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Anonymous: "Paper: Tuning T Cell Affinity Improves Efficacy and Safety of Anti-CD38 & Anti-CD3 Bispecific Antibodies in Monkeys - a Potential Therapy for Multiple Myeloma", , 5 December 2015 (2015-12-05), XP055377940, Retrieved from the Internet: URL:<https://ash.confex.com/ash/2015/webprogram/scheduler/Paper78382.html> [retrieved on 2017-06-01]

DESCRIPTION

[0001] The invention relates to CD38/PD-L1 binding antibodies, targeting CD38 and PD-L1, methods for the production of these molecules, compositions, and uses thereof.

Background of the invention

[0002] Multiple Myeloma (MM) is the third most common haematological malignancy with 114,000 cases globally per year. Despite advances in treatment, MM remains one of the few haematological malignancies with an unmet medical need. Once patients progress through front-line therapy and have relapsed or refractory (r/r) disease, treatment options are very limited. However, in the recent years anti- MM tumor target antigens (TAA) have been developed. One anti-CD38 antibody, daratumumab, has been approved for the treatment of patients with relapsed MM and other anti-CD38 antibodies are currently in development (isatuximab and MOR-202, which is described in U.S. patent US 8,263,746). However, there is a need to improve responses that are currently in the range of 30-35%. It was demonstrated that the activity of anti-CD38 antibodies may be enhanced by immunomodulatory therapeutics (e.g. lenalidomide), which stimulate the immune system of patients. Additionally it was demonstrated that one of the mechanisms of resistance of MM tumor cells to antibody therapies is associated with the increased signalling of checkpoint inhibitor pathways (e.g. PD-1/PD-L1). Therefore, there is an opportunity to enhance cytotoxicity of anti-CD38 antibodies against MM tumor cells and simultaneously activate the immune system by inhibiting checkpoint inhibitor pathways (e.g. PD-1/PD-L1).

[0003] In physiological conditions, PD-L1 plays a major role as guard against autoimmunity by down-regulating the immune system. It is expressed on immune "APC-like" cells (T cells, NK cells, macrophages, myeloid DCs, B cells, epithelial cells, vascular endothelial cells) and tumor cells. PD-L1 binds to its cognate receptors PD-1 and B7-1, and negatively regulates immune cells (T cells, NK cells, etc), by inhibiting their proliferation and activation.

[0004] In pathological conditions, PD-L1 is highly expressed by tumor cells (> 90% MM patients) and is associated with poor prognosis. Blocking antibodies targeting immune checkpoint pathways (anti-PD-1, anti-CTLA-4, anti-PD-L1, etc) have demonstrated remarkable activity in different types of cancer (lung, melanoma etc). Signs of efficacy have been observed in MM, however the activity of this class of promising therapeutics is still suboptimal in MM. One of the reasons could be that molecules, which possess beneficial activity/side effect profile (e.g. anti-PD-L1) require near stoichiometric blocking/saturation of their targets to elicit maximal immunostimulatory effect on T cells.

[0005] Therefore, specific targeting of anti-PD-L1 antibodies to the site of tumors (e.g. targeting CD38+ cancer cells) may help delivering anti-PD-L1 therapeutics to the site where immune stimulation is required, and may result in maximal immune cell stimulation allowing the

complete blocking of PD-L1 on tumor and microenvironment cells. Such targeted immune cell activation at tumor sites may also reduce systemic activation of the immune cells, prevent adverse side effects, and permit higher dosing of therapeutic antibodies.

[0006] Genmab publicly announced studies of daratumumab in combination with atezolizumab in a solid tumor and multiple myeloma (announcement dated March 21, 2016).

[0007] In a poster dated December 5, 2015 at the 57th annual meeting & exposition, Orlando, FL, by American Society of Hematology, Moore et al reported the efficacy of a bispecific antibody anti-CD38 x anti-CD3 in treating multiple myeloma.

Summary of the invention

[0008] To harness the cytotoxic capacity of T cells, BK cells and other immune cells for the treatment of multiple myeloma (MM) and other cancers, preferably CD38+ cancers, bispecific molecules with two binding sites (specific for CD38 and PD-L1 respectively) were designed. The bispecific molecules of the invention remove the inhibition of the immune system associated with the interaction of PD-1 on T cells and NK cells and PD-L1, expressed on tumor and tumor microenvironment cells. Such molecules are useful in treating cancers, especially multiple myeloma or any CD38+ cancers, which overexpress PD-L1, and grow in the microenvironments of PD-L1 expressing immune cells (Plasmacytoid Dendritic Cell, Myeloid-derived Suppressor Cells) that further inhibit T cells and NK cells. The molecules of the invention facilitate the Antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, and complement-dependent cytotoxicity (CDC) of CD38+/PD-L1+ tumor cells as well as PD-L1+ cells of tumor microenvironment cells.

The invention is set out in the appended set of claims.

According to the invention, bispecific CD38/PD-L1 antibodies, comprising anti-CD38 and anti-PD-L1 domains, are engineered. These bispecific CD38/PD-L1 antibodies are capable of simultaneous binding to both antigens.

In a preferred embodiment, bispecific CD38/PD-L1 antibodies are expressed in CHO cells and are purified by affinity chromatography employing Protein A resins. Antibody binding properties are characterized in *in vitro* assays. They simultaneously bind both CD38 and PD-L1 in ELISA assay.

[0009] The bispecific tetravalent four Fab antibodies, having the structure of **Figure 1A or 1B** are designated BiXAb®, a trademark of Biomunex Therapeutics.

[0010] The antibody of the invention is a bispecific and bivalent for CD38 and PD-L1. The antigen-binding bispecific antibodies of the invention are full-length bispecific antibodies consisting of a continuous "composite heavy chain" (made of the natural heavy chain of IgG of mAb1 followed by Linkers and the Fab heavy chain of mAb2), which is constructed of an Fc (Hinge-CH2-CH3) followed by antibody 1 Fab heavy chain (CH1-VH) and the successive Fab

heavy chain (CH1-VH) of antibody 2, the latter joined by a hinge-derived polypeptide linker sequence, and the resulting composite heavy chain during protein expression, associates with the identical second composite heavy chain, while the co-expressed Fab light chains (LC) of antibody 2 and of antibody 1 associate with their cognate heavy chain domains in order to form the final tandem F(ab')₂-Fc molecule; the antibody 1 (Ab1) and the antibody 2 (Ab2) being different and selected from the group consisting of anti-CD38 antibodies (daratumumab, isatuximab, MOR-202 or any other anti-CD38 antibody) and anti-PD-L1 antibodies (atezolizumab, durvalumab, avelumab, MDX-1105 or any other anti-PD-L1 antibody).

The BiXAb[®] antibodies are able to bind bivalently both to CD38 and PD-L1.

[0011] Still another object of the invention is a method for preparing the bispecific antibodies of the invention, said method comprising the following steps: a) culturing in suitable medium and culture conditions a host cell expressing an antibody heavy chain as defined above, and antibody light chains as defined above; and b) recovering said produced antibodies from the culture medium or from said cultured cells.

[0012] The invention makes use of recombinant vectors, in particular expression vectors, comprising polynucleotides encoding the heavy and light chains defined herein, associated with transcription- and translation-controlling elements which are active in the host cell chosen. Vectors which can be used to construct expression vectors in accordance with the invention are known in themselves, and will be chosen in particular as a function of the host cell one intends to use. Preferably, said host cell is transformed with a polynucleotide encoding a heavy chain and two polynucleotides encoding two different light chains. Said polynucleotides can be inserted in a same expression vector, or in separate expression vectors. The method for producing the antibodies of the invention comprises culturing such host-cell and recovering said antigen-binding fragments or antibody from said culture.

Legends to the figures

[0013]

Figures 1A and 1B are schematic representations of a bispecific antibody of the invention, which comprises two heavy chains, and four light chains.

Figure 2 shows a SDS polyacrylamide gel electrophoresis of bispecific antibodies BiXAbs 4218, 4219 and 5104 under reducing conditions.

Figure 3 shows a SDS polyacrylamide gel electrophoresis of BiXAbs 4218, 4219 and 5104 under non-reducing conditions.

Figure 4 shows the ELISA binding assay for BiXAbs 4218 and 4219.

Figure 5 shows the SDS polyacrylamide gel electrophoresis of BiXAb-6567 under reducing and non-reducing conditions. Lane 1: the migration of BiXAb-6567 under reducing conditions;

lane 2: molecular weight markers with the weight of each band indicated; lane 3: the migration of BiXAb-6567 under non-reducing conditions.

Figure 6 shows the Size Exclusion chromatography analysis of BiXAb-6567.

Figure 7 shows the melting profiles of the two parental antibodies (anti-CD38 and anti-PD-L1) and BiXAb-6567 as determined by Digital Scanning Calorimetry.

Figure 8A shows the binding profiles of the two parental antibodies (anti-CD38 and anti-PD-L1) and BiXAb-6567 in a direct CD38 antigen binding ELISA. **Figure 8B** shows the binding profiles of the two parental antibodies (anti-CD38 and anti-PD-L1) and BiXAb-6567 in a direct PD-L1 antigen binding ELISA. **Figure 8C** shows the binding profile of BiXAb-6567 in a dual antigen (PD-L1 and CD38) binding ELISA.

Figures 9A to 9C show Fluorescence-activated cell sorting profiles of the two parental mAbs (anti-CD38 and anti-PD-L1) and BiXAb-6567 on three different cell lines, **9A**: multiple myeloma RPMI-8226, **9B**: CHO cells stably transfected with full-length CD38, and **9C**: ovarian cancer cell line SKOV-3.

Figure 10 shows the titration binding profiles on the CHO-CD38 cell line of the two parental antibodies (anti-CD38 and anti-PD-L1), BiXAb-6567, and the negative control anti-CD20 antibody.

Figure 11 shows the cytotoxic activity profiles of the two parental antibodies (anti-CD38 and anti-PD-L1), BiXAb-6567, and two negative control antibodies, anti-CD20 and anti-HER2, in an ADCC assay employing a multiple myeloma cell line, RPMI-8226, as target cells and unfractionated non-pre-activated mononuclear cells as effector cells.

Figure 12 shows the cytotoxic activity profiles of the two parental antibodies (anti-CD38 and anti-PD-L1), BiXAb-6567, and two negative control antibodies, anti-CD20 and anti-HER2, in an ADCC assay with the CHO-CD38 cell line as target cells and unfractionated non-pre-activated mononuclear cells as effector cells.

Figure 13 shows the cytotoxic activity profiles of the two parental antibodies (anti-CD38 and anti-PD-L1), BiXAb-6567, and two negative control antibodies, anti-CD20 and anti-HER2, in an ADCC assay with the SKOV-3 cell line as target cells and enriched IL-12 pre-activated NK cells as effector cells.

Figure 14 shows the cytotoxic activity profiles of the two parental antibodies (anti-CD38 and anti-PD-L1), BiXAb-6567, and two negative control antibodies, anti-CD20 and anti-HER2, in an ADCC assay with the SKOV-3 cell line as target cells and enriched IL-15 pre-activated NK cells as effector cells.

Detailed description

Definitions:

[0014] The basic structure of a naturally occurring antibody molecule is a Y-shaped tetrameric quaternary structure consisting of two identical heavy chains and two identical light chains, held together by non-covalent interactions and by inter-chain disulfide bonds.

