCTACATCACCTACGACGGCAGCA ACAATAACAACCCAGCCTGAAGAACCAGGATCAGCATC ACCCGGGACACCAGCAAGAACCAGTTCTTCCCTGAAGCT GAACAGCGTGACCAACGAGGACACCGCCACATACTATT

SEQ ID. NO.	Description	Sequence
		GCGCCGACTTCGACTACTGGGGCCAGGGCACCACCCTG ACCGTGTCCAGCGCCAGCACAAAGGGCCCTAGCGTGTT CCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGAA CAGCCGCCCTGGGCTGCCTCGTGAAGGACTACTTCCCCG AGCCCGTGACAGTGTCTTGGAACAGCGGAGCCCTGACA AGCGGCGTGCACACCTTCCCTGCCGTGCTGCAGAGCAG CGGCCTGTACTCCCTGAGCAGCGTGGTCACCGTGCCTAG CAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGT GGAGCCCAAGAGCTGTGATGGCGGAGGAGGGTCCGGA GGCGGTGGCTCCGAGGTGCAGCTGCAGGAATCTGGCCC TGGCCTGGTCAAGCCAAGCCAGAGTCTGAGCCTGACCT GCAGCGTGACCGGCTACAGCATTACCAGCGGCTACTAC TGGAAGTGGATTTCGGCAGTTCCCCGGCAATAAGCTGGA ATGGATGGGCTACATCACCTACGACGGCAGCAACAAC ACAACCCAGCCTGAAGAACCGGATCAGCATCACCCGG GACACCAGCAAGAACCAGTTCTTCTGAAGCTGAACAG CGTGACCACCGAGGACACCGCCACATACTATTGCGCCG ACTTCGACTACTGGGGCCAGGGCACCACCCTGACCGTG TCCAGCGCCAGCACAAAGGGCCCTAGCGTGTTCCCTCT GGCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCC GCCCTGGGCTGCCTCGTGAAGGACTACTTCCCCGAGCCC GTGACAGTGTCTTGGAACAGCGGAGCCCTGACAAGCGG CGTGACACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCT GTACTCCCTGAGCAGCGTGGTCACCGTGCCTAGCAGCA GCCTGGGCACCCAGACCTACATCTGCAACGTGAACCAC AAGCCCAGCAACACCAAAGTGGACAAGAAGGTGGAGC CCAAGAGCTGTGATGGCGGAGGAGGGTCCGGCGGCGGT GGATCCCAGACCGTGGTGACACAGGAACCCAGCCTGAC CGTCTCCCCTGGCGGCACCGTGACCCTGACCTGTGGAA GCAGCACAGGCGCCGTGACCAGCGGCTACTACCCCAAC TGGGTGCAGCAGAAGCCCGGCCAGGCCCTAGAGGACT GATCGGCGGCACCAAGTTTCTGGCCCCTGGCACCCCCG CCAGATTCTCTGGCTCTCTGCTGGGCGGCAAGGCCGCCC TGACACTGTCTGGCGTGCAGCCTGAGGACGAGGCCGAG TACTACTGCGCCCTGTGGTACAGCAACAGATGGGTGTTT GGCGGAGGCACCAAGCTGACCGTGCTGAGCAGCGCTA <u>GC</u> ACCAAGGGCCCCCTCCGTGTTCCCCCTGGCCCCCAGC AGCAAGAGCACCAGCGGCGGCACAGCCGCCCTCGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTC CTGGAACAGCGGAGCCCTGACCTCCGGCGTGCACACCT TCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTG TCCAGCGTGGTCACCGTGCCCTCCAGCAGCCTGGGCAC CCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCA ATACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTG CTGA
66	Murine LIGHT	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA

SEQ ID. NO.	Description	Sequence
	CHAIN CD3 _(2C11) (VHCL)	GCTACCGGTGTGCATTCCGAGGTGCAGCTGGTGGAAAG CGGCGGAGGCCTGGTGCAGCCCCGGCAAGAGCCTGAAGC TGAGCTGCGAGGCCAGCGGCTTCACCTTCAGCGGCTAC GGCATGCACTGGGTGAGACAGGCCCCCTGGCAGAGGACT GGAAAGCGTGGCCTACATCACCAGCAGCAGCATCAACA TTAAGTACGCCGACGCCGTGAAGGGCCGGTTCACCGTG TCCAGGGATAACGCCAAGAACCTGCTGTTCTCCTGCAGAT GAACATCCTGAAGTCCGAGGACACCGCTATGTATTACT GCGCCAGATTTCGACTGGGACAAGAACTACTGGGGCCAG GGCACCATGGTCACAGTGTCTAGCGCTAGCGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTT GAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAA CTTCTATCCCAGAGAGGGCCAAAGTACAGTGGAAGGTGG ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA GCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAA CACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT GAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAGT GTTGA
67	Murine XFAB (CD3 _(2C11))- FAB (MCSP)- FAB (MCSP) (VL-CH1—VH- CH1—VH- CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGACATCCAGATGACCCAGAG CCCCAGCAGCCTGCCTGCCAGCCTGGGCGACAGAGTGA CCATCAACTGCCAGGCCAGCCAGGACATCAGCAACTAC CTGAACTGGTATCAGCAGAAGCCTGGCAAGGCCCCCAA GCTGCTGATCTACTACACCAACAAGCTGGCCGACGGCG TGCCCAGCAGATTCAGCGGCAGCGGCTCCGGCAGAGAC AGCAGCTTCACCATCTCCAGCCTGGAAAGCGAGGACAT CGGCAGCTACTACTGCCAGCAGTACTACAACTACCCCT GGACCTTCGGCCCTGGCACCAAGCTGGAAATCAAGAGC AGCGCTTCCACCAAAGGCCCTTCCGTGTTTCTCTGGCT CCTAGCTCCAAGTCCACCTCTGGAGGCACCGCTGCTCTC GGATGCCTCGTGAAGGATTATTTTCTGAGCCTGTGACA GTGTCCTGGAATAGCGGAGCACTGACCTCTGGAGTGCA TACTTTCCCCGCTGTGCTGCAGTCCTCTGGACTGTACAG CCTGAGCAGCGTGGTGACAGTGCCCAGCAGCAGCCTGG GCACCCAGACCTACATCTGCAACGTGAACCACAAGCCC AGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGT CTTGTGGCGGAGGCGGATCCGGCGGAGGAGGGTCCGAG GTGCAGCTGCAGGAATCTGGCCCTGGCCTGGTCAAGCC AAGCCAGAGTCTGAGCCTGACCTGCAGCGTGACCGGCT ACAGCATTACCAGCGGCTACTACTGGAAGTGGATTTCGG CAGTTCCCCGGAATAAGCTGGAATGGATGGGCTACAT CACCTACGACGGCAGCAACAATAACAACCCAGCCTGA AGAACCGGATCAGCATACCCGGGACACCAGCAAGAAC CAGTTCTTCTGAAAGCTGAACAGCGTGACCACCGAGGA CACCGCCACATACTATTGCGCCGACTTCGACTACTGGGG CCAGGGCACCAACCTGACCGTGTCCAGCGCCAGCACAA

SEQ ID. NO.	Description	Sequence
		AGGGCCCTAGCGTGTTCCTCTGGCCCCCAGCAGCAAG AGCACAAGCGGCGGAACAGCCGCCCTGGGCTGCCTCGT GAAGGACTACTTCCCCGAGCCCGTGACAGTGTCTTGGA ACAGCGGAGCCCTGACAAGCGGCGTGACACCTTCCCT GCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAG CGTGGTCACCGTGCCTAGCAGCAGCCTGGGCACCCAGA CCTACATCTGCAACGTGAACCACAAGCCCAGCAACACC AAAGTGGACAAGAAGGTGGAGCCCAAGAGCTGTGATG GCGGAGGAGGGTCCGGAGGCGGTGGCTCCGAGGTGCA GCTGCAGGAATCTGGCCCTGGCCTGGTCAAGCCAAGCC AGAGTCTGAGCCTGACCTGCAGCGTGACCGGTACAGC ATTACCAGCGGCTACTACTGGAAGTGGATTTCGGCAGTTC CCCGGCAATAAGCTGGAATGGATGGGCTACATCACCTA CGACGGCAGCAACAATAACAACCCAGCCTGAAGAACC GGATCAGCATCACCCGGGACACCAGCAAGAACCAGTTC TTCCTGAAGCTGAACAGCGTGACCACCGAGGACACCGC CACATACTATTGCGCCGACTTCGACTACTGGGGCCAGG GCACCACCTGACCGTGTCCAGCGCCAGCACAAAGGGC CCTAGCGTGTTCCTCTGGCCCCCAGCAGCAAGAGCAC AAGCGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGG ACTACTTCCCCGAGCCCGTGACAGTGTCTTGGAACAGC GGAGCCCTGACAAGCGGCGTGACACCTTCCCTGCCGT GCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGG TCACCGTGCCTAGCAGCAGCCTGGGCACCCAGACCTAC ATCTGCAACGTGAACCACAAGCCCAGCAACACCAAAGT GGACAAGAAGGTGGAGCCCAAGAGCTGTGATTGA
104	Light Chain antiCD33	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGACATCCAGATGACCCAGAG CCCCAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGA CCATCACCTGTGCGGCCAGCGAGAGCGTGGACAACTAC GGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGG CAAGGCCCCCAAGCTGCTGATCTACGCCGCCAGCAATC AGGGCAGCGGCGTGCCCAGCAGATTACAGCGGCTCTGGC AGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCA GCCCCGACGACTTCGCCACCTACTACTGCCAGCAGAGCA AAGAGGTGCCCTGGACCTTCGGCCAGGGCACCAAGGTG GAAATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATC TTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGC TCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAG GCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATC GGGTAAGTCCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTG CGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAA AGAGCTTCAACAGGGGAGAGTGTTAG
105	Light Chain (CD3) _(V9)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGAGGTGCAGCTGGTCGAGAG

SEQ ID. NO.	Description	Sequence
	(VH-CL)	CGGAGGCGGCCTGGTGCAGCCTGGCGGCAGCCTGAGAC TGAGCTGCGCCGCCAGCGGCTACAGCTTCACCGGCTAC ACCATGAACTGGGTCCGGCAGGCACCTGGCAAGGGACT GGAATGGGTGGCCCTGATCAACCCCTACAAGGGCGTGA GCACCTACAACCAGAAGTTCAAGGACCGGTTACCATC AGCGTGGACAAGAGCAAGAACACCGCCTATCTGCAGAT GAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACT GCGCCAGAAGCGGCTACTACGGCGACAGCGACTGGTAC TTCGACGTGTGGGGCCAGGGCACCCCTCGTGACCGTGTCT AGCGCTAGCGTGGCTGCACCATCTGTCTTCATCTTCCCG CCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGA CAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAA GTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGTTGA
106	Fab(CD33)- CrossFab (CD3 _(v9)) (VH-CH1—VL- CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCCAGGTGCAGCTGGTGCAGTCT GGCGCCGAAGTGAAGAAACCCGGCAGCAGCGTGAAGG TGTCCTGCAAGGCCAGCGGCTACACCTTCACCGACTAC AACATGCACTGGGTCCGCCAGGCCCCAGGCCAGGGACT GGAATGGATCGGCTACATCTACCCCTACAACGGCGGCA CCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCATC ACCGCCGACGAGAGCACCAACACCGCCTACATGGAAGT GAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTACT GCGCCAGAGGCAGACCCGCCATGGACTACTGGGGCCAG GGCACCCCTGGTGACAGTGTCCAGCGCCAGCACAAAGGG CCCTAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCA CAAGCGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAG GACTACTTCCCCGAGCCCGTGACAGTGTCTTGGAACAG CGGAGCCCTGACAAGCGGCGTGACACCTTCCCTGCCG TGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTG GTCACCGTGCCTAGCAGCAGCCTGGGCACCCAGACCTA CATCTGCAACGTGAACCACAAGCCCAGCAACACCAAAG TGGACAAGAAGGTGGAGCCCAAGAGCTGTGATGGCGG AGGAGGGTCCGGAGGCGGTGGATCCGACATCCAGATGA CCCAGAGCCCCTCTAGCCTGAGCGCCAGCGTGGGCGAC AGAGTGACCATCACCTGTGCGGGCCAGCCAGGACATCAG AAACTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGG CCCCAAGCTGCTGATCTACTACACCTCTAGACTGGAAA GCGGCGTGCCAGCCGGTTTAGCGGCAGCGGCTCCGGC ACCGACTACACCCTGACCATCAGCAGCCTGCAGCCCGA GGACTTCGCCACCTACTACTGCCAGCAGGGCAACACAC TCCCCTGGACCTTCGGCCAGGGCACCAAGGTGGAGATC AAGTCCAGCGCTAGCACCAAGGGCCCCTCCGTGTTCCC