[0015] In mammalian species, there are five types of heavy chains: μ , δ , ϵ , γ , and α , which determine the class (isotype) of immunoglobulin: IgA, IgD, IgE, IgG, and IgM, respectively. The heavy chain N-terminal variable domain (VH) is followed by a constant region, containing three domains (numbered CH1, CH2, and CH3 from the N-terminus to the C-terminus) in heavy chains γ , α , and δ , while the constant region of heavy chains μ and ϵ is composed of four domains (numbered CH1, CH2, CH3 and CH4 from the N-terminus to the C-terminus). The CH1 and CH2 domains of IgA, IgG, and IgD are separated by a flexible hinge, which varies in length between the different classes and in the case of IgA and IgG, between the different subtypes: IgG1, IgG2, IgG3, and IgG4 have respectively hinges of 15, 12, 62 (or 77), and 12 amino acids, and IgA1 and IgA2 have respectively hinges of 20 and 7 amino acids.

[0016] There are two types of light chains: λ and κ , which can associate with any of the heavy chains isotypes, but are both of the same type in a given antibody molecule. Both light chains appear to be functionally identical. Their N-terminal variable domain (VL) is followed by a constant region consisting of a single domain termed **CL**.

[0017] The heavy and light chains pair by protein/protein interactions between the CH1 and CL domains, and via VH /VL interactions and the two heavy chains associate by protein/protein interactions between their CH3 domains. The structure of the immunoglobulin molecule is generally stabilized by interchains disulfide bonds between the CH1 and CL domains and between the hinges.

[0018] The antigen-binding regions correspond to the arms of the Y-shaped structure, which consist each of the complete light chain paired with the VH and CH1 domains of the heavy chain, and are called the **Fab fragments** (for Fragment antigen binding). Fab fragments were first generated from native immunoglobulin molecules by papain digestion which cleaves the antibody molecule in the hinge region, on the amino-terminal side of the interchains disulfide bonds, thus releasing two identical antigen-binding arms. Other proteases such as pepsin, also cleave the antibody molecule in the hinge region, but on the carboxy-terminal side of the interchains disulfide bonds, releasing fragments consisting of two identical Fab fragments and remaining linked through disulfide bonds; reduction of disulfide bonds in the F(ab')₂ fragments generates Fab' fragments.

[0019] The part of the antigen binding region corresponding to the VH and VL domains is called the **Fv** fragment (for Fragment variable); it contains the CDRs (complementarity determining regions), which form the antigen-binding site (also termed paratope).

[0020] The effector region of the antibody which is responsible of its binding to effector molecules or cells, corresponds to the stem of the Y-shaped structure, and contains the paired CH2 and CH3 domains of the heavy chain (or the CH2, CH3 and CH4 domains, depending on the class of antibody), and is called the Fc (for Fragment crystallisable) region.

[0021] Due to the identity of the two heavy chains and the two light chains, naturally occurring antibody molecules have two identical antigen-binding sites and thus bind simultaneously to two identical epitopes.

[0022] An antibody "**specifically binds**" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. "Specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0023] The terms "**subject**," "**individual**," and "**patient**" are used interchangeably herein and refer to a mammal being assessed for treatment and/or being treated. Subjects may be human, but also include other mammals, particularly those mammals useful as laboratory models for human disease, e.g. mouse, rat, rabbit, dog, etc.

[0024] The term "**treatment**" or "**treating**" refers to an action, application or therapy, wherein a subject, including a human being, is subjected to medical aid with the purpose of improving the subject's condition, directly or indirectly. Particularly, the term refers to reducing incidence, or alleviating symptoms, eliminating recurrence, preventing recurrence, preventing incidence, improving symptoms, improving prognosis or combination thereof in some embodiments. The skilled artisan would understand that treatment does not necessarily result in the complete absence or removal of symptoms. For example, with respect to cancer, "treatment" or "treating" may refer to slowing neoplastic or malignant cell growth, proliferation, or metastasis, preventing or delaying the development of neoplastic or malignant cell growth, proliferation, or metastasis, or some combination thereof.

Design of the preferred bispecific antibodies:

[0025] The invention provides bispecific tetravalent antibodies, comprising two binding sites to each of their targets, and a functional Fc domain allowing the activation of effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, and complement-dependent cytotoxicity (CDC).

[0026] The antibodies of the invention are full-length antibodies. They preferably comprise heavy chains and light chains from human immunoglobulins, preferably IgG, still preferably IgG1.

[0027] The light chains preferably are Kappa light chains.

[0028] In a preferred embodiment, the linker of the invention connects two pairs of IgG Fab domains in a tetra-Fab bispecific antibody format, the amino acid sequence of which comprises the heavy chain sequences of at least two Fab joined by a linker, followed by the native hinge sequence, followed by the IgG Fc sequence, coexpressed with the appropriate IgG light chain sequences.

[0029] An example of the antibodies of the invention, which have an IgG-like structure, is illustrated in **Figures 1A and 1B**.

[0030] The bispecific antibodies of the invention comprise

- a continuous heavy chain constructed of an Fc (Hinge-CH₂-CH₃)
- followed by antibody 1 Fab heavy chain (CH₁-VH) and the successive Fab heavy chain (CH₁-VH) of antibody 2, the latter joined by a hinge-derived polypeptide linker sequence,
- and during protein expression the resulting heavy chain assembles into dimers while the co-expressed antibody 1 and antibody 2 light chains (VL-CL) associate with their cognate heavy chains in order to form the final tandem F(ab)[']₂-Fc molecule, the antibody 1 (Ab₁) and the antibody 2 (Ab₂) being different.

[0031] Ab₁ and Ab₂, being different, independently are selected from the group consisting of an anti-CD38 antibody (such as daratumumab) and an anti-PD-L1 antibody (such as atezolizumab).

[0032] Daratumumab binds a unique CD38 epitope at the C-terminal region of human CD38, amino acids 233 to 246 and 267 to 280, with amino acids in positions 272 and 274 being particularly important for binding. Advantageously, Ab₁ and/or Ab₂ may be antibodies that bind to the same epitope, or overlapping epitope (e.g. with an overlap of at least 4 amino acids) with respect to daratumumab.

In another embodiment, Ab₁ and/or Ab₂ may be antibodies that bind to the same epitope, or overlapping epitope with respect to atezolizumab.

[0033] In a particular embodiment, the bispecific molecule is a bispecific antibody which comprises, preferably consists of, a) two heavy chains, each comprising, preferably consisting of, SEQ ID NO:1 and b) four light chains, two comprising, preferably consisting of, SEQ ID NO:2, the two others comprising, preferably consisting of, SEQ ID NO: 3. Such bispecific antibody is designated BiXAb-4218.

[0034] In another particular embodiment, the bispecific molecule is a bispecific antibody which comprises, preferably consists of, a) two heavy chains, each comprising, preferably consisting of, SEQ ID NO:4 and b) four light chains, two comprising, preferably consisting of, SEQ ID NO:5, the two others comprising, preferably consisting of, SEQ ID NO: 6. Such bispecific

antibody is designated BiXAb-4219.

[0035] In another particular embodiment, the bispecific molecule is a bispecific antibody which comprises, preferably consists of, a) two heavy chains, each comprising, preferably consisting of, SEQ ID NO:7 and b) four light chains, two comprising, preferably consisting of, SEQ ID NO:8, the two others comprising, preferably consisting of, SEQ ID NO: 9. Such bispecific antibody is designated BiXAb-5104.

[0036] In a preferred embodiment, the bispecific molecule is a bispecific antibody which comprises, preferably consists of, a) two heavy chains, each comprising, preferably consisting of, SEQ ID NO:10 and b) four light chains, two comprising, preferably consisting of, SEQ ID NO:11, the two others comprising, preferably consisting of, SEQ ID NO: 12. Such bispecific antibody is designated BiXAb-6567.

[0037] The heavy chain (SEQ ID NO:10) comprises

- VH of daratumumab (SEQ ID NO:22)
- CH1 domain (human IgG1 of G1m(3) allotype with mutations L124Q and S188V) of daratumumab Fab (SEQ ID NO:23)
- AP linker (SEQ ID NO:15)
- VH of atezolizumab (SEQ ID NO: 24)
- CH1 domain (human IgG1 of G1m(3) allotype with the mutation T192D) of atezolizumab Fab (SEQ ID NO:25)
- Hinge of human IgG1 (SEQ ID NO:26)
- CH2 domain of human IgG1 (SEQ ID NO:27)
- CH3 domain of human IgG1 of G1m(3) allotype (SEQ ID NO:28)

[0038] Light chain SEQ ID NO: 11 comprises

- VL of daratumumab (SEQ ID NO :29)
- CKappa domain of daratumumab with mutations V133T and S176V (SEQ ID NO :30)
Light chain SEQ ID NO: 12 comprises
- VL of atezolizumab (SEQ ID NO : 31)
- CKappa domain of atezolizumab with mutations S114A and N137K (SEQ ID NO :32)

Design of the linkers

[0039] The polypeptide linker, also designated "hinge-derived polypeptide linker sequence" or "pseudo hinge linker", comprises all or part of the sequence of the hinge region of one or more immunoglobulin(s) selected among IgA, IgG, and IgD, preferably of human origin. Said

polypeptide linker may comprise all or part of the sequence of the hinge region of only one immunoglobulin. In this case, said immunoglobulin may belong to the same isotype and subclass as the immunoglobulin from which the adjacent CH1 domain is derived, or to a different isotype or subclass. Alternatively, said polypeptide linker may comprise all or part of the sequences of hinge regions of at least two immunoglobulins of different isotypes or subclasses. In this case, the N-terminal portion of the polypeptide linker, which directly follows the CH1 domain, preferably consists of all or part of the hinge region of an immunoglobulin belonging to the same isotype and subclass as the immunoglobulin from which said CH1 domain is derived.

Optionally, said polypeptide linker may further comprise a sequence of from 2 to 15, preferably of from 5 to 10 N-terminal amino acids of the CH2 domain of an immunoglobulin.

[0040] The polypeptide linker sequence typically consists of less than 80 amino acids, preferably less than 60 amino acids, still preferably less than 40 amino acids.

[0041] In some cases, sequences from native hinge regions can be used; in other cases point mutations can be brought to these sequences, in particular the replacement of one or more cysteine residues in native IgG1, IgG2 or IgG3 hinge sequences by alanine or serine, in order to avoid unwanted intra-chain or inter-chains disulfide bonds.

In a particular embodiment, the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO:13), wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid. In particular, the polypeptide linker sequence may comprise or consist of a sequence selected from the group consisting of

EPKSCDKTHTSPPAPAPPELLGGPGGPPGPGGGG (SEQ ID NO: 14);

EPKSCDKTHTSPPAPAPPELLGGPAAPPAPAPAGG (SEQ ID NO: 15);

EPKSCDKTHTSPPAPAPPELLGGPAAPPGPAPGGG (SEQ ID NO:16);

EPKSCDKTHTCPPCPAPPELLGGPSTPPTPSPSGG (SEQ ID NO:17) and

EPKSCDKTHTSPPSPAPPELLGGPSTPPTPSPSGG (SEQ ID NO:18).

A non-limitative example of a hinge-derived polypeptide linker which can be used in a multispecific antigens-binding fragment of the invention is a polypeptide having SEQ ID NO: 17. Said polypeptide consists of the full length sequence of human IgG1 hinge, followed by the 9 N-terminal amino-acids of human IgG1 CH2 (APPELLGGPS, SEQ ID NO: 19), by a portion of the sequence of human IgA1 hinge (TPPTSPSPS, SEQ ID NO: 20), and by the dipeptide GG, added to provide supplemental flexibility to the linker. In another preferred embodiment, the hinge-derived polypeptide linker sequence is SEQ ID NO: 15 or SEQ ID NO:18.

[0042] In a particular embodiment, X₁, X₂ and X₃, identical or different, are Threonine (T) or Serine (S).

In another particular embodiment, X₁, X₂ and X₃, identical or different, are selected from the group consisting of Ala (A), Gly (G), Val (V), Asn (N), Asp (D) and Ile (I), still preferably X₁, X₂ and X₃, identical or different, may be Ala (A) or Gly (G).

Alternatively, X₁, X₂ and X₃, identical or different, may be Leu (L), Glu (E), Gln (Q), Met (M),

Lys (K), Arg (R), Phe (F), Tyr (T), His (H), Trp (W), preferably Leu (L), Glu (E), or Gln (Q).

In a particular embodiment, X₄ and X₅, identical or different, are any amino acid selected from the group consisting of Serine (S), Cysteine (C), Alanine (A), and Glycine (G).

In a preferred embodiment, X₄ is Serine (S) or Cysteine (C).

In a preferred aspect, X₅ is Alanine (A) or Cysteine (C).

[0043] In a particular embodiment, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid other than Threonine (T) or Serine (S). Preferably X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A), Gly (G), Val (V), Asn (N), Asp (D) and Ile (I).

Alternatively, X₆, X₇, X₈, X₉, X₁₀, identical or different, may be Leu (L), Glu (E), Gln (Q), Met (M), Lys (K), Arg (R), Phe (F), Tyr (T), His (H), Trp (W), preferably Leu (L), Glu (E), or Gln (Q).

In a preferred embodiment, X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A) and Gly (G).

In still a preferred embodiment, X₆ and X₇ are identical and are preferably selected from the group consisting of Ala (A) and Gly (G).

[0044] In a preferred embodiment, the polypeptide linker sequence comprises or consists of sequence SEQ ID NO: 13, wherein

X₁, X₂ and X₃, identical or different, are Threonine (T), Serine (S);

X₄ is Serine (S) or Cysteine (C);

X₅ is Alanine (A) or Cysteine (C);

X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A) and Gly (G).

[0045] In another preferred embodiment, the polypeptide linker sequence comprises or consists of sequence SEQ ID NO: 13, wherein

X₁, X₂ and X₃, identical or different, are Ala (A) or Gly (G);

X₄ is Serine (S) or Cysteine (C);

X₅ is Alanine (A) or Cysteine (C);

X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A) and Gly (G).

Production of the bispecific antibodies:

[0046] The skilled person may refer to international patent application WO2013/005194, for general techniques of expressing multispecific antibodies.

[0047] Also herein described is a polynucleotide comprising a sequence encoding a protein chain of the antibody of the invention. Said polynucleotide may also comprise additional sequences: in particular it may advantageously comprise a sequence encoding a leader sequence or signal peptide allowing secretion of said protein chain. Host-cells transformed with said polynucleotide are also disclosed.

[0048] Typically, the amino acid sequences of different anti-CD38 and anti-PDL-1 monoclonal antibodies are used to design the DNA sequences, optionally after codon optimization for mammalian expression. For the heavy chain, the DNAs encoding signal peptides, variable region and constant CH1 domain of Fab1 followed the hinge linker and variable region and constant CH1 domain of Fab2 with flanking sequences for restriction enzyme digestion are synthesized. For the light chain, the DNAs encoding signal peptides and variable and constant Kappa regions are synthesized.

[0049] Nucleic acids encoding heavy and light chains of the antibodies of the invention are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Such control sequences include a signal sequence, a promoter, an enhancer, and a transcription termination sequence. Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences.

In one example, both the heavy and light chain-coding sequences (e.g., sequences encoding a VH and a VL, a VH-CH1 and a VL-CL, or a full-length heavy chain and a full-length light chain) are included in one expression vector. In another example, each of the heavy and light chains of the antibody is cloned into an individual vector. In the latter case, the expression vectors encoding the heavy and light chains can be co-transfected into one host cell for expression of both chains, which can be assembled to form intact antibodies either *in vivo* or *in vitro*. Alternatively, the expression vector encoding the heavy chain and that or those encoding the light chains can be introduced into different host cells for expression each of the heavy and light chains, which can then be purified and assembled to form intact antibodies *in vitro*.

[0050] In a particular embodiment, a host cell is co-transfected with three independent expression vectors, such as plasmids, leading to the coproduction of all three chains (namely the heavy chain HC, and two light chains LC1 and LC2, respectively) and to the secretion of the bispecific antibody.

More especially the three vectors may be advantageously used in a following molecular ratio of 2:1:1 (HC : LC1 : LC2).

[0051] The recombinant vectors for expression the antibodies described herein typically contain a nucleic acid encoding the antibody amino acid sequences operably linked to a

promoter, either constitutive or inducible. The vectors can be suitable for replication and integration in prokaryotes, eukaryotes, or both. Typical vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the nucleic acid encoding the antibody. The vectors optionally contain generic expression cassettes containing at least one independent terminator sequence, sequences permitting replication of the cassette in both eukaryotes and prokaryotes, i.e., shuttle vectors, and selection markers for both prokaryotic and eukaryotic systems.

[0052] Bispecific antibodies as described herein may be produced in prokaryotic or eukaryotic expression systems, such as bacteria, yeast, filamentous fungi, plant, insect (e.g. using a baculovirus vector), and mammalian cells. It is not necessary that the recombinant antibodies of the invention are glycosylated or expressed in eukaryotic cells; however, expression in mammalian cells is generally preferred. Examples of useful mammalian host cell lines are human embryonic kidney line (293 cells), baby hamster kidney cells (BHK cells), Chinese hamster ovary cells/- or + DHFR (CHO, CHO-S, CHO-DG44, Flp-in CHO cells), African green monkey kidney cells (VERO cells), and human liver cells (Hep G2 cells).

[0053] Mammalian tissue cell culture is preferred to express and produce the polypeptides because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various Cos cell lines, HeLa cells, preferably myeloma cell lines (such as NS0), or transformed B-cells or hybridomas.

[0054] In a most preferred embodiment, the bispecific antibodies of the invention are produced by using a CHO cell line, most advantageously CHO-S or CHO-DG-44 cell lines or their derivatives.

Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, or cytomegalovirus.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example calcium phosphate treatment or electroporation may be used for other cellular hosts. (See generally Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989). When heavy and light chains are cloned on separate expression vectors, the vectors are co-transfected to obtain expression and assembly of intact immunoglobulins.

Host cells are transformed or transfected with the vectors (for example, by chemical transfection or electroporation methods) and cultured in conventional nutrient media (or modified as appropriate) for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0055] The expression of the antibodies may be transient or stable.

Preferably, the bispecific antibodies are produced by the methods of stable expression, in

which cell lines stably transfected with the DNA encoding all polypeptide chains of a bispecific antibody, such as BiXAb-6567, are capable of sustained expression, which enables manufacturing of therapeutics. For instance stable expression in a CHO cell line is particularly advantageous.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be further isolated or purified to obtain preparations that are substantially homogeneous for further assays and applications. Standard protein purification methods known in the art can be used. For example, suitable purification procedures may include fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, high-performance liquid chromatography (HPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), ammonium sulfate precipitation, and gel filtration (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., 1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses.

[0056] In vitro production allows scale-up to give large amounts of the desired bispecific antibodies of the invention. Such methods may employ homogeneous suspension culture, e.g. in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, e.g. in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges.

Mutated derivatives and mutations:

[0057] The polypeptide sequences that bind CD38 may derive from any anti-CD38 antibody, e.g. selected from the group consisting of daratumumab, isatuximab, MOR-202 or their mutated derivatives.

The polypeptide sequences that bind PD-L1 may derive from any anti-PD-L1 antibody, e.g. selected from the group consisting of atezolizumab, durvalumab, avelumab, MDX-1105 or their mutated derivatives.

The term **"mutated derivative", "mutant", or "functional variant"** designates a sequence that differs from the parent sequence to which it refers by deletion, substitution or insertion of one or several amino acids. Preferably the mutated derivative preferably show at least 80%, preferably at least 85%, still preferably at least 90% homology sequence with the native sequence. In a particular embodiment, the mutations do not substantially impact the function of the antibody.

Mutated derivatives, or functional variants, can comprise a VH chain that comprises an amino acid sequence at least 85% (e.g., 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to any of the reference sequences recited herein, a VL chain that has an amino acid sequence at least 85% (e.g., 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to any of the reference sequences recited herein, or both. These variants are capable of binding to CD38 and PD-L1. In some examples, the variants possess similar antigen-binding affinity relative to the reference antibodies described above (e.g., having a K_D less than 1×10^{-7} M, 10^{-8} M, preferably less than 1×10^{-9} or 1×10^{-10} M).

The affinity of the binding is defined by the terms k_a (associate rate constant), k_d (dissociation rate constant), or K_D (equilibrium dissociation). Typically, specifically binding when used with respect to an antibody refers to an antibody that specifically binds to ("recognizes") its target(s) with an affinity (K_D) value less than 10^{-7} M, preferably less than 10^{-8} M, e.g., less than 10^{-9} M or 10^{-10} M. A lower K_D value represents a higher binding affinity (i.e., stronger binding) so that a K_D value of 10^{-9} indicates a higher binding affinity than a K_D value of 10^{-8} .

The **"percent identity"** of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

In other embodiments, the functional variants described herein can contain one or more mutations (e.g., conservative substitutions) which preferably do not occur at residues which are predicted to interact with one or more of the CDRs.

It is herein described mutated derivatives, or functional variants, which are substantially identical to the reference antibody.

The term **"substantially identical"** or **"insubstantial"** means that the relevant amino acid sequences (e.g., in framework regions (FRs), CDRs, VH, or VL domain) of a variant differ insubstantially (e.g., including conservative amino acid substitutions) as compared with a reference antibody such that the variant has substantially similar binding activities (e.g., affinity, specificity, or both) and bioactivities relative to the reference antibody. Such a variant may include minor amino acid changes, e.g. 1 or 2 substitutions in a 5 amino acid sequence of a specified region. Generally, more substitutions can be made in FR regions, in contrast to CDR regions, as long as they do not adversely impact the binding function of the antibody (such as reducing the binding affinity by more than 50% as compared to the original antibody). In some embodiment, the sequence identity can be about 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher, between the original and the modified antibody. In some embodiments, the modified antibody has the same binding specificity and has at least 50% of the affinity of the original antibody.

Conservative substitutions will produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with another residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art. For example, amino acid substitutions can be used to identify important residues of the molecule sequence, or to increase or decrease the affinity of the molecules described herein. Variants comprising one or more conservative amino acid substitutions can be prepared according to methods for

altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

The present disclosure also provides antibody variants with improved biological properties of the antibody, such as higher or lower binding affinity, or with altered ADCC properties on CD38 and/or PD-L1 expressing cells.

Amino acid sequence variants of the antibody can be prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or via 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 is made to achieve the final construct, provided that the final construct possesses the desired characteristics. Nucleic acid molecules encoding amino acid sequence variants of the antibody can be prepared by a variety of methods known in the art. These methods include oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant (natural) version of the antibody. In one embodiment, the equilibrium dissociation constant (KD) value of the antibodies of the invention is less than 10^{-7} M, particularly less than 10^{-8} M, 10^{-9} M or 10^{-10} M. The binding affinity may be determined using techniques known in the art, such as ELISA or biospecific interaction analysis (e.g. using surface plasmon resonance), or other techniques known in the art.

[0058] Any of the molecules described herein can be examined to determine their properties, such as antigen-binding activity, antigen-binding specificity, and biological functions, following routine methods.

Any of the molecules described herein can be modified to contain additional nonproteinaceous moieties that are known in the art and readily available, e.g., by PEGylation, hyperglycosylation. Modifications that can enhance serum half-life are of interest.