SEQ ID. NO.	Description	Sequence
		CCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAG CCGCCCTCGGCTGCCTGGTCAAGGACTACTTCCCCGAGC CCGTGACCGTGTCTTGAACAGCGGAGCCCTGACCTCC GGCGTGACACCTTCCCCGCCGTGCTGCAGAGCAGCGG CCTGTACAGCCTGTCCAGCGTGGTACACCGTGCCCTCCAG CAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACC ACAAGCCCAGCAATACCAAGGTGGACAAGAAGGTGGA GCCCAAGAGCTGCTGA
107	MCSP CDR1 VH	GGCTACTCCATCACCAGTGGTTATTACTGGAAC
108	MCSP CDR2 VH	TACATAACCTACGACGGTAGCAATAACTACAACCCATC TCTCAAAAAT
109	MCSP CDR3 VH	TTTGACTAC
110	MCSP CDR1 VL	AGTGCAAGTCAGGGCATTAGAAATTATTTAAAC
111	MCSP CDR2 VL	TACACATCAAGTTTACACTCA
112	MCSP CDR3VL	CAGCAGTATAGTAAGCTTCCTTGGACG
113	GA201 CDR1 VH	GACTACAAGATACAC
114	GA201 CDR2 VH	TATTTCAACCCTAACAGCGGTTATAGTACCTACGCACAG AAGTTCCAGGGC
115	GA201 CDR3 VH	CTATCCCCAGGCGGTTACTATGTTATGGATGCC
116	GA201 CDR1 VL	CGGGCAAGTCAGGGCATTAAACAATTACTTAAAT
117	GA201 CDR2 VL	AATACCAACAACCTTGCAGACA
118	GA201 CDR3 VL	TTGCAGCATAATAGTTTTCCACG
119	GA201 VH	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAA GCCTGGGTCTCGGTGAAGGTCTCCTGCAAGGCCTCTGG TTTCACATTCACTGACTACAAGATACTGGGTGCGACA GGCCCCTGGACAAGGGCTCGAGTGGATGGGATATTTCA ACCCTAACAGCGGTTATAGTACCTACGCACAGAAGTTC CAGGGCAGGGTCACCATTACCGCGGACAAATCCACGAG CACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGG ACACGGCCGTGTATTACTGTGCGAGACTATCCCCAGGC GGTACTATGTTATGGATGCCTGGGGCCAAGGGACCAC CGTGACCGTCTCCTCA
120	GA201 VL	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCA TCTGTGCGGAGACCGGGTCACCATCACCTGCCGGGCAAG TCAGGGCATTAAACAATTACTTAAATTGGTACCAGCAGA AGCCAGGGAAAGCCCCTAAGCGCCTGATCTATAATACC

SEQ ID. NO.	Description	Sequence
		AACAACCTTGCAGACAGGCGTCCCATCAAGGTTTCAGCGG CAGTGGATCCGGGACAGAATTCCTCTCACCATCAGCA GCCTGCAGCCTGAAGATTTTGCCACCTATTACTGCTTGC AGCATAATAGTTTTCCACGTTTGGCCAGGGCACCAAG CTCGAGATCAAG
121	3F2 CDR1 VH	AGCTACGCCATGAGC
122	3F2 CDR2 VH	GCCATCTCCGGCAGCGGAGGCAGCACCTACTACGCCGA CAGCGTGAAG
123	3F2 CDR3 VH	TATTGCGCCAAGGGATGGTTCGGC
124	3F2 CDR1 VL	AGAGCCAGCCAGAGCGTGACCAGCAGCTACCTG
125	3F2 CDR2 VL	AACGTGGGCAGCAGACGGGCC
126	3F2 CDR3 VL	TGCCAGCAGGGCATCATGCTGCCCCC
127	3F2 VH	GAGGTGCAGCTGCTGGAATCTGGAGGCGGCCTGGTGCA GCCTGGCGGCAGCCTGAGACTGTCTTGCGCCGCCAGCG GCTTCACCTTCAGCAGCTACGCCATGAGCTGGGTCCGAC AGGCTCCTGGCAAGGGACTGGAATGGGTGTCCGCCATC TCCGGCAGCGGAGGCAGCACCTACTACGCCGACAGCGT GAAGGGCCGGTTCACCATCAGCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGA GGATACCGCCGTGTATTATTGCGCCAAGGGATGGTTCG GCGGCTTCACTACTGGGGCCAGGGAACCCTGGTGACA GTGTCCAGC
128	3F2 VL	GAGATCGTGCTGACCCAGTCTCCCGGCACCCTGAGCCT GAGCCCTGGCGAGAGAGCCACCCTGAGCTGCAGAGCCA GCCAGAGCGTGACCAGCAGCTACCTGGCCTGGTATCAG CAGAAGCCCCGGCCAGGCCCCCAGACTGCTGATCAACGT GGGCAGCAGACGGGGCCACCGGCATCCCCGATAGATTCA GCGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATC AGCCGGCTGGAACCCGAGGACTTCGCCGTGTACTACTG CCAGCAGGGCATCATGCTGCCCCCACCCTTCGGCCAGG GCACCAAGGTGGAATCAAG
129	CH1A1A CDR1 VH	GAGTTCGGCATGAAC
130	CH1A1A CDR2 VH	TGGATCAACACCAAGACCGGCGAGGCCACCTACGTGGA AGAGTTCAAGGGC
131	CH1A1A CDR3 VH	TGGGACTTCGCCTATTACGTGGAAGCCATGGACTAC
132	CH1A1A CDR1 VL	AAGGCCAGTGCGGCTGTGGGTACGTATGTTGCG
133	CH1A1A CDR2 VL	TCGGCATCCTACCGCAAAAGG
134	CH1A1A CDR3 VL	CACCAATATTACACCTATCCTCTATTACG
135	CH1A1A VH	CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA ACCTGGAGCTAGTGTGAAGGTGTCCTGCAAGGCCAGCG GCTACACCTTCACCGAGTTCGGCATGAACTGGGTCCGA

SEQ ID. NO.	Description	Sequence
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136	CH1A1A VL	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCA TCTGTGGGAGACAGAGTCACCATCACTTGCAAGGCCAG TGCGGCTGTGGGTACGTATGTTGCGTGGTATCAGCAGA AACCAGGGAAAGCACCTAAGCTCCTGATCTATTCGGCA TCCTACCGCAAAAGGGGAGTCCCATCAAGGTTCAAGTGG CAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA GTCTGCAACCTGAAGATTTGCAACTTACTACTGTCACC AATATTACACCTATCCTCTATTCACGTTTGGCCAGGGCA CCAAGCTCGAGATCAAG
137	Anti-CD33 CDR1 VH	GGCTACACCATCACCGACAGCAACATCCAC
138	Anti-CD33 CDR2 VH	TACATCTACCCCTACAACGGCGGCACCGACTACAACCA G
139	Anti-CD33 CDR3 VH	GGCAACCCCTGGCTGGCCTAT
140	Anti-CD33 CDR1 VL	CGGGCCAGCGAGAGCCTGGACAACCTACGGCATCCGGTT TCTGACC
141	Anti-CD33 CDR2 VL	GCCGCCAGCAACCAGGGCAGC
142	Anti-CD33 CDR3 VL	CAGCAGACCAAAGAGGTGCCCTGGTCC
143	Anti-CD33 VH	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA ACCCGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGCG GCTACACCATCACCGACAGCAACATCCACTGGGTCCGA CAGGCCCTGGGCAGAGCCTGGAATGGATCGGCTACAT CTACCCCTACAACGGCGGCACCGACTACAACCAGAAGT TCAAGAACCGGGCCACCCTGACCGTGGACAACCCACC AACACCGCCTACATGGAAGTGAAGCAGCCTGCGGAGCGA GGACACCGCCTTCTACTACTGCGTGAACGGCAACCCCT GGCTGGCCTATTGGGGCCAGGGAACCCTGGTCACCGTG TCTAGC
144	Anti-CD33 VL	GACATCCAGCTGACCCAGAGCCCCAGCACCCCTGTCTGC CAGCGTGGGCGACAGAGTGACCATCACCTGTCGGGCCA GCGAGAGCCTGGACAACCTACGGCATCCGGTTTCTGACC TGGTTCCAGCAGAAGCCCCGGCAAGGCCCCCAAGCTGCT GATGTACGCCGCCAGCAACCAGGGCAGCGGCGTGCCAA GCAGATTCAGCGGCAGCGGCTCCGGCACCGAGTTCACC CTGACCATCAGCAGCCTGCAGCCCGACGACTTCGCCAC CTACTACTGCCAGCAGACCAAAGAGGTGCCCTGGTCCT

SEQ ID. NO.	Description	Sequence
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150	(scFv) ₂ antiMCSP/anti huCD3 (LC007(VL- VH)—V9(VH- VL))	ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCCACA GCCACCGGTGTGCATTCCGACATCGTGCTGACCCAGAG CCCCAGCAGCCTGAGCGCCAGCCTGGGCGACAGAGTGA CCATCAGCTGCAGCGCCTCCCAGGGCATCAGAACTAC CTGAACTGGTATCAGCAGCGGCCCGACGGCACCGTGAA GCTGCTGATCTACTACACCAGCTCCCTGCACAGCGGCGT GCCAGCAGATTTTCAGGCAGCGGCAGCGGCACTGACT ACAGCCTGACCATCTCCAACCTGGAACCCGAGGACATT GCCACCTACTACTGCCAGCAGTACAGCAAGCTGCCCTG GACCTTCGGCGGAGGACCAAGCTGGAAATCAAGGGCG GAGGCGGATCCGGCGGAGGTGGAAGTGGCGGCGGAGG CTCTGAGGTGCAATTGCAGGAAAGCGGCCCTGGCCTGG TGAAACCCAGCCAGAGCCTGAGCCTGACCTGCAGCGTG ACCGGCTACTCCATCACCAGCGGCTACTACTGGAAGT GATCAGACAGTTCCCCGGAAACAAGCTGGAATGGATGG GCTACATCACCTACGACGGCAGCAACAACCTACAACCCC AGCCTGAAGAACCGGATCAGCATCACCCGGGACACCAG CAAGAACCAGTTCTTCCTGAAGCTGAACAGCGTGACCA CCGAGGATACCGCCACCTATTACTGTGCCGACTTCGACT ACTGGGGCCAGGGCACCAACCTGACCGTGTTCATCCGGT GGCGGCGGATCCGAAGTGCAGCTGGTGGAGTCTGGCGG TGGACTGGTGCAGCCAGGCGGCTCCCTGAGACTGAGCT GCGCCGCTCCGGCTACAGCTTCACCGGCTACACCATG AATTGGGTCCGCCAGGCCCTGGAAAGGGACTGGAATG GGTGGCCCTGATCAACCCCTACAAGGGCGTGAGCACCT ACAACCAGAAGTTCAAGGACCGGTTACCATCAGCGTG GACAAGAGCAAGAACACAGCCTACCTGCAGATGAATC CCTGAGAGCCGAGGATACCGCCGTGTATTACTGTGCCC GCAGCGGCTACTACGGCGACTCCGACTGGTACTTCGAC GTGTGGGGGCAGGGAACCTGGTCACCGTGTCCAGCGT GGAAGGCGGCAGCGGAGGATCTGGCGGCTCTGGCGGA AGCGGCGGAGTGGACGATATCCAGATGACACAGTCCCC CAGCTCCCTGAGCGCCAGCGTGGGCGACAGAGTGACCA TCACCTGTCGGGCCAGCCAGGACATCCGGAATTATCTC AATTGGTATCAGCAGAAACCTGGCAAAGCTCCTAACT GCTGATCTACTACACCTCCCGGCTGGAAAGCGGCGTGC CCAGCAGATTTTCCGGCAGCGGGAGCGGCACCGATTAC ACACTGACCATCAGCAGCCTGCAGCCCGAGGACTTTGC CACCTACTATTGCCAGCAGGGCAACACCCTGCCCTGGA CCTTTGGGCAGGGCACAAAGGTGGAGATCAAGCACCAC CACCATCACCCTGA
154	Light Chain antiCD33	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGACATCCAGCTGACCCAGAG CCCCTCCACACTCTCTGCCTCAGTGGGCGATAGGGTCAC CATTACTTGCAGAGCTAGCGAGTCCCTGGACAACTACG GAATCCGCTTCCTTACATGGTTTCAGCAGAAGCCTGGAA