[0059] Throughout the present description, amino acid sequences are defined according to Kabat et al, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[0060] Mutations can be located in constant domains. The bispecific antibodies indeed advantageously comprise Fab fragments having mutations at the interface of the CH1 and CL domains, said mutations facilitate cognate pairing of heavy chain/light chain and preventing their mispairing.

In a preferred embodiment, bispecific antibodies are described herein, which comprise

- two Fab fragments with different mutated CH1 and mutated CL domains consisting of
 1. a) Fab fragment having mutated CH1 and mutated C-Kappa domains derived

- from a human IgG1/Kappa, and the VH and VL domains of Ab1,
2. b) Fab fragment having mutated CH1 and mutated C-Kappa domains derived from a human IgG1/Kappa and the VH and VL domains of Ab2,
 3. c) a mutated light chain constant domain which is derived from human Kappa constant domain,
- the Fab fragments being tandemly arranged in the following order
- the C-terminal end of the mutated CH1 domain of Ab1 Fab fragment being linked to the N-terminal end of the VH domain of Ab2 Fab fragment through a polypeptide linker,
 - the hinge region of a human IgG1 linking the C-terminal end of mutated CH1 domain of Ab2 fragment to the N-terminal of the CH2 domain,
 - the dimerized CH2 and CH3 domains of a human IgG1.

[0061] In particular examples, bispecific antibodies are described, wherein the Fab CH1 domain of one of Ab1 or Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the threonine residue at position 192 of said CH1 domain with an aspartic acid and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the asparagine residue at position 137 of said CL domain with a lysine residue and substitution of the serine residue at position 114 of said CL domain with an alanine residue, and/or wherein the Fab CH1 domain of one or the other of Ab1 or Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the leucine residue at position 124 of said CH1 domain with a glutamine and substitution of the serine residue at position 188 of said CH1 domain with a valine residue, and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the valine residue at position 133 of said CL domain with a threonine residue and substitution of the serine residue at position 176 of said CL domain with a valine residue.

[0062] The antibodies of the invention may be glycosylated or not, or may show a variety of glycosylation profiles. In a preferred embodiment, antibodies are unglycosylated on the variable region of the heavy chains, but are glycosylated on the Fc region.

[0063] Certain mutated derivatives may use humanized forms of the reference antibody. In a humanization approach, complementarity determining regions (CDRs) and certain other amino acids from donor mouse variable regions are grafted into human variable acceptor regions and then joined to human constant regions. See, e.g. Riechmann et al., Nature 332:323-327 (1988); U.S. Pat. No. 5,225,539.

Therapeutic uses:

[0064] The bispecific antibody of the invention is useful as a medicament, in particular in

treating a cancer.

[0065] The term "cancer" as used herein includes any cancer, especially a hematological malignancy, and any other cancer characterized by CD38 or PD-L1 expression or overexpression, and especially those cancers characterized by co-expression of both CD38 and PD-L1.

[0066] Examples of cancers are lymphoma or leukemia, such as Non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), or multiple myeloma (MM), breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, colorectal cancer, pancreatic cancer, lung cancer, leiomyoma.

[0067] The bispecific molecule as defined herein may be formulated in a composition, e.g. a pharmaceutical composition, containing a bispecific molecule as defined herein, formulated together with a pharmaceutical carrier.

[0068] As used herein, a "pharmaceutical carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, or isotonic and absorption delaying agents, that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).

[0069] The composition can be administered by a variety of methods known in the art. The route and/or mode of administration will vary depending upon the desired results.

[0070] To administer the bispecific antibody of the invention by certain routes of administration, it may be necessary to coat the bispecific antibody of the invention with, or co-administer the bispecific antibody of the invention with a material to prevent its inactivation. For example, the bispecific antibody of the invention may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutical carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art.

[0071] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, or sorbic acid. It may also be desirable to include isotonic agents, such as sodium chloride into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents, which delay absorption.

[0072] Actual dosage levels of the active ingredients in the pharmaceutical compositions described herein may be varied so as to obtain an amount of the active ingredient, which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0073] The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. For example the bispecific antibody of the invention can be administrated at a dosage of 0.2-20mg/kg from 3 times/week to 1 time/month.

[0074] The present invention, thus generally described above, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting the instant invention.

Examples

Example 1. Preparation of bispecific antibodies BiXAb-4218, BiXAb-4219 and BiXAb-5104

Gene synthesis

[0075] The amino acid sequences of different anti-CD38 and anti-PDL-1 monoclonal antibodies were used to design the DNA sequences after codon optimization for mammalian expression using GeneScript program. For the heavy chain, the DNAs encoding signal peptides, variable region and constant CH1 domain of Fab1 followed the hinge linker and variable region and constant CH1 domain of Fab2 with flanking sequences for restriction enzyme digestion were synthesized by GeneScript. For the light chain, the DNAs encoding signal peptides and variable and constant Kappa regions were synthesized by GeneScript.

[0076] PCR reactions using PfuTurbo Hot Start were carried out to amplify the inserts which were then digested by NotI + Apal and NotI + HindIII for heavy and light chains, respectively. The double digested heavy chain fragments were ligated with NotI + Apal digested Evitria's proprietary expression vector in which the human IgG1 CH1 + hinge + CH2 + CH3 domains were already inserted. The double digested light chain fragments were ligated with NotI + HindIII treated Evitria's proprietary vector. Plasmid DNAs were verified by double strand DNA sequencing.

Expression, Purification and Characterization

[0077] For a 50 mL scale expression, a total of 50 µg of plasmid DNAs in Evitria's proprietary vector (25 µg heavy chain + 12.5 µg of each light chain, LC1 and LC2) were mixed in 1.5 mL Eppendorf tube, 1 mL of CHO SFM medium containing 25 µL of 3 mg/mL PEI pH7.0 was added, incubated at RT for 20 min. The mixture of DNA-PEI was loaded into 49 mL of FreeStyle™ CHO-S cells at $1-2 \times 10^6$ cells/mL in 125mL shaking flask. Cells were shaken for 6 more days. The supernatant was harvested by centrifuging cells at 3,000 rpm for 15 min. The harvested supernatant was purified by Protein A resin. Electrophoresis was performed under reducing conditions and non-reducing conditions employing Gel Biorad Stain-Free 4 - 15% gels and the corresponding running buffer. Samples were prepared by combining the purified BiXAb® antibodies with 2X SDS sample buffer and heating for 5 min at 95°C. Preparation of reduced samples included the addition of NuPAGE reducing agent prior to heating. The apparent MW was determined using Ladder Precision Plus Protein Unstained Standards (Biorad). **Figure 2** presents the SDS-PAGE pattern of CD38/PD-L1 antibodies under reducing conditions. Two bands corresponding to the composite heavy chain and two co-migrating light chains are observed and are of the expected molecular weight. **Figure 3** presents the SDS-PAGE pattern of CD38/PD-L1 antibodies under non-reducing conditions.

[0078] The dominant band at 250 kDa corresponds to the complete CD38/PD-L1 BiXAb® molecule as expected.

[0079] For Dual Antigen Binding Plate ELISA Assay the following reagents were used: Recombinant human CD38, Fc-tagged (Creative BioMart); biotinylated Human PD-L1, Avi Tag (AcroBiosystems); Streptavidin-HRP, (Biotechne RD-Systems). Human CD38-Fc fusion protein was coated with 100 µL/well at 2 µg/mL in 1× PBS pH7.4 in Maxisorp plates at 4°C overnight. The plates were washed 5 times with 1× PBS containing 0.05% Tween-20 (1× PBST), then blocked with 3% non-fat milk/1× PBST at 200 µL/well with shaking at RT for 1 hr. 100 µL/well of BiXAb® 4218 and BiXAb® 4219 at 1 mg/ ml stock solution starting at 1/500 dilution in 1× PBS at 1:3 series dilutions were added. The plates were incubated at RT for 1 hr with shaking, followed by 5 washes with 1X PBST. 100 µL/well of 1 µg/mL Biotin-human PD-L1 protein in 1X PBS was added and plates were shaken at RT for 1 hr. After 5 washes with 1× PBST, 100 µL/well of 0.1 µg/mL of Streptavidin-conjugated HRP in 1× PBS was added. The plates were shaken at RT for 1 hr followed by 5 washes with 1× PBST. 100 µL/well TMB substrate in 1× PBS was added for color development. The data were collected at 405 nm for 0.1 sec per well on a Victor II multifunction plate reader. **Figure 4** demonstrates the dual antigen binding profiles of two CD38/PD-L1 BiXAbs®. This profile confirms that both types of binding domains of these molecules (anti-CD38 domains and anti-PD-L1 domains) bind their cognate antigen targets.

Example 2. Preparation of bispecific antibody of the invention BiXAb-6567

Gene synthesis

[0080] The amino acid sequences of anti-CD38 (daratumumab) and anti-PDL1 (atezolizumab) were used to design the DNA sequences, after codon optimization for mammalian expression, using the GeneScript program. These antibodies are referred to as the "parental" anti-CD38 and the "parental" anti-PD-L1 mAbs.

[0081] The DNA construct of the heavy chain was designed as such: signal peptide (SEQ ID NO:21), followed by sequence SEQ ID NO:10 [consisting of the variable region, followed by the constant CH1 domain of Fab1 (anti-CD38), in which mutations Leu to Gln and Ser to Val at Kabat positions 124 and 188 were introduced, respectively, followed by the linker, followed by the variable region, followed by the constant CH1 domain of Fab2 (anti-PD-L1), in which mutation Thr to Asp at Kabat position 192 was introduced]; flanking sequences for restriction enzyme digestion were introduced on both ends of the heavy chain DNA construct. The DNA construct for the light chain was designed as such: signal peptide (SEQ ID NO:21), followed by the variable region, followed by the constant Kappa region. For the anti-CD38 light chain, mutations were introduced at Kabat positions 143 (Leu to Gln) and 188 (Ser to Val) in the constant Kappa domain. For the anti-PDL1 light chain, mutations at Kabat positions 133 (Val to Thr) and 176 (Ser to Val) were introduced into the constant Kappa domain. All DNA constructs were synthesized by Gene Art.

[0082] PCR reactions, using PfuTurbo Hot Start, were carried out to amplify the inserts, which were then digested with NotI and Apal, and NotI and HindIII for heavy and light chains, respectively. The double digested heavy chain fragments were ligated with NotI and Apal treated pcDNA3.1 expression vector (Invitrogen) into which the human IgG1 hinge followed by the CH2-CH3 domains were already inserted. The double-digested light chain fragments were ligated with NotI and HindIII treated pcDNA3.1 expression vector (Invitrogen). Plasmid DNAs were verified by double strand DNA sequencing.

Expression and Purification

[0083] The bispecific antibody BiXAb-6567 was produced employing transient gene expression by co-transfecting 3 genes coded on separate vectors in a 2:1:1 = HC:LC1:LC2 molecular ratio (1 continuous heavy chain (HC) and 2 light chains (LC)) in CHO-S cells adapted to serum-free medium in suspension (CHO SFM-II medium, Life Technologies™). Typically, for 50 mL scale expression, a total of 50 µg of plasmid DNA (25 µg heavy chain, 12.5 µg of anti-CD38 light chain and 12.5 µg of anti-PD-L1 light chain) were mixed in a 1.5 mL Eppendorf tube, then 1 mL of CHO SFM medium containing 25 µL of 3 mg/mL PEI transfection reagent pH7.0 (Polyplus) was added, and the reaction incubated at room temperature for 20min. The DNA-PEI mixture

was subsequently added to 49 mL of Life Technologies' Invitrogen FreeStyle™ CHO-S cells at 1~2x 10⁶/mL in a 125mL shake flask. Cells were shaken for 6 days. The supernatant was harvested by centrifugation at 3,000 rpm for 15 min. The expression titer of BiXAb-6567 in the supernatant was determined using ForteBio's protein A biosensors (Octet® Systems). BiXAb-6567 was then purified on protein A affinity resin (MabSelect SuRe, GE Healthcare Life Sciences). The antibody was eluted from protein A using 0.1 M glycine pH 3.5, and the eluate was neutralized by 1 M TRIS. The purified antibody, in Dulbecco's PBS (Lonza), was sterile-filtered (0.2 µM sterile filters, Techno Plastic Products AG), and the final concentration determined by reading the optical density (OD) at 280 nm (Eppendorf BioSpectrometer®).