SEQ ID. NO.	Description	Sequence
	(Myelotarg)	AAGCACCAAAGCTGCTCATGTATGCCGCTTCTAATCAA GGCAGTGGTGTGCCCAGCCGGTTCTCCGGGTCTGGCTCA GGAACCGAATTTACTCTGACCATTAGCTCCTTGCAGCCT GATGACTTCGCAACATACTATTGTCAGCAGACCAAGGA GGTCCCATGGTCTTTTGGTCAAGGCACAAAAGTGGAGG TTAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTG TTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGT AACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGG ACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGA AGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA GCTTCAACAGGGGAGAGTGTTAG
155	Light Chain CD3 _(v9) (VL-CH1)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGATATTCAGATGACCCAGAG CCCCAGCTCTCTGAGCGCCAGCGTGGGCGACAGAGTGA CCATCACCTGTCTGGGCCAGCCAGGACATCAGAACTAC CTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGATCTACTACACCAGCAGACTGGAAAGCGGCG TGCCCTCCAGATTTTCCGGCAGCGGCTCCGGCACCGACT ACACCCTGACCATCAGCAGCCTGCAGCCCGAGGATTTC GCCACATATTACTGCCAGCAGGGCAATACCCTGCCCTG GACCTTCGGACAGGGCACAAAAGTGGAAATCAAGAGC AGCGCTTCCACCAAAGGCCCTTCCGTGTTTCTCTGGCT CCTAGCTCCAAGTCCACCTCTGGAGGCACCGCTGCTCTC GGATGCCTCGTGAAGGATTATTTTCTGAGCCTGTGACA GTGTCCTGGAATAGCGGAGCACTGACCTCTGGAGTGCA TACTTTCCCCGCTGTGCTGCAGTCCTCTGGACTGTACAG CCTGAGCAGCGTGGTGACAGTGCCCAGCAGCAGCCTGG GCACCCAGACCTACATCTGCAACGTGAACCACAAGCCC AGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGT CTTGTTGA
156	Fab (CD33 _(Myelotarg))- XFab (CD3 _(v9)) (VH-CH1— VH-CL)	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACA GCTACCGGTGTGCATTCCGAGGTGCAGCTGGTGCAGTCT GGCGCCGAAGTGAAGAAACCCGGCAGCAGCGTGAAGG TGTCTTGCAAGGCCAGCGGCTACACCATCACCGACAGC AACATCCACTGGGTGCGCCAGGCCCTGGCCAGTCTCT GGAATGGATCGGCTACATCTACCCCTACAACGGCGGCA CCGACTACAACCAGAAGTTCAAGAACCGGGCCACCCTG ACCGTGGACAACCCCAACCAATACCGCCTACATGGAAGT GAGCAGCCTGCGGAGCGAGGACACCGCCTTCTACTACT GCGTGAACGGCAACCCCTGGCTGGCCTATTGGGGCCAG GGAACACTCGTGACCGTGTCCAGCGCTAGCACCAAGGG CCCTAGCGTGTTCCCTCTGGCCCCTAGCAGCAAGAGCAC CTCTGGCGGAACAGCCGCCCTGGGCTGCCTCGTGAAGG ACTACTTTCCCGAGCCCGTGACAGTGTCTGGAAGTCTG

SEQ ID. NO.	Description	Sequence
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While there are shown and described presently preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims.

Claims

1. A bispecific antibody comprising at least two fab fragments, wherein
the first fab fragment comprises at least one antigen binding site specific for a first
antigen; and the second fab fragment comprises at least one antigen binding site specific
for a second antigen wherein either the variable regions or the constant regions of the
second Fab heavy and light chain are exchanged; and wherein the bispecific antibody is
devoid of a Fc domain.
2. The bispecific antibody of claim 1, additionally comprising a third fab fragment.
3. The bispecific antibody of claim 2, wherein the third fab fragment comprises at
least one antigen binding site specific for the first or second antigen.
4. The bispecific antibody of claim 2, wherein the third fab fragment comprises at
least one antigen binding site specific for the first antigen.
5. The bispecific antibody of any of claims 2 to 4, wherein the third fab fragment is
connected to the first fab fragment.
6. The bispecific antibody of claim 5, wherein the C-terminus of the third Fab
fragment is connected to the N-terminus of the first Fab fragment.
7. The bispecific antibody of claims 2 to 4, wherein the third Fab fragment is
connected to the second Fab fragment.
8. The bispecific antibody of claim 7, wherein the N-terminus of the third Fab
fragment is connected to the C-terminus of the first Fab fragment.
9. The bispecific antibody of any of claims 1 to 8, wherein the fab fragments are
connected via a peptide linker.
10. The bispecific antibody of claim 9, wherein the peptide linker is a (G4S)₂ linker.
11. A pharmaceutical composition comprising a bispecific antibody of claims 1 to 10.
12. A prokaryotic or eukaryotic host cell comprising vectors comprising nucleic acid
molecules encoding the light chains and heavy chains of the bispecific antibody
of any of claims 1 to 10.
13. A method of producing an antibody comprising culturing the host cell of claim 12
so that the antibody is produced.

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14. An immunoconjugate comprising the antibody of any of claims 1 to 10 and a cytotoxic agent.
15. The invention as described herein, especially with reference to the foregoing examples.

Fig. 1 a)

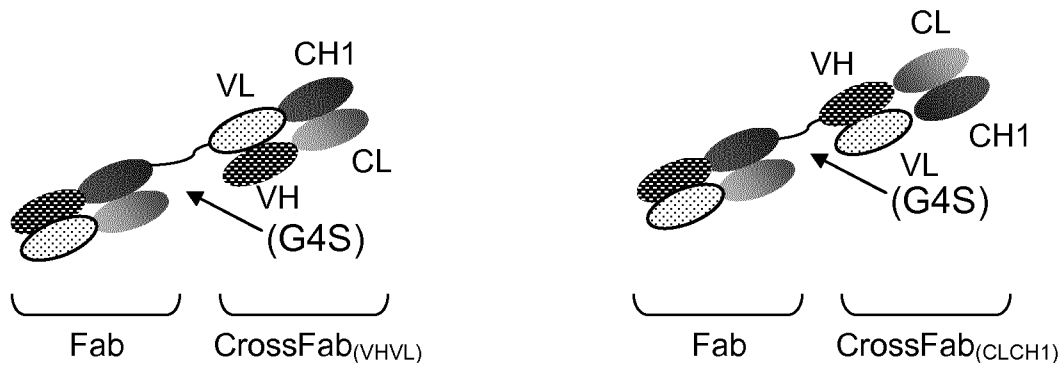


Fig. 1 b)



Fig. 1c)

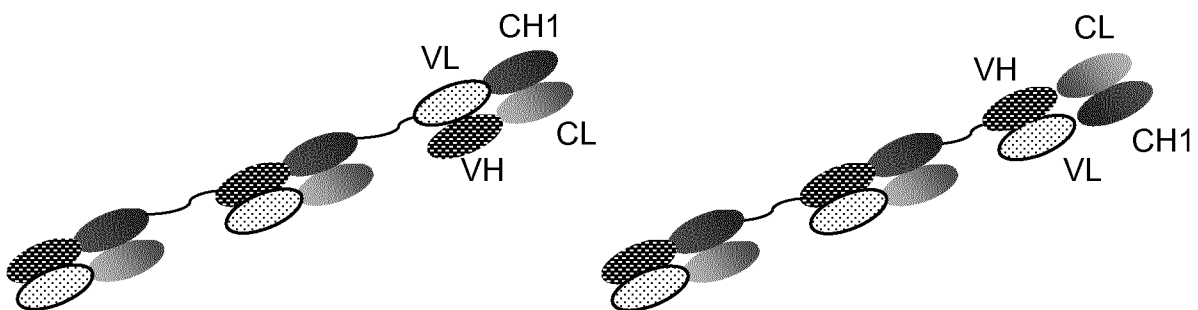


Fig. 1 d)

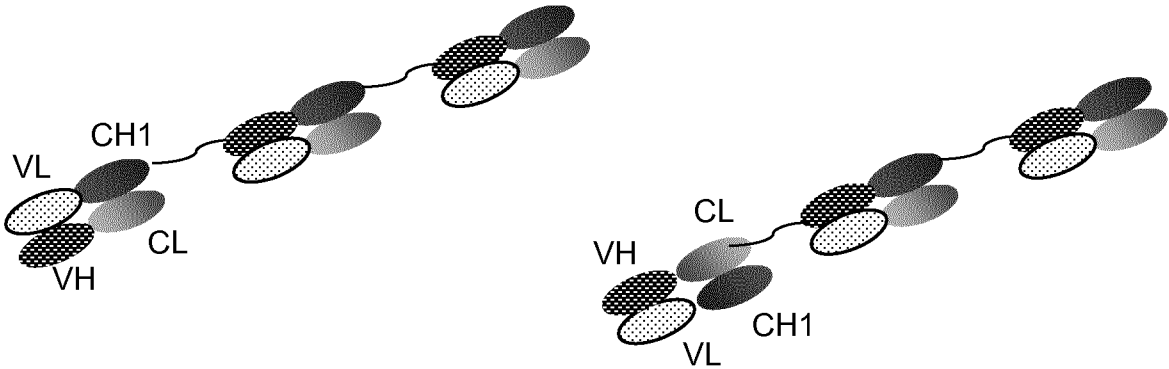


Fig. 1e)