[0084] BiXAab-6567 typically exhibited good expression titer (> 180 mg / liter) in transient CHO expression. This level of expression is comparable to the level of expression seen with conventional monoclonal antibodies.

SDS polyacrylamide gel electrophoresis

[0085] In order to evaluate the quality of purified BiXAb-6567, we performed SDS-PAGE (Experion™ automated electrophoresis system, BioRad). In the presence of sodium dodecyl sulfate (SDS) in the running buffer, the rate at which an antibody migrates in the gel depends primarily on its size, enabling molecular weight determination. This assay was performed under non-reducing conditions and under reducing conditions; the latter permits disruption of the disulfide bonds, and hence visualization of individual polypeptide chains (the light chains and the heavy chain). The SDS-PAGE data are presented in **Figure 5**. Under non-reducing conditions, the quaternary structure of the antibody is maintained, and the molecular weight observed should represent the sum of the molecular weights of the different heavy and light chains. The bispecific antibody of the invention (BiXAb-6567) consists of six chains: two heavy chains and four light chains. The theoretical molecular weight of BiXAb-6567 is 244.40 kDa, not accounting for post-translational modifications (PTM), e.g. N-glycosylation in the Fc at asparagine 297. The gel was calibrated using a mixture of standards of known molecular weight. The non-reducing data exhibit a major band running close to the 250 kDa molecular weight standard, which is in accordance with the calculated molecular weight and the expected glycosylation of two asparagines at position 297 in the Fc domain. Under reducing conditions, dithiothreitol (DTT) further denatures BiXAb-6567 by reducing the disulfide linkages and breaking the quaternary structure, and thus the six polypeptide chains should migrate separately in the gel according to their molecular weight. The two identical heavy chains of BiXAb-6567 co-migrate as a single band, and the two pairs of light chains, due to their nearly identical molecular weight, co-migrated as the second band. Therefore, the data exhibit two major bands, at approximately 75 kDa and 25 kDa, based on the mobility of the molecular weight standards. Each heavy chain possessed one N-glycosylation site at asparagine 297, which explains the broadness of the higher molecular weight band and the observed molecular weight slightly higher than calculated (75.44 kDa); this broadening is typical for glycosylated proteins. The calculated molecular weights of the light chains of anti-CD38 (23.40 kDa) and

anti-PD-L1 (23.36 kDa) are very similar, and thus resulted in their co-migration.

[0086] In conclusion, the SDS-PAGE of BiXAb-6567 exhibited the expected profiles, under both non-reducing and reducing conditions, and was in agreement with the calculated theoretical molecular weights, when accounting for the existence of an N-glycosylation site in the heavy chain.

Size Exclusion chromatography analysis

[0087] Protein aggregation is frequently observed in engineered protein molecules. We performed analytical size exclusion chromatography (SEC) to assay the high molecular weight species content of the single-step affinity-purified BiXAb-6567 preparation (see Expression and Purification of variants). We employed an SEC-s3000 (300x 7.8 mm) column (BioSep) and an AktaPurifier 10 system (GE Healthcare); the assay was conducted at a flow rate of 1 mL/min using PBS buffer pH 7.4.

[0088] The SEC chromatogram presented in **Figure 6** demonstrated that the main peak corresponded to the expected size of the monomeric BiXAb-6567; this peak represented 98.2% of the total sample. In addition, a small peak corresponding to higher molecular weight species (possibly dimers) was observed; this peak represented 1.8% of the total sample. Thus, we concluded that the percentage content of higher molecular weight species is minor, and is similar to conventional monoclonal antibodies produced in CHO expression systems. The narrow and symmetric shape of the monomeric peak suggested that BiXAb-6567 was correctly assembled and was represented by a single species.

Example 3. Characterization of BiXAb-6567 by Differential Scanning Calorimetry

[0089] Differential Scanning Calorimetry (DSC) was used to compare the thermal stability of BiXAb-6567, the parental anti-CD38 mAb, and the parental anti-PD-L1 mAb. A Microcal™ VP-Capillary DSC system (Malvern Instruments) was used to perform differential scanning calorimetry experiments.

[0090] All samples were centrifuged (20,000x g, 5 min, 4 °C), and their protein content was quantitated prior to the DSC analysis using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific) employing the IgG analysis program. For assay, all samples were diluted in PBS to a final concentration of 1mg/mL

[0091] The pre-equilibration time was 3 min, and the resulting thermograms were acquired between 20 and 110 °C at a scan rate of 60 °C/h, a filtering period of 25 sec, and medium feedback. Prior to sample analysis, 5 buffer/buffer scans were measured to stabilize the instrument, and a buffer/buffer scan was performed between each protein/buffer scan. The

data were fit to a non-2-state unfolding model, with the pre- and post- transition adjusted by subtraction of the baseline.

[0092] The DSC curves presented in **Figure 7** (covering the 50 to 100 °C range) demonstrated the manner in which individual Fv regions can lead to different Fab unfolding profiles; this experiment also demonstrated that the Fv regions dictate the apparent stabilities of the Fabs. The DSC profile of the anti-CD38 mAb exhibited two transitions: a large peak having a Cp max of 170 Kcal/mole/oC and a Tm1 of 70.9 oC, corresponding to the unfolding of both CH2 and Fab domains, and a small peak having a Cp max of 20 Kcal/mole/oC and a Tm2 of 81.5oC, corresponding to the unfolding of the CH3 domain. The DSC profile of the anti-PD-L1 mAb exhibited two transitions: a small peak having a Cp max of 20 Kcal/mole/oC and a Tm1 of 69.9 oC, corresponding to the unfolding of the CH2 domain, and a large peak having a Cp max of 160 Kcal/mole/oC and a Tm2 of 83.4 oC, corresponding to the unfolding of both CH3 and Fab domains.

[0093] The DSC profile of BiXAb-6567 also exhibited two transitions with two large peaks. The first peak had a Cp max of 130 Kcal/mole/oC and a Tm1 of 71.5 oC, and corresponded to the unfolding of the CH2 and Fab domains of the anti-CD38 mAb; the second peak had a Cp max of 170 Kcal/mole/oC and a Tm2 of 81.5 oC, and corresponded to the unfolding of the CH3 and Fab domains of the anti-PD-L1 mAb. Thus, the DSC profile of BiXAb-6567 resembled the superposition of the two DSC profiles of the two parental mAbs, and illustrated the excellent assembly and stability of BiXAb-6567. The Tonset of BiXAb-6567 (63.3 oC) was similar to that of the parental mAbs (anti-CD38 Tonset=63.5 oC and anti-PD-L1 Tonset=63.2 oC), indicating that BiXAb-6567 possessed stability properties similar to those of the parental antibodies. The calculated ΔH of BiXAb-6567 was 1560 kcal/mole, reflecting the larger size of the bispecific molecule relative to the two parental antibodies (anti-CD38 ΔH =963 kcal/mole and anti-PD-L1 ΔH =820 kcal/mole).

Definitions:

[0094] Tm or denaturation/melting temperature is the point at which the concentration of the unfolded and folded species is equal, and is the midpoint of the unfolding transition. As a parameter, it describes the susceptibility of the protein to thermal denaturation, and thus it relates to the stability of the protein. The higher the Tm the more stable the protein.

[0095] Tonset is the temperature at which the unfolding transition begins. The values for this parameter are usually 5 to 10 °C lower than the Tm. It is also a parameter describing protein stability, but with relevance to the resistance to thermal denaturation.

[0096] ΔH is the calorimetric enthalpy of unfolding, and reflects the disruption of intramolecular interactions in the protein (i.e. breaking of intra- and inter- domain interactions). The thermal unfolding process is endothermic, and thus yields positive enthalpy values. The calorimetric enthalpy (ΔH) is the area under the thermal unfolding transition peak.

Example 4. Cell free binding properties of BiXAb-6567**Direct CD38 antigen-binding plate ELISA assay**

[0097] 100 μ L of either parental mAb, anti-CD38 or anti-PDL1, each at a concentration of 3 μ g/mL, prepared by dilution with PBS pH 7.4, were used to coat Maxisorp plates at 4°C overnight. Also, BiXAb-6567, at a concentration of 5 μ g/mL, prepared by dilution with PBS pH 7.4, was used to coat Maxisorp plates at 4°C overnight. The plates were washed 5 times with 1x PBS containing 0.05% Tween-20 (PBST), and then blocked with 200 μ L/well 1% BSA in 1x PBS at room temperature for 2 hrs. The plates were subsequently washed 5 times with 1x PBST. A seven-point 3-fold dilution series of recombinant CD38 His/Flag-tagged (Creative Biomart) in 1x PBS, starting at 1 μ g/mL, was prepared; 100 μ L of each dilution step was added per assay well. The plates were incubated at room temperature for 1 hr, and washed 5 times with 1x PBST. 100 μ L/well of anti-Flag-tag antibody-conjugated HRP (Abcam), diluted 10,000-fold in 1x PBS, was added and the plates were incubated at room temperature for 1 hr. After 5 washes with 1x PBST, 100 μ L/well of TMB substrate in 1x PBS was added for colorimetric readout, and the plates incubated for 15 min at room temperature for color development. The assay data were collected employing a Victor2 microplate reader (Perkin Elmer) at 650nm.

[0098] BiXAb-6567 exhibited a dose-dependent binding curve very similar to that of the parental anti-CD38 antibody (**Figure 8A**). The EC₅₀ of CD38 binding for both antibodies were as follows: EC₅₀[BiXAb-6567] = 171 ng/mL and EC₅₀[anti-CD38] = 199 ng/mL. This result suggested that BiXAb-6567 possessed correctly assembled anti-CD38 Fab domains, since it exhibited binding similar to that of the parental anti-CD38 mAb. The parental anti-PDL1 mAb, used as a negative control, did not exhibit any binding, as expected.

Direct PDL1 antigen binding plate ELISA assay.

[0099] 100 μ L of biotinylated human PD-L1 protein (AcroBiosystems) at a concentration of 1 μ g/mL, prepared by dilution with 1x PBS pH7.4, was used to coat Maxisorp plates at 4°C overnight. The plates were washed 5 times with PBST, and then blocked with 200 μ L/well 1% BSA in 1x PBS at room temperature for 2 hrs. The plates were subsequently washed 5 times with 1x PBST. Seven-point 3-fold dilution series of either the anti-CD38 mAb (starting at 0.3 mg/mL), or the anti-PD-L1 mAb (starting at 0.3 mg/mL), or BiXAb-6567 (starting at 0.5 mg/mL) in 1x PBS were prepared; 100 μ L of each dilution step was added per assay well. The plates were incubated at room temperature for 1 hr and washed 5 times with 1x PBST. 100 μ L/well of anti-human antibody (IgG H&L)-conjugated HRP (Abliance), diluted 5,000-fold in 1x PBS, was added, and the plates were incubated at room temperature for 1 hr. After 5 washes with 1x PBST, 100 μ L/well of TMB substrate in 1x PBS was added for colorimetric readout, and the

plates incubated for 15 min at room temperature for color development. The assay data were collected employing a Victor2 microplate reader (Perkin Elmer) at 650nm.

[0100] BiXAb-6567 exhibited a dose-dependent binding curve very similar to that of the parental anti-PD-L1 antibody (**Figure 8B**). The EC₅₀ of PD-L1 binding for both antibodies were as follows: EC₅₀[BiXAb-6567] = 93 ng/mL and EC₅₀[anti-PD-L1] = 72 ng/mL. This result suggested that BiXAb-6567 possessed correctly assembled anti-PD-L1 Fab domains, since it exhibited binding similar to that of the parental anti-PD-L1 mAb. The parental anti-CD38 mAb, used as a negative control, did not exhibit any binding, as expected.

Dual antigen-binding ELISA assay

[0101] 100 µL of recombinant human Fc-tagged CD38 (Creative BioMart), at 2 µg/mL prepared by dilution with 1x PBS pH7.4, was used to coat Maxisorp plates at 4°C overnight. The plates were washed 5 times with 1x PBST, and then blocked with 200 µL/well 1% BSA in 1x PBS at room temperature for 2 hrs. The plates were washed 5 times with 1x PBST. A seven-point three-fold dilution series in 1x PBS of BiXAb-6567 (starting at 1 µg/mL) was prepared, and 100 µL of each dilution step was added per assay well. The plates were incubated at room temperature for 1 hr, and subsequently washed 5 times with 1x PBST. 100 µL/well of 1 µg/mL biotinylated human PD-L1 (AcroBiosystems) in 1x PBS was added, and the plates were incubated at room temperature for 1 hr. After 5 washes with 1x PBST, 100 µL/well of 0.1 µg/mL of streptavidin-conjugated HRP (Biotechne) prepared by dilution with 1x PBS was added. The plates were incubated at room temperature for 1 hr. After 5 washes with 1x PBST, 100 µL/well of TMB substrate in 1x PBS was added for colorimetric readout, and the plates incubated for 15 min at room temperature for color development. The assay data were collected employing a Victor2 microplate reader (Perkin Elmer) at 650nm.

[0102] BiXAb-6567 exhibited a dose-dependent binding curve in the dual ELISA format, suggesting that it possessed correctly assembled anti-CD38 and anti-PD-L1 Fab domains (**Figure 8C**). This demonstrated that BiXAb-6567 is a bispecific antibody capable of binding CD38 and PD-L1 simultaneously with EC₅₀ = 144 ng/ mL. Neither of the two parental mAbs, anti-CD38 or anti-PDL1, exhibited any binding in this dual ELISA format, as expected.

Example 5. Determination of relative binding activity by Fluorescence-activated cell sorting (FACS)

[0103] CHO-CD38 cells (CHO cells stably transfected with full length human CD38) were cultured in DMEM-Glutamax-I medium supplemented with 100 µg/ml penicillin, 100 µg/ml streptomycin, 10% fetal calf serum and 500 µg/ml geneticin. SKOV-3 cells and RPMI-8226 cells were cultured in RPMI 1640-Glutamax-I medium, supplemented with 100 µg/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum.

[0104] 3×10^5 cells (CHO-CD38, or SKOV-3, or RPMI-8226) per each sample were used. Cells were washed $1 \times$ with the PBA solution (PBS supplemented with 1%BSA and 0.05% Na-azide). For the determination of the FACS profiles, the cells were stained with the respective antibodies at a concentration of $50 \mu\text{g/ml}$ in a volume of $30 \mu\text{l}$. For the titration of BiXAb-6567 and the parental anti-CD38 antibody, and subsequent determination of the binding parameters, CHO-CD38 cells were stained with the respective antibodies at the indicated concentrations in a volume of $30 \mu\text{l}$. Cells were incubated for 30 min on ice and then washed 2 times with 1 ml of PBA solution. Cells were incubated with fluorescently labelled anti-human kappa or anti-human IgG Fc gamma specific secondary antibodies on ice in the dark for 30 min, and then washed 2 times with 1 ml PBA solution; lastly, cells were re-suspended in a final volume of $500 \mu\text{l}$ PBA solution. Samples were assayed using either an Epics-XL or a Navios flow cytometer (Beckman Coulter). 10.000 events were acquired in each experiment.

[0105] The binding profiles of BiXAb-6567 and the parental anti-CD38 and anti-PD-L1 parental antibodies are presented in **Figures 9A-C**. We chose to test a multiple myeloma cell line, RPMI-8226, which expresses high levels of CD38 and negligible levels of PD-L1 (**Figure 9A**); a CHO-CD38 cell line that expressed a very high level of CD38 due to stably transfected full length CD38 (**Figure 9B**); and an ovarian cancer cell line SKOV-3, which is known to express PD-L1 (**Figure 9C**). These profiles exhibited a single peak for BiXAb-6567 that was very similar to the profiles of both parental antibodies on the 3 cell lines. This suggested that BiXAb-6567 is correctly folded and possesses binding attributes similar to those of the parental antibodies. As expected, CHO-CD38 expressed only CD38 and no PD-L1, whereas SKOV-3 expressed only PD-L1 and no CD38.

[0106] In order to quantitatively confirm that the binding properties of BiXAb-6567 are similar to those of the parental anti-CD38 antibody, a titration of BiXAb-6567 and the anti-CD38 parental antibody was performed employing CHO-CD38 cells, as presented in **Figure 10**. The EC_{50} of BiXAb-6567 was determined to be 17.1 nM and that of the parental anti-CD38 was 8.5 nM , confirming the similar binding properties of the anti-CD38 Fab domains in BiXAb-6567 and in the parental anti-CD38 antibody. Negative controls in this experiment, anti-PD-L1 and anti-CD20 antibodies, demonstrated no binding to CHO-CD38 cells, as expected.

Example 6. Antibody Dependent Cell-mediated Cytotoxicity (ADCC) with unfractionated non-preactivated mononuclear cells (MNC)

[0107] CHO-CD38, SKOV-3, and RPMI-8226 cells were cultured as described in Example 5 above. For preparation of MNC the following procedure was employed. Freshly drawn peripheral blood was anti-coagulated with citrate. Subsequently, 5ml of Ficoll-Paque PLUS solution was layered with 6 ml anti-coagulated whole blood. Samples were centrifuged for 20 min at 2,500 rpm at RT with no subsequent centrifuge breaking. MNC were collected from the plasma / Ficoll interface. The MNC cell suspension was diluted 1:10 in PBS and centrifuged for 5 minutes at 1,800 rpm at room temperature. The supernatant was removed, and the

erythrocytes were lysed by addition of 45 ml ice-cold distilled water to the cell suspension for 30 seconds, after which 5 ml of 10x PBS was added. The cells were centrifuged for 5 min at 1800 rpm at room temperature and washed with 1x PBS three times to remove platelets. Finally cells were re-suspend in 5 ml cell culture medium. Cell numbers were adjusted to achieve 40:1= Effector cell : Tumor cell ratio in the ADCC assays.

[0108] For the ADCC ⁵¹Chromium release assay, 1×10⁶ target cells (RPMI 8226, SKOV-3, or CHO-CD38) were incubated with 100μCi ⁵¹ Chromium in 200 μl PBS for 2 hours at 37°C and 5% CO₂. After 2 hours incubation, cells were washed three times with 7 ml of medium and finally re-suspended at a concentration of 0.1 × 10⁶ cells/ml. Target cells (5,000 cells/well) and MNC in the presence of antibodies were incubated in a 96-well micro-titer plate (200μl assay volume) for 3 hours at 37°C and 5% CO₂. For the determination of maximal target cell lysis (= maximal cpm) Triton X-100 was added. To determine basal ⁵¹Chromium release (= basal cpm) target cells were not further manipulated. After 4hr incubation, micro-titer plates were centrifuged for 5 min at 2000 rpm and 25 μl supernatant was mixed with 125 μl of Optiphase Supermix (Perkin Elmer) and incubated in a shake incubator for 1 min. Samples were assayed in a MicroBeta TriLux (Perkin Elmer) beta-counter instrument. Target cell lysis was calculated using the following formula:

$$\% \text{ lysis} = (\text{experimental cpm} - \text{basal cpm}) / (\text{maximal cpm} - \text{basal cpm}) \times 100.$$

[0109] All of the measurements were performed in triplicate.

[0110] ADCC assays of CD38+ cells (RPMI-8226 and CHO-CD38) were performed employing non-pre-activated MNC as effector cells (**Figures 11 and 12**) The assays showed potent cytotoxicity of BiXAb-6567 and the anti-CD38 antibody on RPMI-8226 cells with EC₅₀ of 0.8 nM and 0.3 nM, respectively; on CHO-CD38 cells, the cytotoxicity of BiXAb-6567 and the anti-CD38 antibody had EC₅₀ of 0.2 nM and 0.07 nM, respectively. Anti-PD-L1 showed minimal activity on both cells lines; two negative control mAbs, anti-CD20 and anti-HER2, did not facilitate any lysis, as expected. These results demonstrate the potent ADCC activity of BMX-6567 against CD38+ cells, which is similar to that of the parental anti-CD38 antibody.

Example 7. ADCC with enriched pre-activated NK cells

[0111] SKOV3 cells, RPMI 8226, and CHO-CD38 cells were cultured as described in Example 5. MNC were prepared as described in Example 6. NK cells were isolated from MNC by negative selection employing the "NK cell isolation kit, human" (Miltenyi) according to the manufacturer's instructions. NK cells were cultivated over night at a seeding density of 2×10⁶ cells / ml in RPMI medium supplemented with 10% fetal calf serum. IL-12 or IL-15 was added to a final concentration of 10 ng/ml. ADCC assays were performed as outlined in Example 5 with the exception that the Effector cell : Tumor cell ratio was kept at 10:1 and the duration of the reaction was reduced to 3 hr.

[0112] The ADCC properties of the anti-PD-L1 moiety of the BiXAb-6567 were assayed on the PD-L1+ cell line SKOV-3 employing either IL-12 or IL-15 pre-activated enriched NK cells. The results are presented in **Figures 13 and 14**. This experiment compared the ADCC properties of BiXAb-6567 with those of the parental anti-PD-L1 antibody; as a positive control an anti-HER2 antibody was employed, and as negative controls an anti-CD20 antibody and the parental anti-CD38 antibody were employed since SKOV-3 cells are PD-L1+/HER2+/CD20-/CD38-. **Figures 13 and 14** demonstrate the potent ADCC activity of BiXAb-6567 and the parental anti-PD-L1 antibodies, independently of whether IL-12 or IL-15 was employed in culturing the NK cells. The EC50 of BiXAb-6567 and the parental anti-PD-L1 antibodies were 0.007 nM and 0.03 nM, respectively, when IL-12 was used. The profiles were even more similar when IL-15 was employed; however the curve fits did not converge, thus preventing the calculation of EC50 values. These results demonstrate the potent ADCC activity of BMX-6567 against PD-L1+ cells, which is similar to that of the parental anti-PD-L1 antibody.