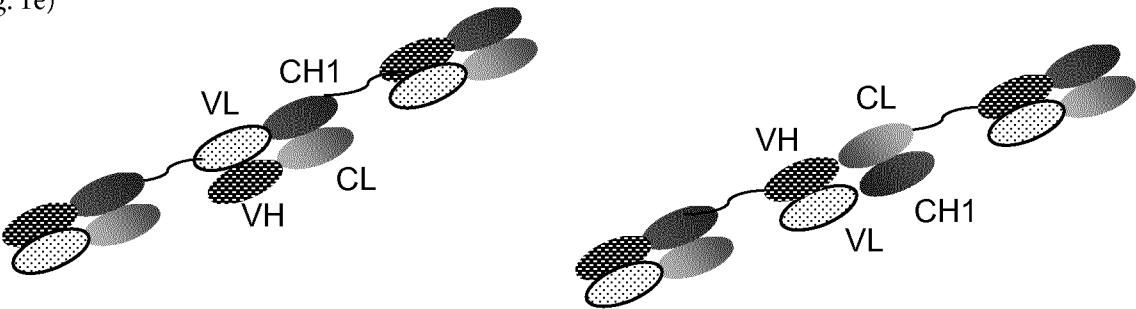


Fig. 2

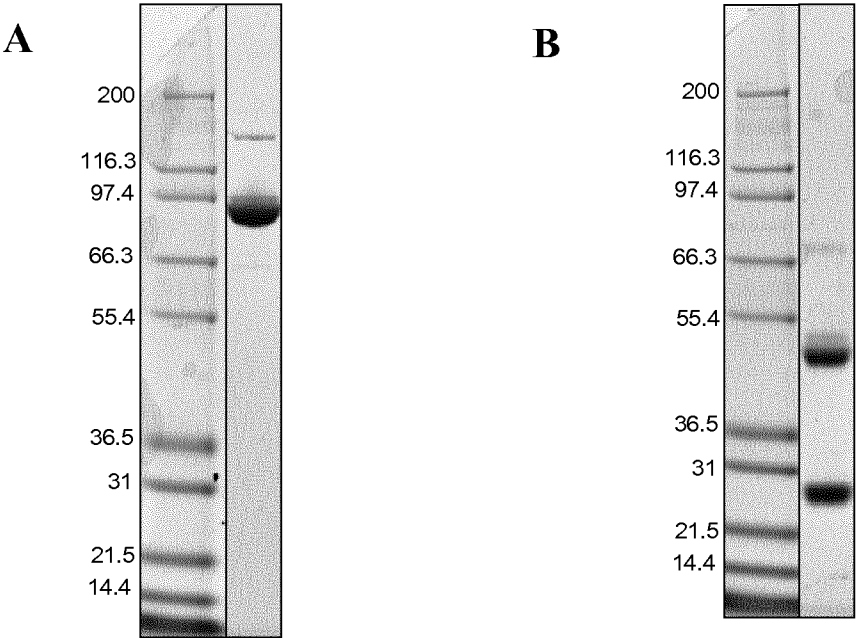


Fig. 3

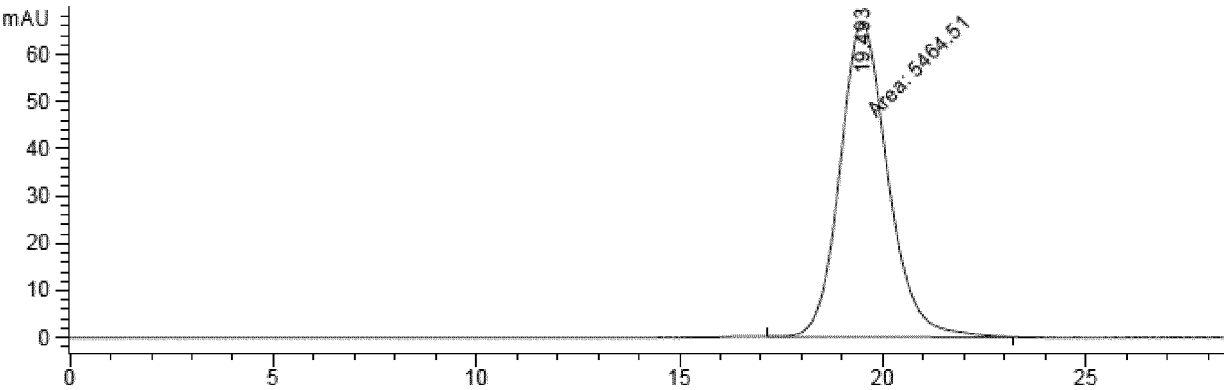


Fig. 4

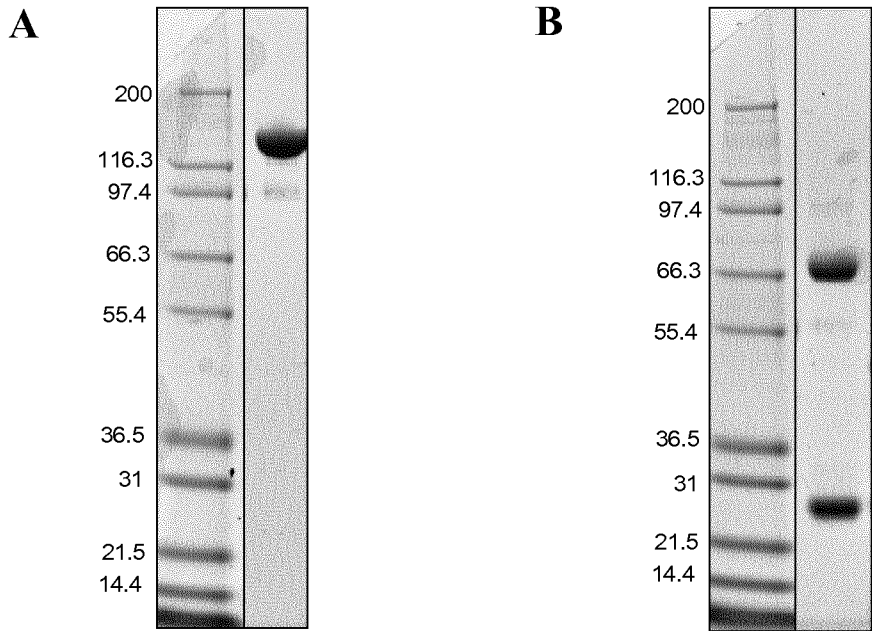


Fig. 5

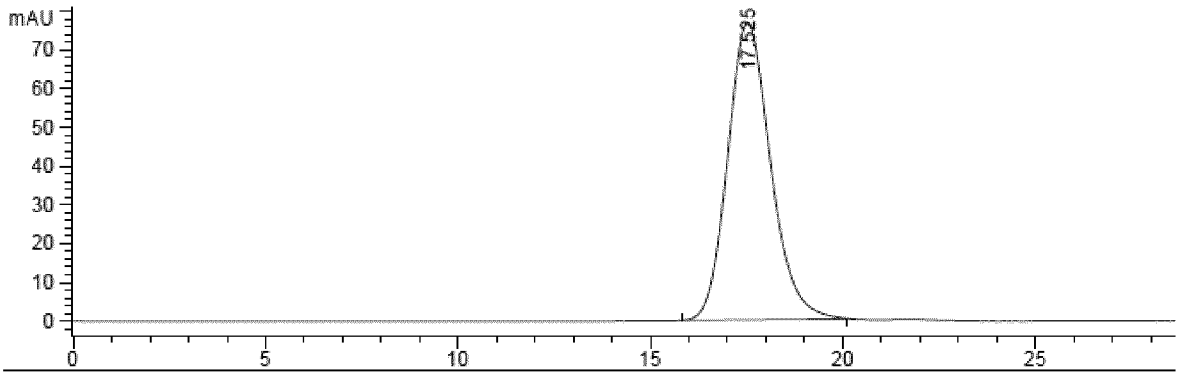


Fig. 6

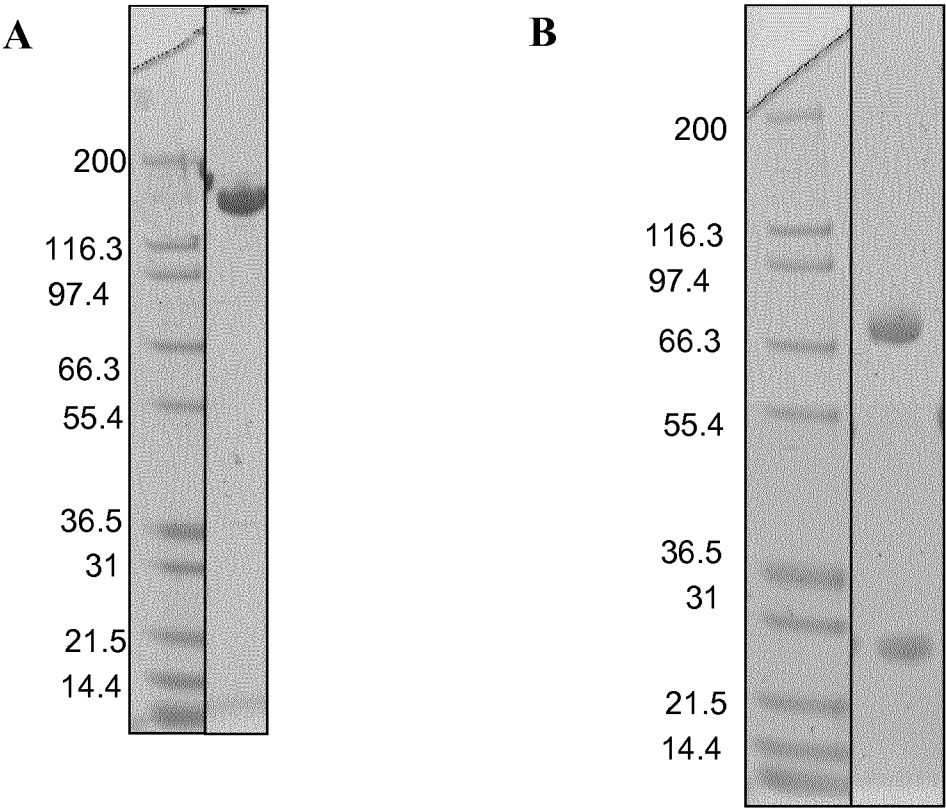


Fig. 7

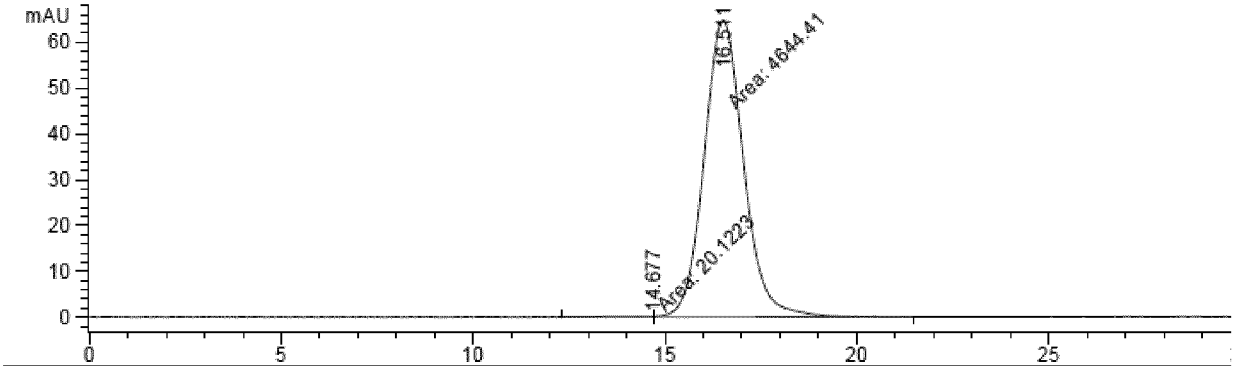


Fig. 8

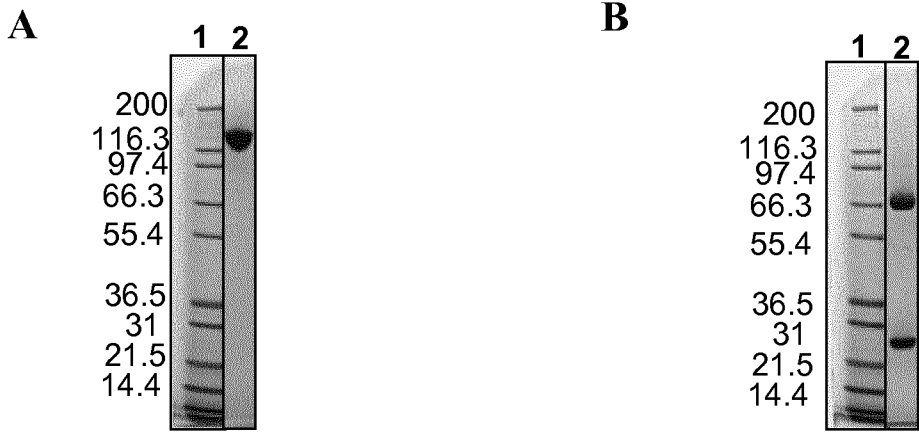


Fig. 9

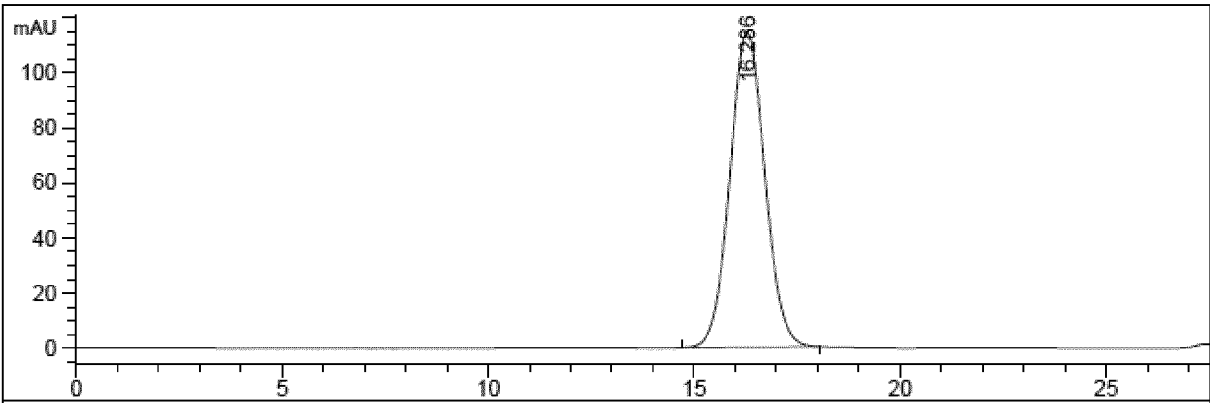


Fig. 10

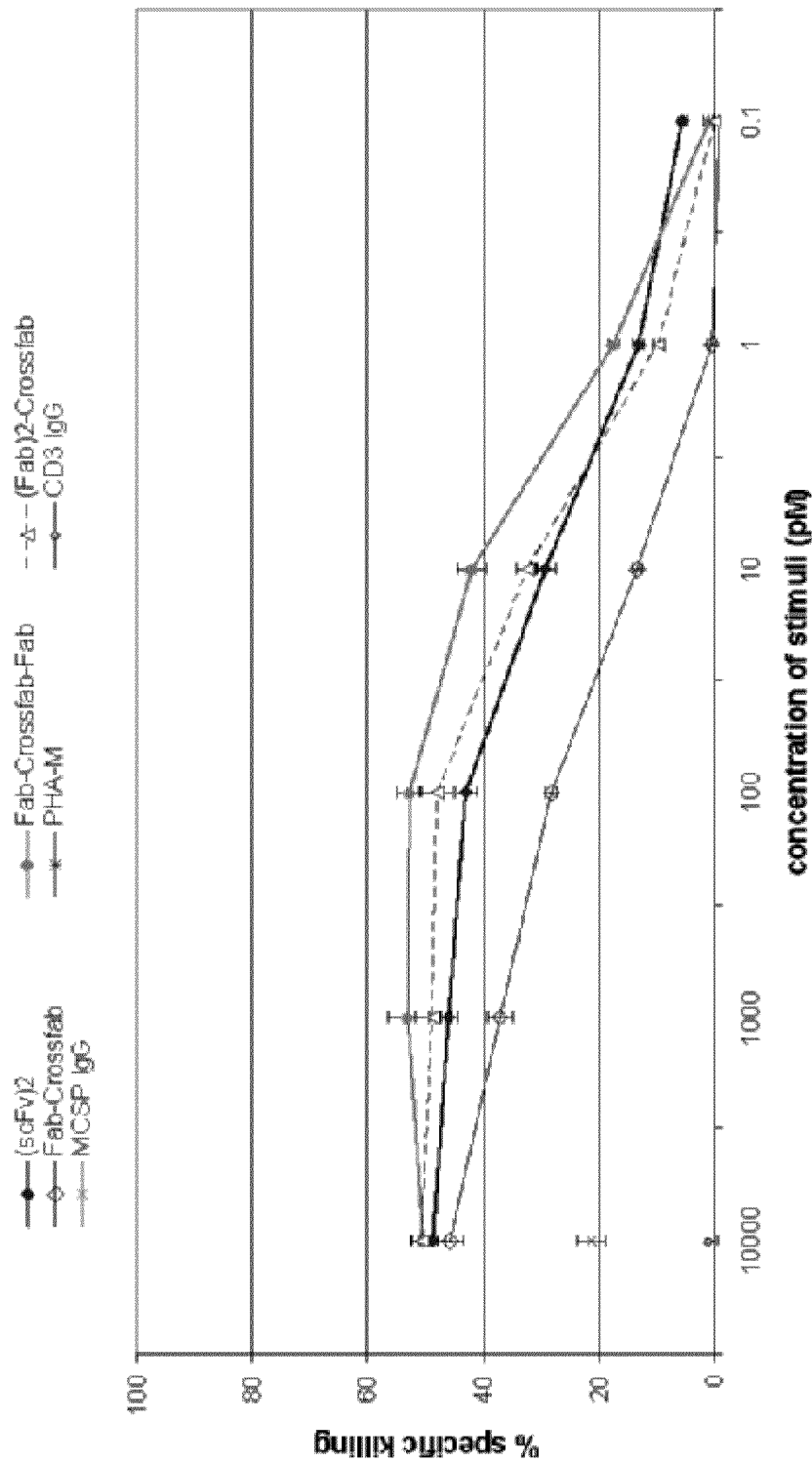


Fig. 11

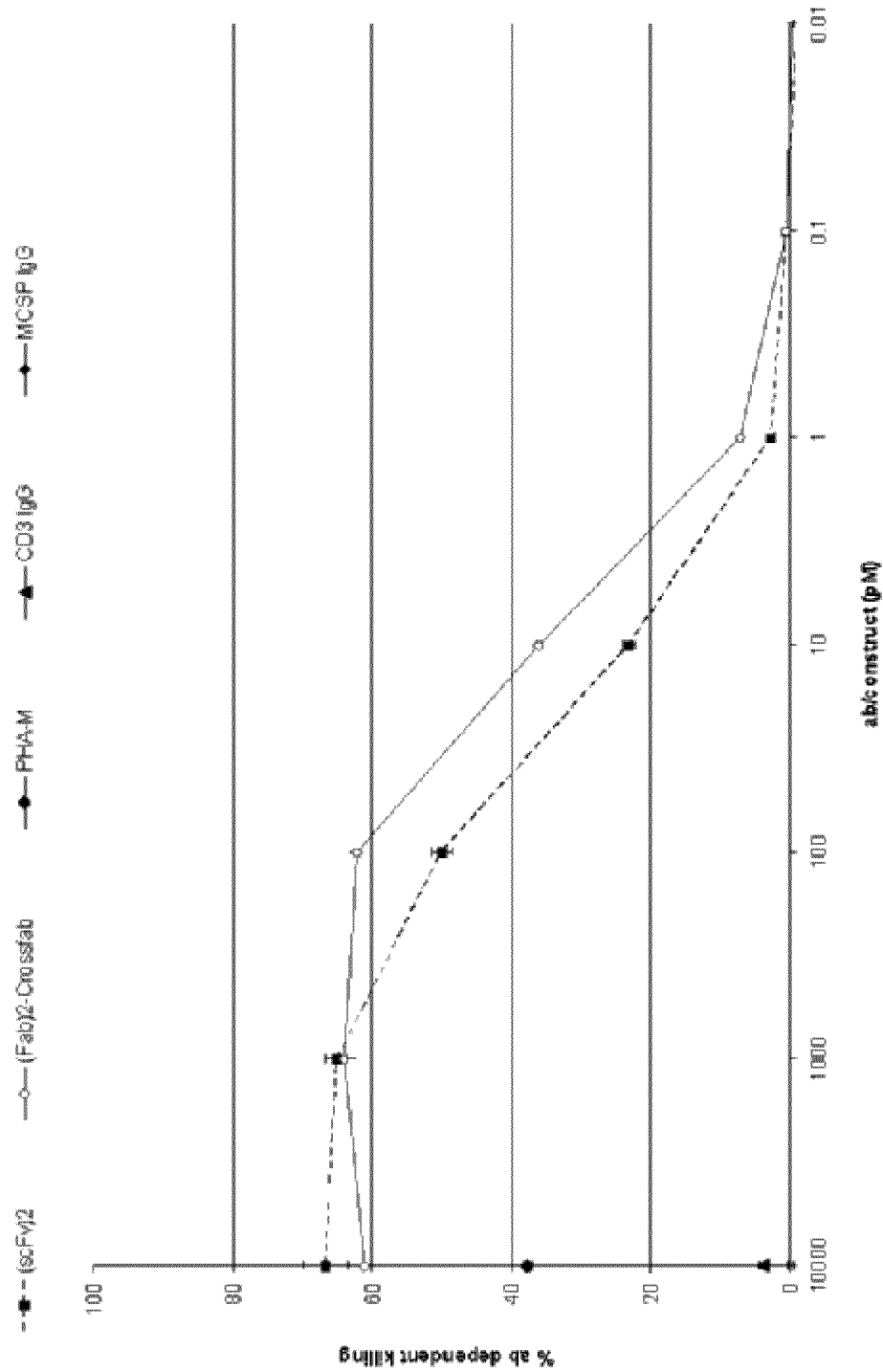


Fig. 12

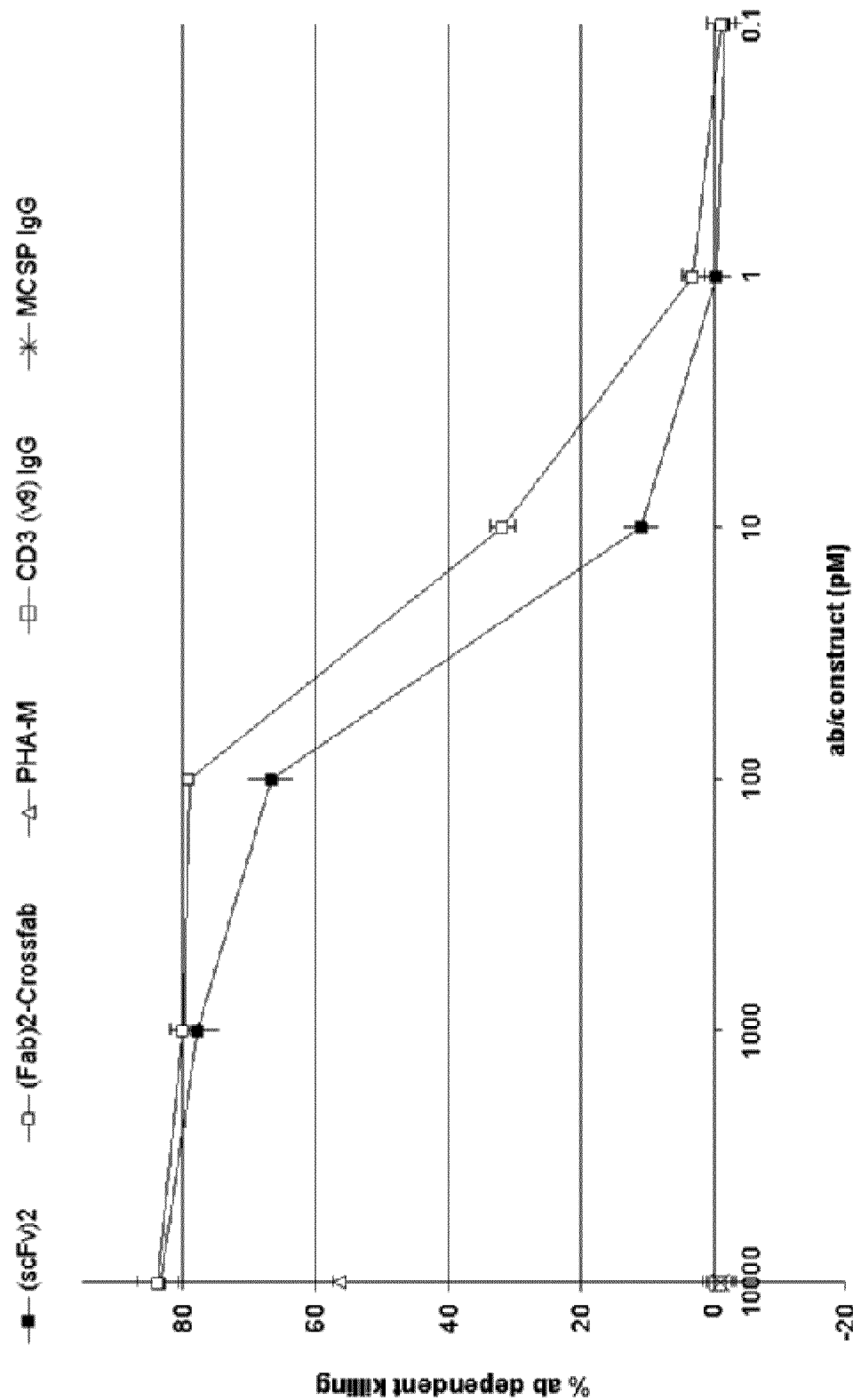


Fig. 13

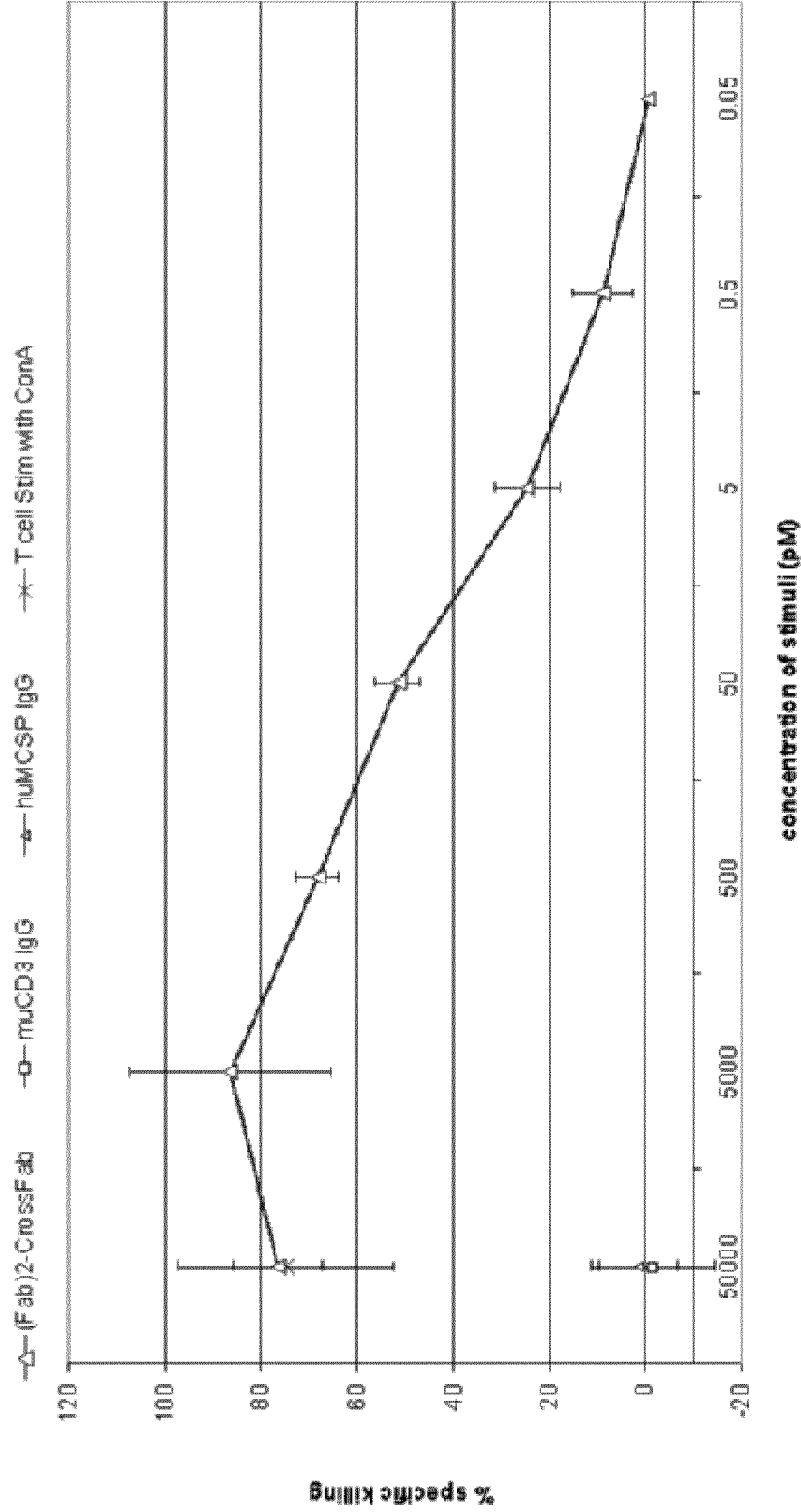


Fig. 14

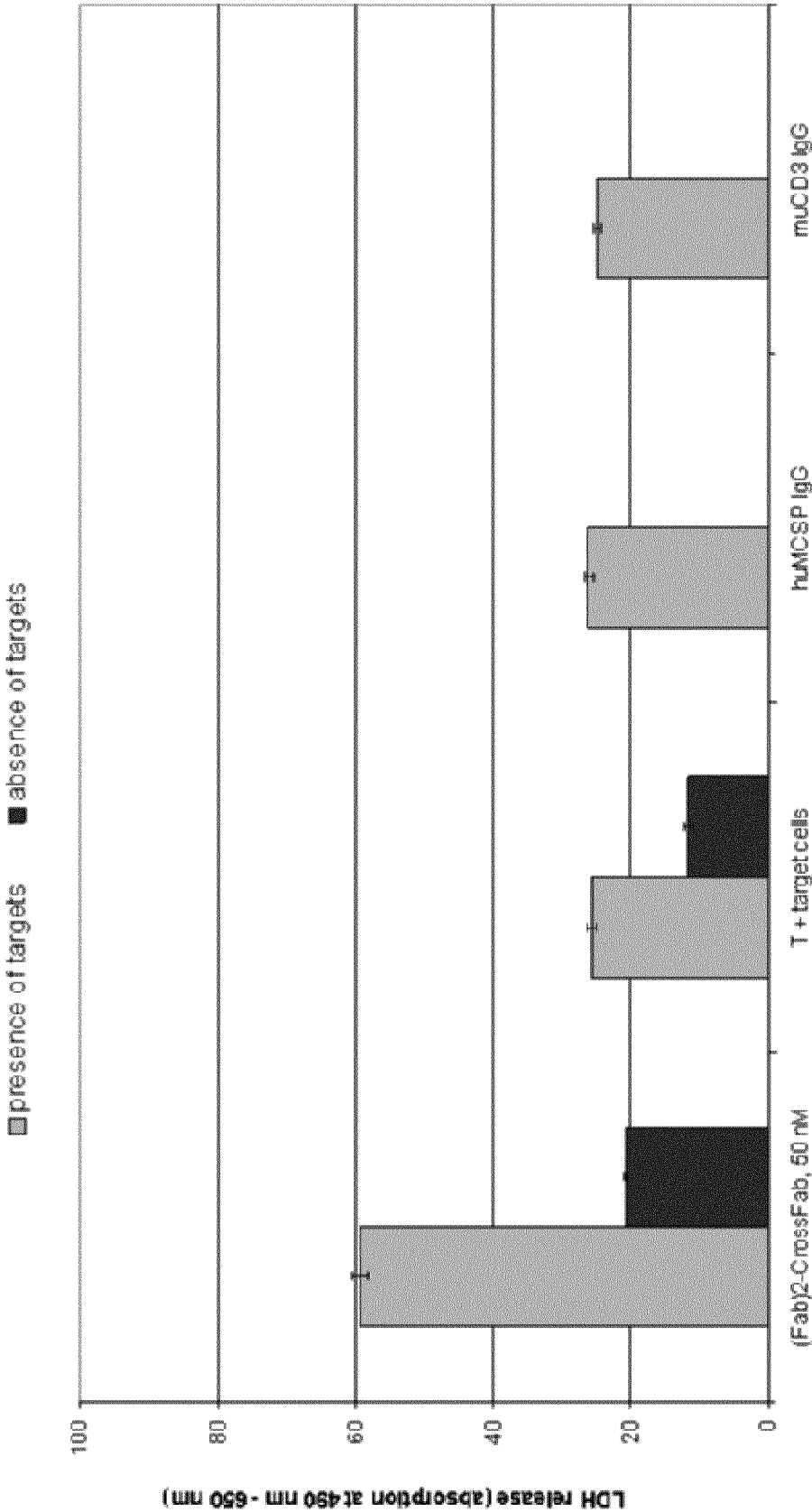


Fig.15 a)

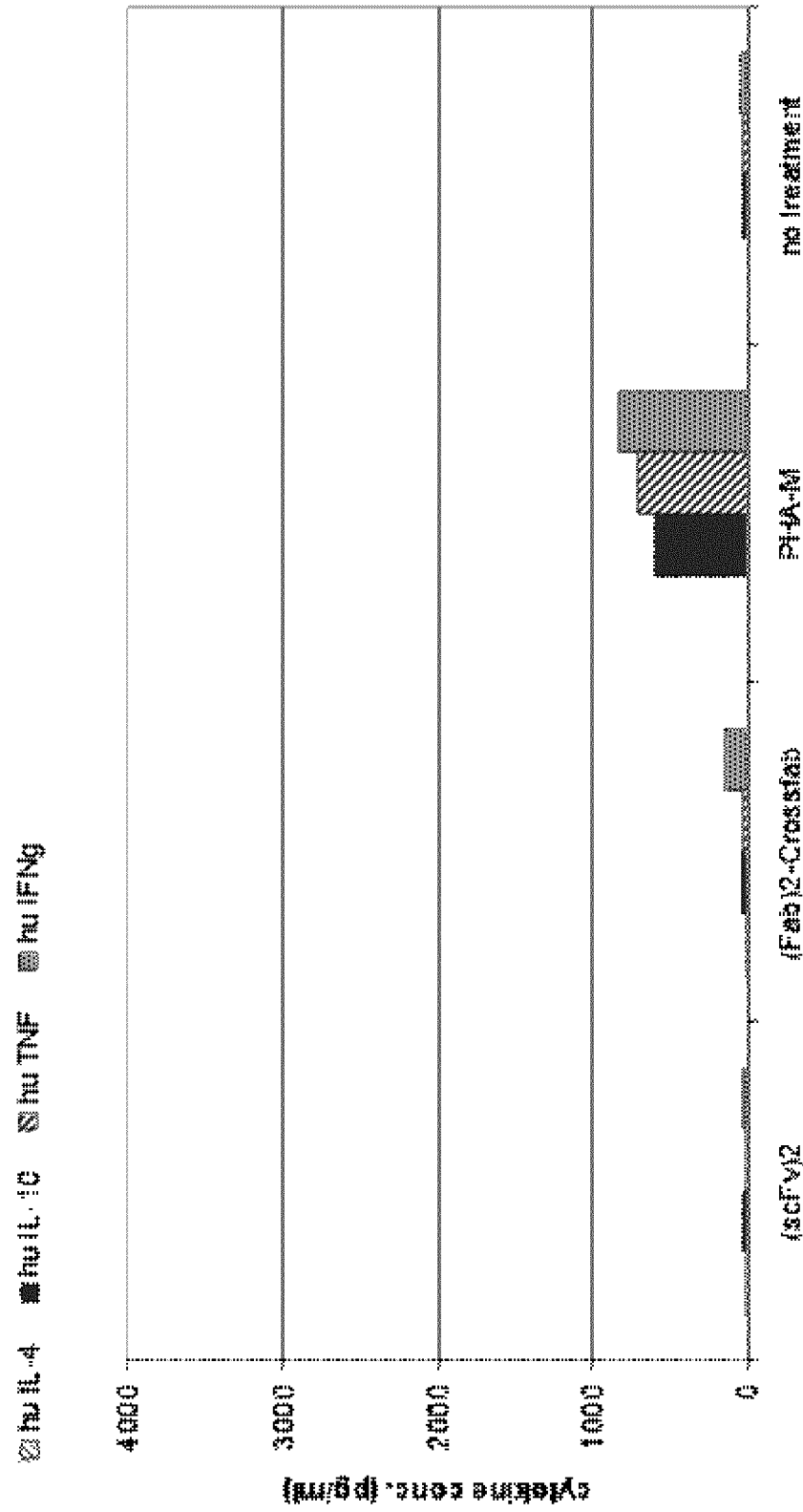


Fig. 15 b)

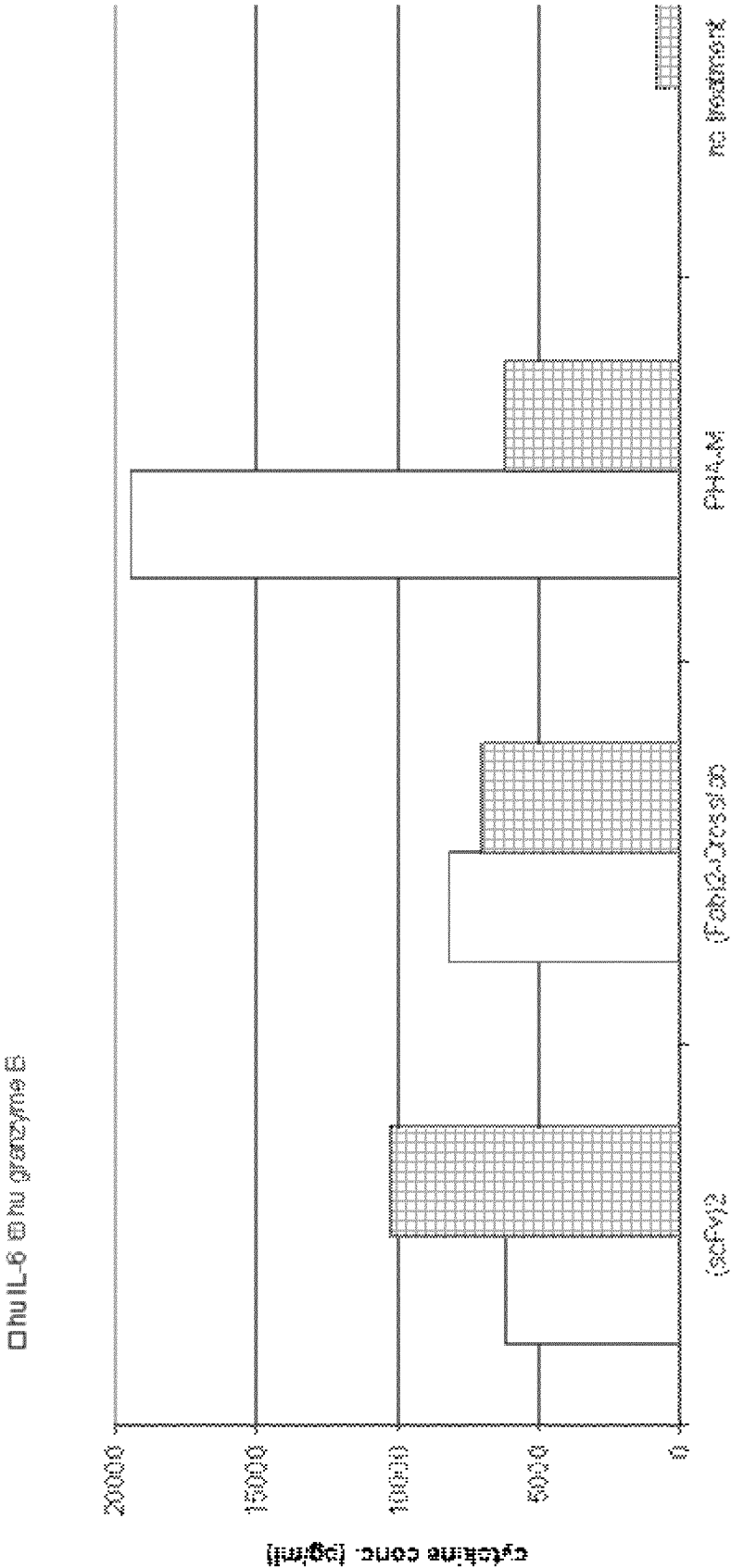


Fig. 15 c)

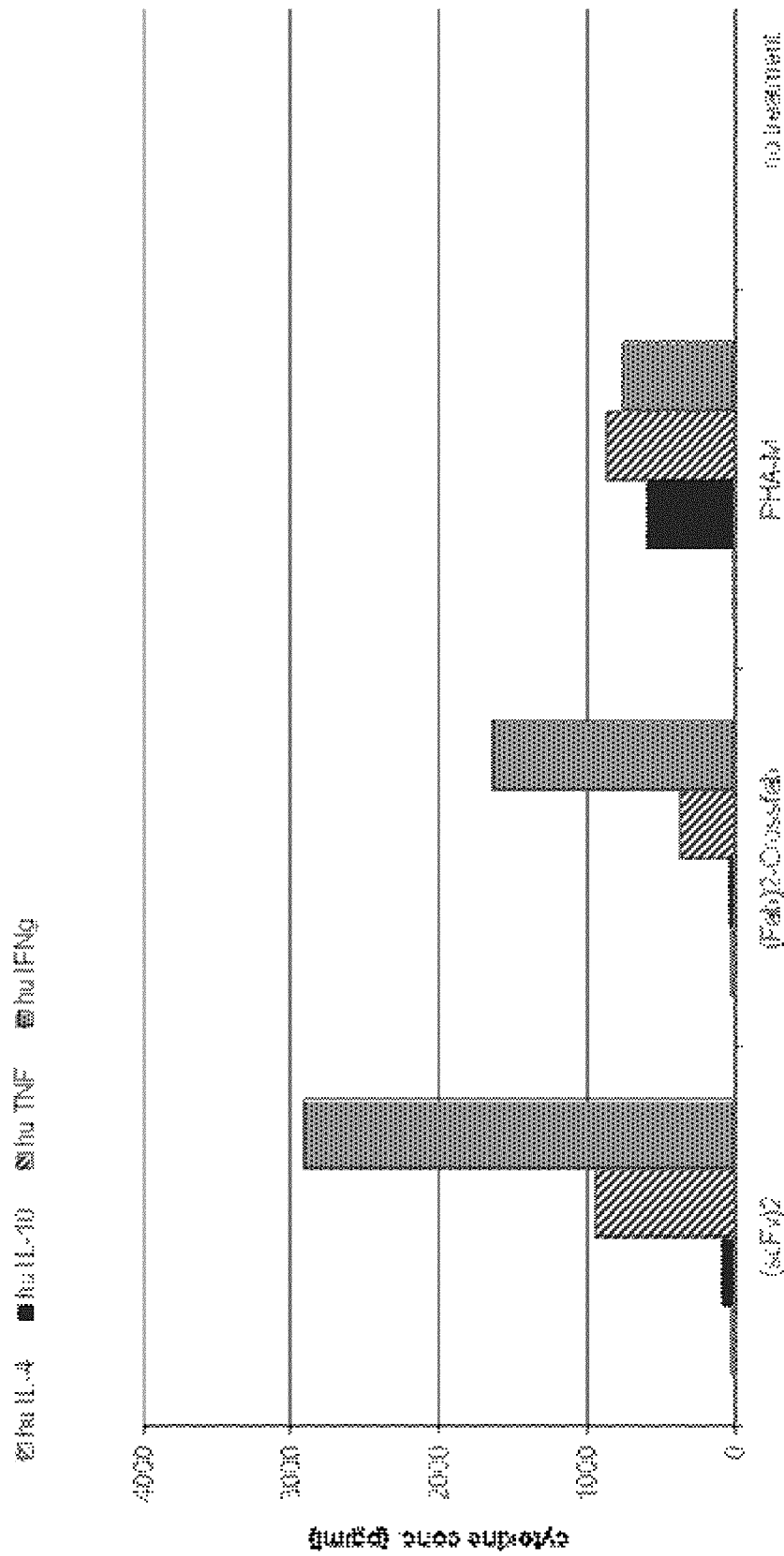


Fig. 16

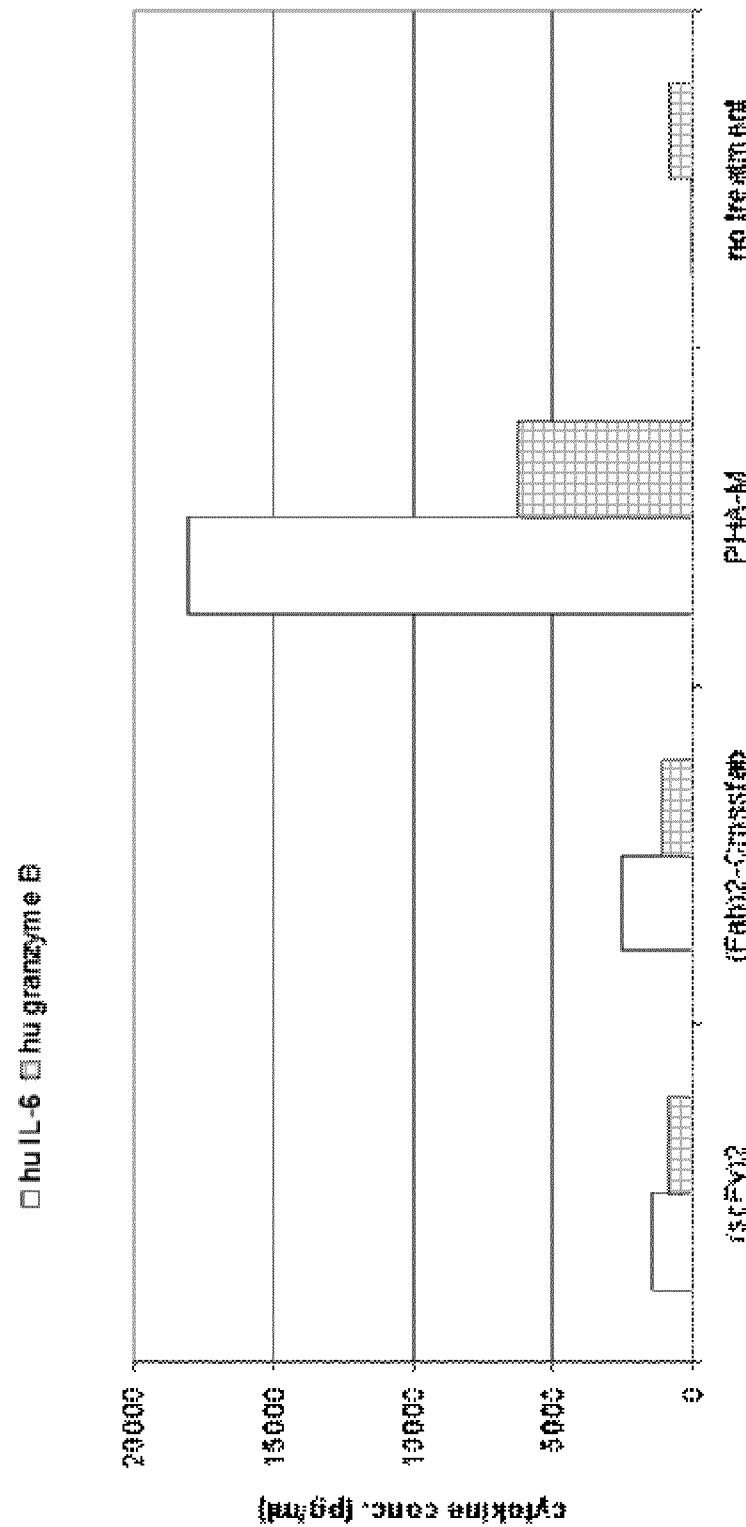


Fig. 17

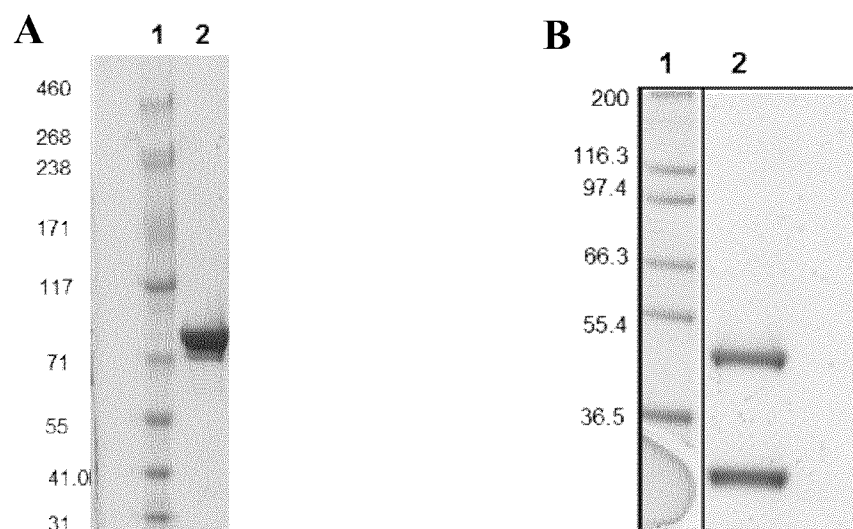


Fig. 18

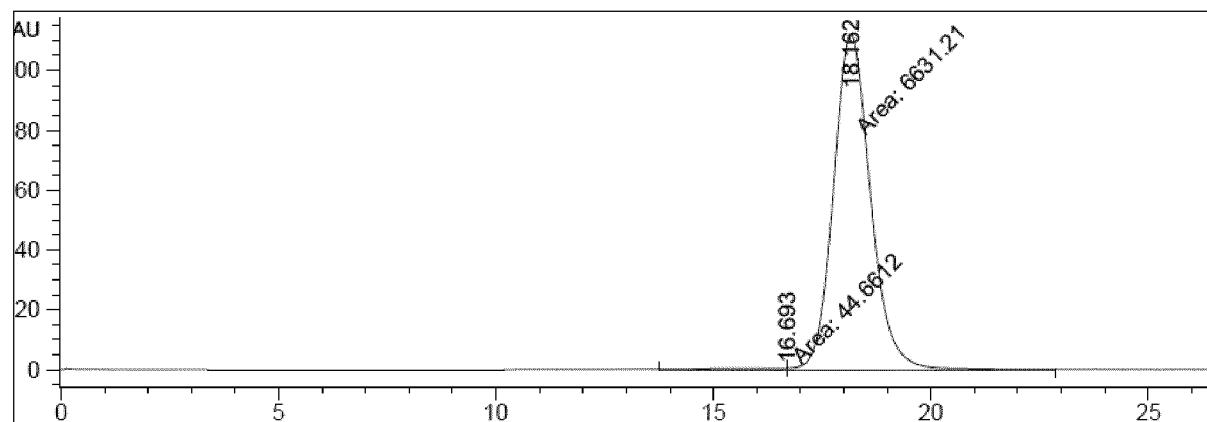
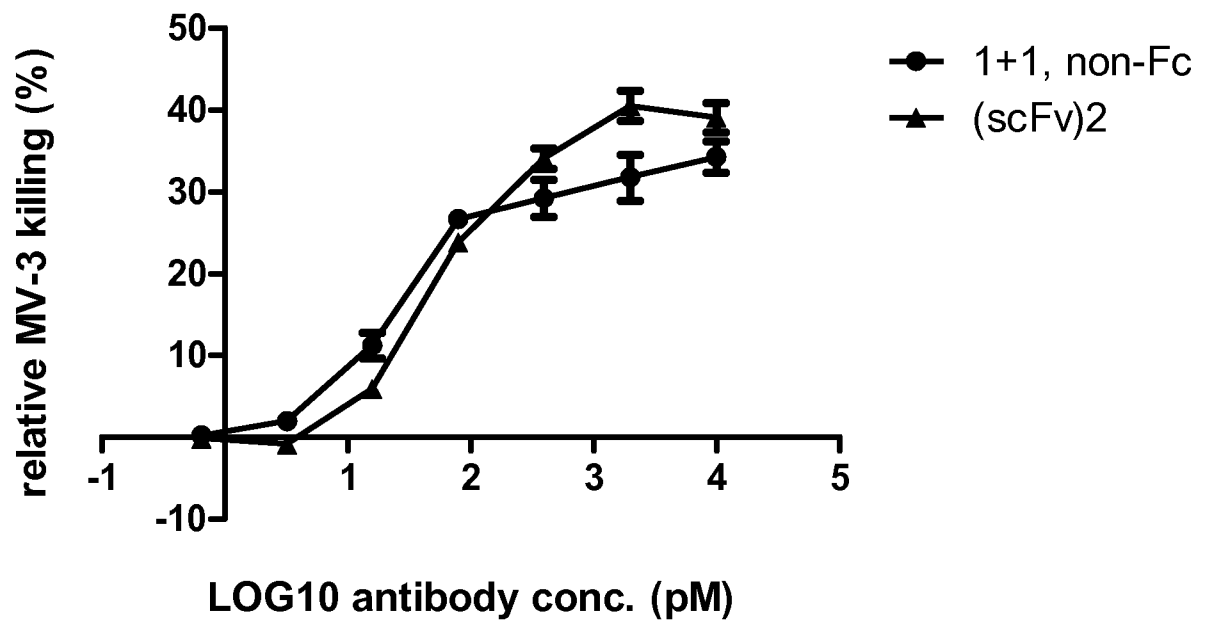


Fig. 19



	1+1, non-Fc	(scFv)2
EC50 (pM)	25.35	57.1

Fig. 20 a)

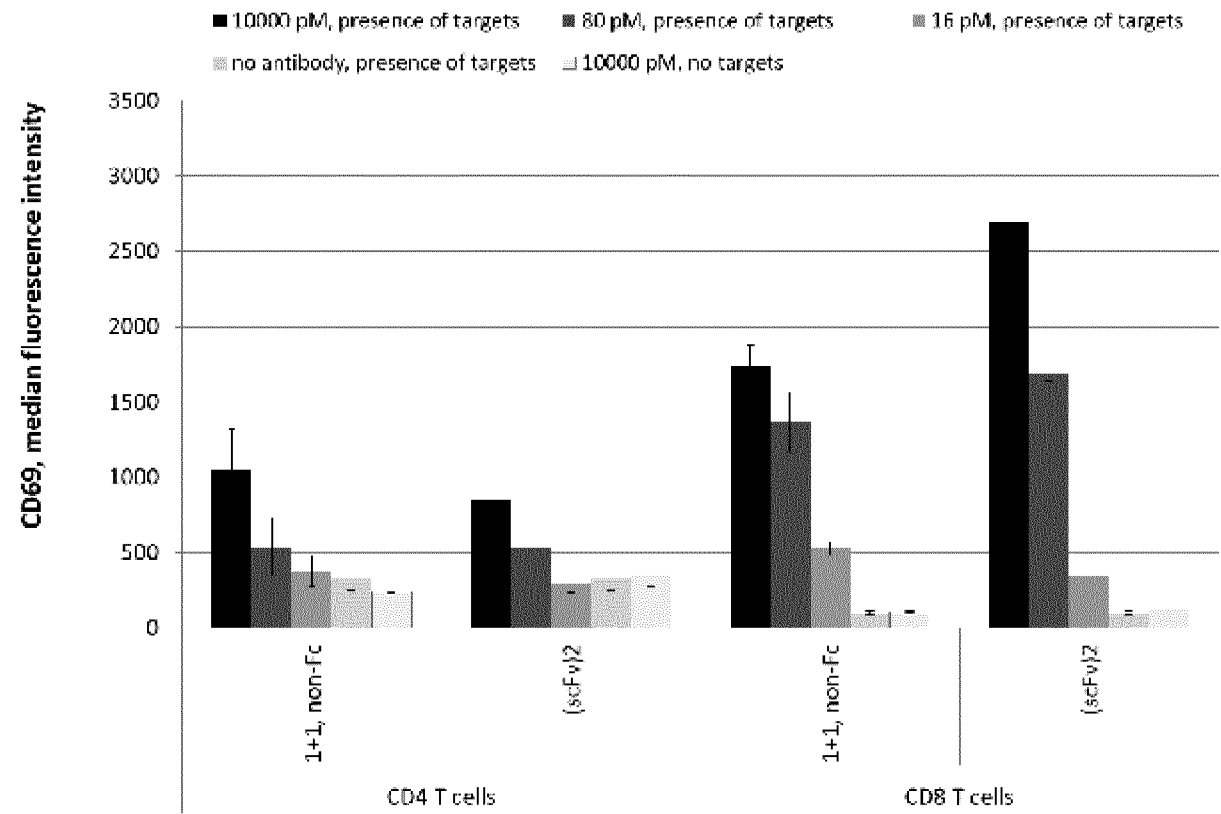


Fig. 20 b)

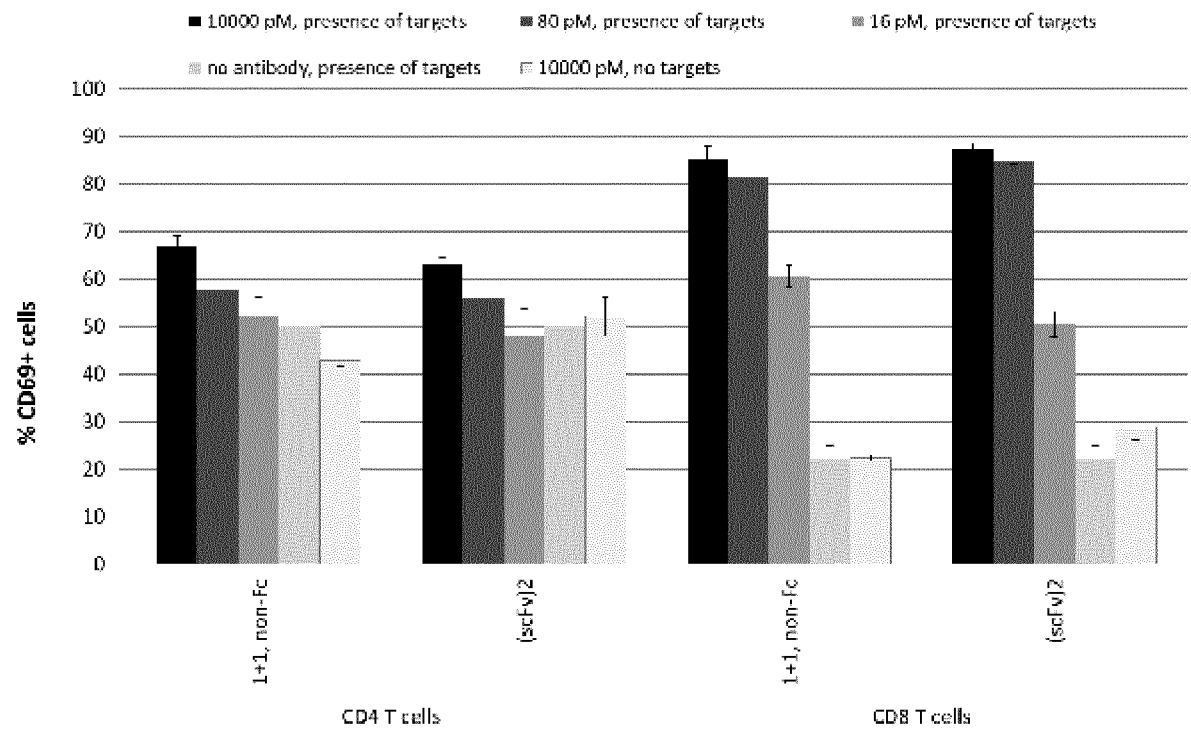


Fig. 21

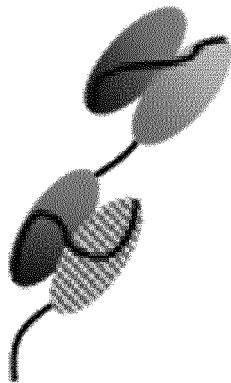


Fig. 22

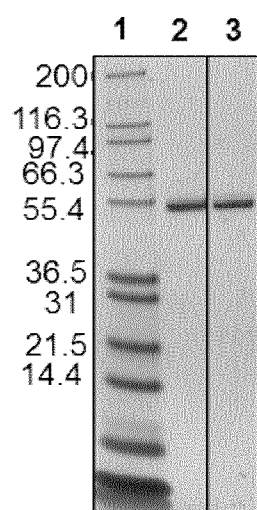
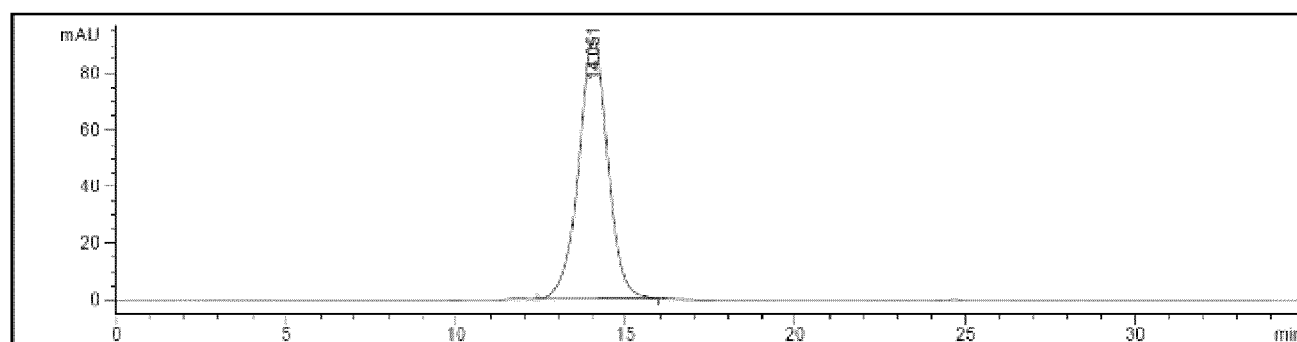


Fig. 23



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/066219

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/30 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/080254 A1 (HOFFMANN LA ROCHE [CH]; KLEIN CHRISTIAN [DE]; SCHAEFER WOLFGANG [DE]) 2 July 2009 (2009-07-02)	1,11-15
Y	page 12; figure 2a page 4, line 17 - line 19	2-10
Y	----- WO 2010/115551 A1 (ROCHE GLYCART AG [CH]; BOSSENMAIER BIRGIT [DE]; BRINKMANN ULRICH [DE];) 14 October 2010 (2010-10-14) figures 3a,3b	2-10
A	----- WO 2010/136172 A1 (HOFFMANN LA ROCHE [CH]; CROASDALE REBECCA [DE]; KLEIN CHRISTIAN [CH];) 2 December 2010 (2010-12-02) the whole document ----- -/-	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 October 2012

Date of mailing of the international search report

22/11/2012

Name and mailing address of the ISA/

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Fax: (+31-70) 340-3016

Authorized officer

Wagner, René

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/066219

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W. SCHAEFER ET AL: "Immunoglobulin domain crossover as a generic approach for the production of bispecific IgG antibodies", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 108, no. 27, 5 July 2011 (2011-07-05), pages 11187-11192, XP55003817, ISSN: 0027-8424, DOI: 10.1073/pnas.1019002108 the whole document -----	1-15
A	CHAN L A ET AL: "Variable region domain exchange in human IgGs promotes antibody complex formation with accompanying structural changes and altered effector functions", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 41, no. 5, 1 July 2004 (2004-07-01), pages 527-538, XP002519620, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2004.03.034 the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/066219

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009080254	A1	02-07-2009	AR 069797 A1 17-02-2010
		AU 2008340695 A1 02-07-2009	
		CA 2709347 A1 02-07-2009	
		CN 101903405 A 01-12-2010	
		EP 2238173 A1 13-10-2010	
		JP 2011507489 A 10-03-2011	
		KR 20100087397 A 04-08-2010	
		PE 15202009 A1 25-09-2009	
		TW 200932270 A 01-08-2009	
		US 2009175851 A1 09-07-2009	
		WO 2009080254 A1 02-07-2009	
WO 2010115551	A1	14-10-2010	AR 076194 A1 26-05-2011
		AR 076195 A1 26-05-2011	
		AU 2010233993 A1 08-09-2011	
		AU 2010233995 A1 08-09-2011	
		CA 2757426 A1 14-10-2010	
		CA 2757669 A1 14-10-2010	
		CN 102361883 A 22-02-2012	
		CN 102361884 A 22-02-2012	
		EP 2417160 A1 15-02-2012	
		EP 2417164 A1 15-02-2012	
		JP 2012522523 A 27-09-2012	
		JP 2012522525 A 27-09-2012	
		KR 20110124368 A 16-11-2011	
		KR 20110126748 A 23-11-2011	
		SG 175078 A1 28-11-2011	
		SG 175080 A1 28-11-2011	
		TW 201039848 A 16-11-2010	
		TW 201039849 A 16-11-2010	
		US 2010254988 A1 07-10-2010	
		US 2010254989 A1 07-10-2010	
		WO 2010115551 A1 14-10-2010	
		WO 2010115553 A1 14-10-2010	
WO 2010136172	A1	02-12-2010	AR 076797 A1 06-07-2011
		AU 2010252284 A1 17-11-2011	
		CA 2761233 A1 02-12-2010	
		CN 102448985 A 09-05-2012	
		EP 2435473 A1 04-04-2012	
		KR 20120012838 A 10-02-2012	
		PE 05402012 A1 09-05-2012	
		SG 176219 A1 29-12-2011	
		TW 201100543 A 01-01-2011	
		US 2010322935 A1 23-12-2010	
		WO 2010136172 A1 02-12-2010	