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Met His Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Asp Tyr Tyr Gly Ser Asn Ser Leu Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Lys Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Thr Pro Pro Thr Pro Ser Pro Ser Gly Gly Glu Asn Leu Tyr
245 250 255

Phe Gln Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
260 265 270

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
275 280 285

Ser Arg Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
290 295 300

Glu Trp Val Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val
305 310 315 320

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
325 330 335

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
340 345 350

Tyr Tyr Cys Ala Arg Glu Gly Gly Trp Phe Gly Glu Leu Ala Phe Asp
355 360 365

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
 370 375 380

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 385 390 395 400

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 405 410 415

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 420 425 430

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
 435 440 445

Val Glu Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
 450 455 460

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
 465 470 475 480

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 485 490 495

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 500 505 510

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 515 520 525

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 530 535 540

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
 545 550 555 560

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 565 570 575

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 580 585 590

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 595 600 605

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
 610 615 620

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 625 630 635 640

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 645 650 655

Thr Pro Pro Val Leu Asp Ser Asp Glv Ser Phe Phe Leu Trv Ser Lvs

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          660          665          670
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    675                      680                      685

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    690                      695                      700

Ser Leu Ser Pro Gly Lys
    705                      710

<210> 8
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> LC1

<400> 8
Asp Ile Val Met Thr Gln Ser His Leu Ser Met Ser Thr Ser Leu Gly
  1              5              10              15

Asp Pro Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Val
    20              25              30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Arg Leu Ile
    35              40              45

Tyr Ser Ala Ser Tyr Arg Tyr Ile Gly Val Pro Asp Arg Phe Thr Gly
    50              55              60

Ser Gly Ala Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
    65              70              75              80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Pro Pro Tyr
    85              90              95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
    100             105             110

Pro Ala Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
    115             120             125

Thr Ala Ser Val Val Cys Leu Leu Glu Asn Phe Tyr Pro Arg Glu Ala
    130             135             140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
    145             150             155             160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
    165             170             175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
    180             185             190

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Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

Phe Asn Arg Gly Glu Cys
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<210> 9

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<223> LC2

<400> 9

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Asp Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Leu Pro
 85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
 100 105 110

Ala Pro Ala Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Lys Asn Phe Tyr Pro Arg Glu
 130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
 145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
 165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
 180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
 195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 10

<211> 702

<212> PRT

<213> Artificial Sequence

<220>

<223> BiXAB BMX101-AP-ML1-CC1

<400> 10

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Asn Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Gln Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Val Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Ser Pro Pro Ala Pro Ala Pro Glu Leu Leu
225 230 235 240

225 230 235 240
 Gly Gly Pro Ala Ala Pro Pro Ala Pro Ala Pro Ala Gly Gly Glu Val
 245 250 255
 Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
 260 265 270
 Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser Trp Ile
 275 280 285
 His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Trp
 290 295 300
 Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly
 305 310 315 320
 Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
 325 330 335
 Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 340 345 350
 Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 355 360 365
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 370 375 380
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 385 390 395 400
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 405 410 415
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 420 425 430
 Gly Leu Tyr Ser Leu Ser Ser Val Val Asp Val Pro Ser Ser Ser Leu
 435 440 445
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 450 455 460
 Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 465 470 475 480
 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 485 490 495
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 500 505 510
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 515 520 525
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 530 535 540

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Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
545                550                555                560

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
                    565                570                575

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                    580                585                590

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
595                600                605

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
610                615                620

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
625                630                635                640

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                    645                650                655

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                    660                665                670

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
675                680                685

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
690                695                700

<210> 11
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Daratumumab LC

<400> 11
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1          5          10          15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
          20          25          30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
          35          40          45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
          50          55          60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65          70          75          80

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Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Thr Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Val
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 12

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Atezolizumab-LC

<400> 12

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala

100	105	110
Pro Ala Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly		
115	120	125
Thr Ala Ser Val Val Cys Leu Leu Lys Asn Phe Tyr Pro Arg Glu Ala		
130	135	140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln		
145	150	155
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser		
165	170	175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr		
180	185	190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser		
195	200	205
Phe Asn Arg Gly Glu Cys		
210		

<210> 13

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 1

<220>

<221> misc_feature

<222> (4)..(4)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (8)..(8)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (10)..(11)

<223> Xaa can be any naturally occurring amino acid

<220>

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<222> (14)..(14)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (24)..(25)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (28)..(28)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (30)..(30)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (32)..(32)

<223> Xaa can be any naturally occurring amino acid

<400> 13

Glu	Pro	Lys	Xaa	Cys	Asp	Lys	Xaa	His	Xaa	Xaa	Pro	Pro	Xaa	Pro	Ala
1				5					10					15	

Pro	Glu	Leu	Leu	Gly	Gly	Pro	Xaa	Xaa	Pro	Pro	Xaa	Pro	Xaa	Pro	Xaa
			20					25					30		

Gly Gly

<210> 14

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 2

<400> 14

Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ala	Pro	Ala
1				5					10					15	

Pro	Glu	Leu	Leu	Gly	Gly	Pro	Gly	Gly	Pro	Pro	Gly	Pro	Gly	Pro	Gly
			20					25					30		

Gly Gly

<210> 15

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 2

<400> 15

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ala Pro Ala
 1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ala Ala Pro Pro Ala Pro Ala Pro Ala
 20 25 30

Gly Gly

<210> 16

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 3

<400> 16

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ala Pro Ala
 1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ala Ala Pro Pro Gly Pro Ala Pro Gly
 20 25 30

Gly Gly

<210> 17

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 4

<400> 17

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Thr Pro Pro Thr Pro Ser Pro Ser
 20 25 30

Gly Gly

<210> 18

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 6

<400> 18

Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala
1				5					10					15	

Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Thr	Pro	Pro	Thr	Pro	Ser	Pro	Ser
			20					25					30		

Gly Gly

<210> 19

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Nter IgG1 CH2

<400> 19

Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
1				5				

<210> 20

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Portion of human IgA1 hinge

<400> 20

Thr	Pro	Pro	Thr	Pro	Ser	Pro	Ser
1				5			

<210> 21

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> signal peptide

<400> 21

Met	Asn	Phe	Gly	Leu	Arg	Leu	Ile	Phe	Leu	Val	Leu	Thr	Leu	Lys	Gly
1				5					10					15	

Val Gln Cys

<210> 22

<211> 122

<212> PRT

<213> Homo sapiens

<400> 22

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Asn Ser Phe
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
          50          55          60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
          85          90          95

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr Trp
          100          105          110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
          115          120

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<210> 23

<211> 98

<212> PRT

<213> Artificial Sequence

<220>

<223> CH1 domain of daratumumab

<400> 23

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Gln Ala Pro Ser Ser Lys
1          5          10          15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
          20          25          30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
          35          40          45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
          50          55          60

Leu Val Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65          70          75          80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
          85          90          95

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Arg Val

<210> 24

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> VH of atezolizumab

<400> 24

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
 20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
 100 105 110

Leu Val Thr Val Ser Ser
 115

<210> 25

<211> 98

<212> PRT

<213> Artificial Sequence

<220>

<223> CH1 domain of atezolizumab

<400> 25

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Asp Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val

<210> 26

<211> 15

<212> PRT

<213> Homo sapiens

<400> 26

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> 27

<211> 110

<212> PRT

<213> Homo sapiens

<400> 27

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105 110

<210> 28

<211> 107

<212> PRT

<213> Homo sapiens

<400> 28

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu

1				5						10						15
Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	
			20					25					30			
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	
		35					40					45				
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	
	50					55					60					
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	
65					70					75					80	
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	
				85					90					95		
Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys						
			100					105								

<210> 29
<211> 107
<212> PRT
<213> Homo sapiens

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<400> 29
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1             5             10             15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
 20             25             30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35             40             45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
 50             55             60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65             70             75             80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
 85             90             95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100             105

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<210> 30
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CKappa domain of daratumumab

<400> 30

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Thr Cys Leu Leu Asn Asn Phe
 20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 50 55 60

Thr Tyr Ser Leu Val Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105

<210> 31

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> VL of atezolizumab

<400> 31

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 32

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> CKappa domain of atezolizumab

<400> 32

Arg Thr Val Ala Ala Pro Ala Val Phe Ile Phe Pro Pro Ser Asp Glu
 1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Lys Asn Phe
 20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- [US8263746B \[0002\]](#)
- [WO2013005194A \[0046\]](#)
- [US5225539A \[0063\]](#)

Non-patent literature cited in the description

- **MOORE** 57th annual meeting & exposition, Orlando, FL American Society of Hematology 2015 1205 [0007]
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press 1989 0000 [0054]
- **SCOPES** Protein Purification Springer-Verlag 1982 0000 [0055]
- **KARLINALTSCHUL** Proc. Natl. Acad. Sci. USA, 1990, vol. 87, 2264-68 [0057]
- **KARLINALTSCHUL** Proc. Natl. Acad. Sci. USA, 1993, vol. 90, 5873-77 [0057]
- **ALTSCHUL et al.** J. Mol. Biol., 1990, vol. 215, 403-10 [0057]
- **ALTSCHUL et al.** Nucleic Acids Res., 1997, vol. 25, 173389-3402 [0057]
- Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press 1989 0000 [0057]
- Current Protocols in Molecular Biology John Wiley & Sons, Inc. [0057]
- **KABAT et al.** Sequences of Proteins of Immunological Interest Public Health Service, National Institutes of Health 1991 0000 [0059]
- **RIECHMANN et al.** Nature, 1988, vol. 332, 323-327 [0063]

Patentkrav

- 1.** Bispecifikt molekyle omfattende mindst et anti-CD38-domæne og mindst et anti-PD-L1-domæne, hvilke samtidigt kan binde til henholdsvis CD38- og PD-L1-antigener, hvilket bispecifikt molekyle er et fuld længde antistof omfattende to
- 5 tungkæder og fire letkæder,
- hvor hver tungkæde omfatter
- a. en Fc-region omfattende hinge-CH2-CH3-domæner,
- b. hvilken Fc-region er bundet til Fab-tungkæde (CH1-VH) af antistof 1 (Ab1),
- 10 c. som er bundet til nævnte Fab-tungkæde (CH1-VH) af antistof 2 (Ab2), via en hinge-afledt polypeptidlinkersekvens, hvor nævnte polypeptidlinkersekvens binder N-terminus af nævnte Fab-tungkæde VH-domæne af Ab1 med C-terminus af nævnte CH1-domæne af Ab2,
- og de fire letkæder omfatter Fab-letkæder (CL-VL) af Ab1 og Fab-letkæder
- 15 (CL-VL) af Ab2 associeret med deres beslægtede tungkædedomæner; idet Ab1 og Ab2 er forskellige og valgt fra gruppen bestående af anti-CD38-antistoffer og anti-PD-L1-antistoffer.
- 2.** Det bispecifikke molekyle ifølge krav 1, hvor Ab1 er et anti-CD38-antistof, og
- 20 Ab2 er et anti-PD-L1-antistof.
- 3.** Det bispecifikke molekyle ifølge krav 1, hvor Ab1 er et anti-PD-L1-antistof, og Ab2 er et anti-CD38-antistof.
- 25 **4.** Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 3, hvor anti-CD38-antistoffet er valgt fra gruppen bestående af daratumumab, isatuximab og MOR-202.
- 5.** Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 4, hvor
- 30 anti-PD-L1-antistoffet er valgt fra gruppen bestående af atezolizumab, durvalumab, avelumab og MDX-1105.
- 6.** Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 5, hvor CH1- og CL-domænerne af Ab1 har en sekvens forskellig fra CH1- og CL-

domænerne af Ab2.

7. Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 5, hvor Fab-CH1-domænet af et af Ab1 eller Ab2 er et muteret domæne, som stammer
 5 fra CH1-domænet af en immunoglobulin ved substitution af threonin-resten ved position 192 af nævnte CH1-domæne med en asparaginsyre, og det beslægtede CL-domæne er et muteret domæne, som stammer fra CL-domænet af en immunoglobulin ved substitution af asparagin-resten ved 137 af nævnte CL-domæne med en lysin-rest og substitution af serin-resten ved position 114 af
 10 nævnte CL-domæne med en alanin-rest, og/eller hvor Fab-CH1-domænet af det ene eller det andet af Ab1 eller Ab2 er et muteret domæne, som stammer fra CH1-domænet af en immunoglobulin ved substitution af leucin-resten ved position 124 af nævnte CH1-domæne med en glutamin og substitution af serin-resten ved position 188 af nævnte CH1-domæne med en valin-rest, og det beslægtede CL-
 15 domæne er et muteret domæne, som stammer fra CL-domænet af en immunoglobulin ved substitution af valin-resten ved position 133 af nævnte CL-domæne med en threonin-rest og substitution af serin-resten ved position 176 af nævnte CL-domæne med en valin-rest.

8. Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 7, som omfatter, fortrinsvis består af a) to tungkæder, som hver omfatter, fortrinsvis bestående af, SEQ ID NO:10 og b) fire letkæder, to omfattende, fortrinsvis bestående af, SEQ ID NO:11, idet de andre to omfatter, fortrinsvis bestående af, SEQ ID NO: 12.

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9. Fremgangsmåde til fremstilling af det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 8, idet nævnte fremgangsmåde omfatter følgende trin:
 a. dyrkning i et egnet medium og ved egnede dyrkningsbetingelser af en værtselle, som udtrykker en antistof-tungkæde som defineret i et hvilket
 30 som helst af kravene 1 til 8, og en antistof-letkæde som defineret i et hvilket som helst af kravene 1 til 8, og
 b. genvinding af nævnte fremstillede antistoffer fra dyrkningsmediet eller fra nævnte dyrkede celler.

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10. Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 8, til anvendelse som et medikament.

11. Det bispecifikke molekyle ifølge et hvilket som helst af kravene 1 til 8, til
5 anvendelse til behandling af en kræftform, fortrinsvis et multipelt myelom, lymfom eller leukæmi.

DRAWINGS

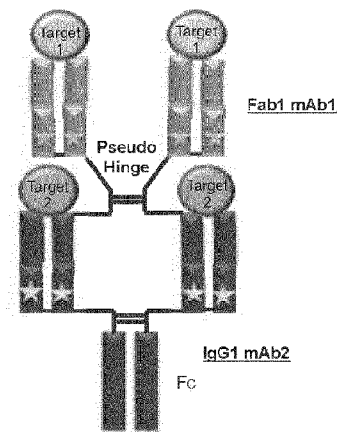


Figure 1A

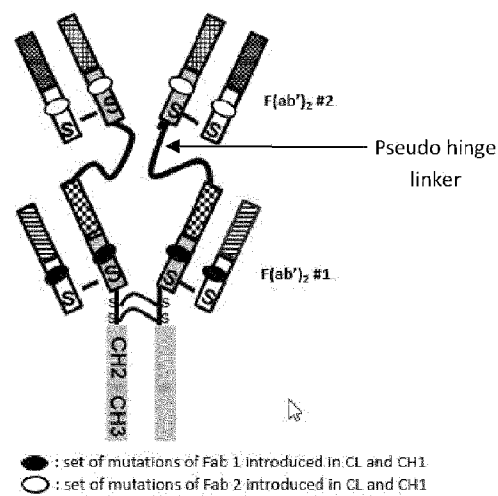
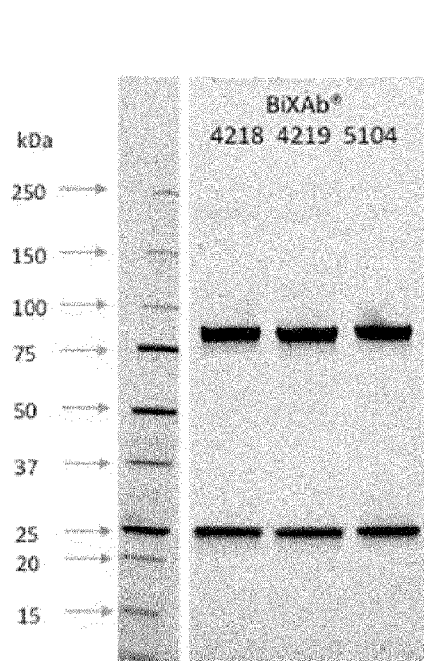
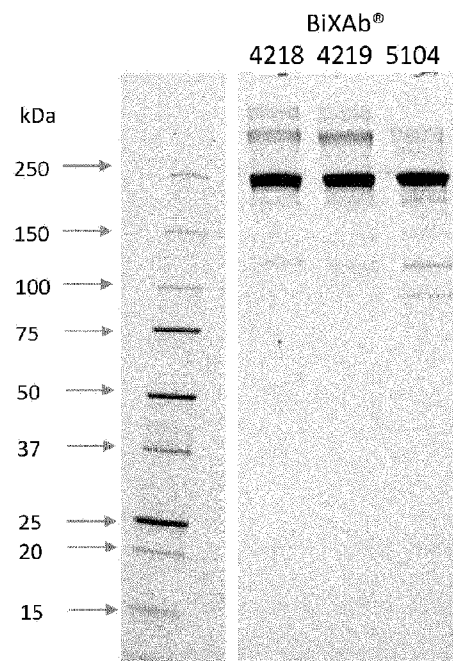


Figure 1B



SDS-PAGE under reducing conditions

Figure 2



SDS-PAGE under non-reducing conditions

Figure 3

Binding ELISA

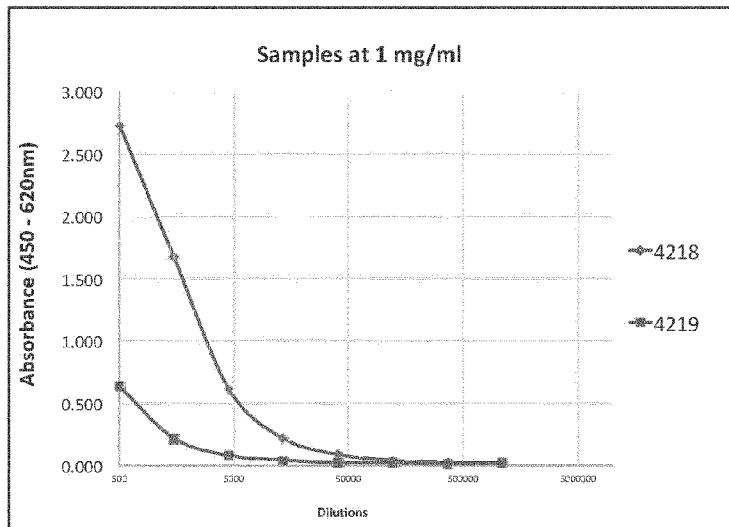


Figure 4

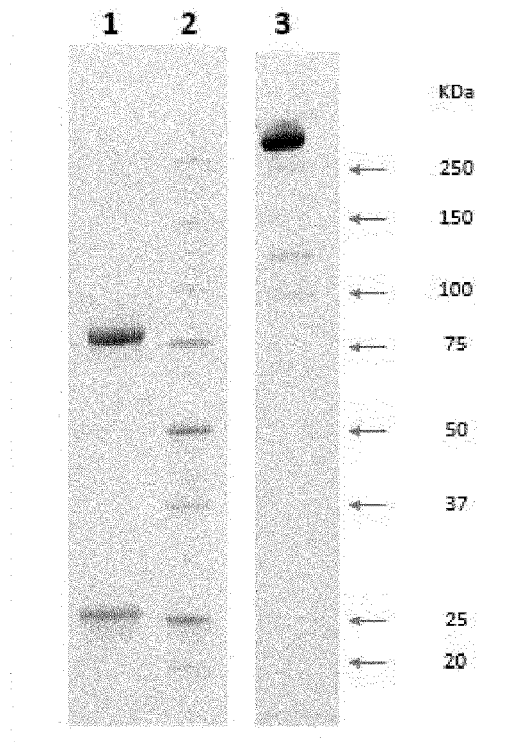


Figure 5

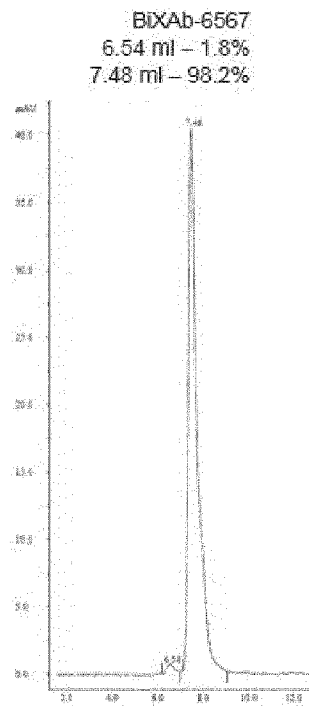


Figure 6

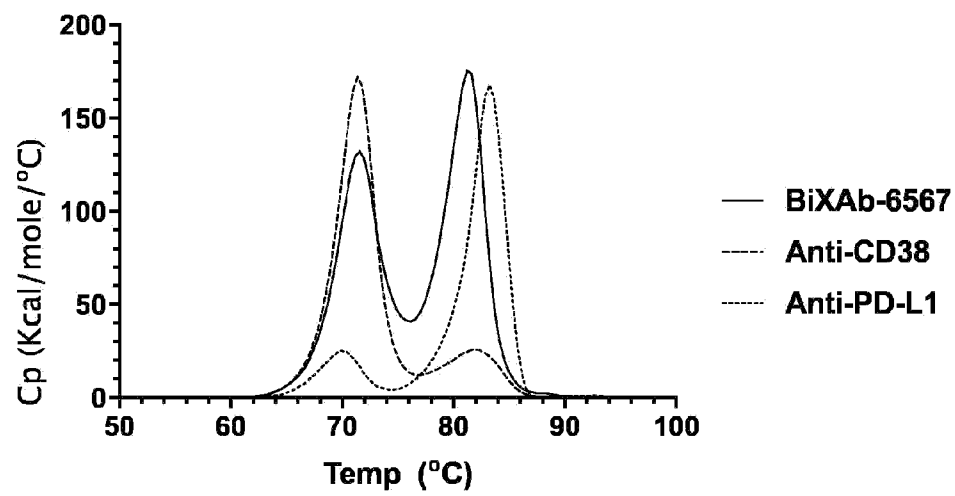


Figure 7

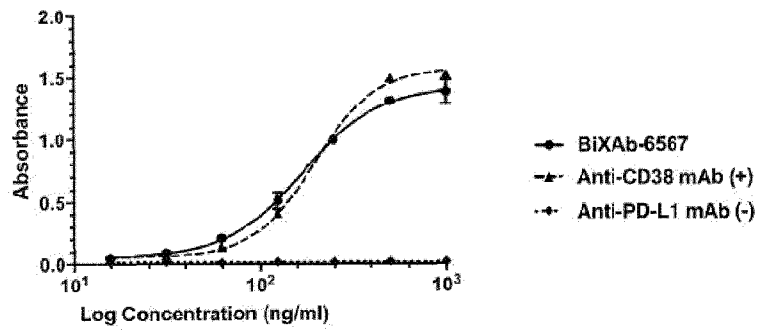


Figure 8A

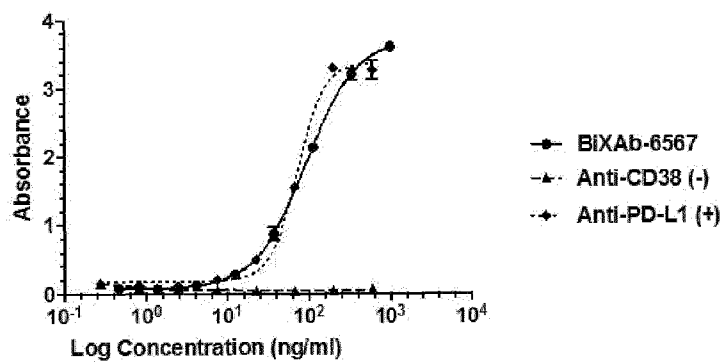


Figure 8B

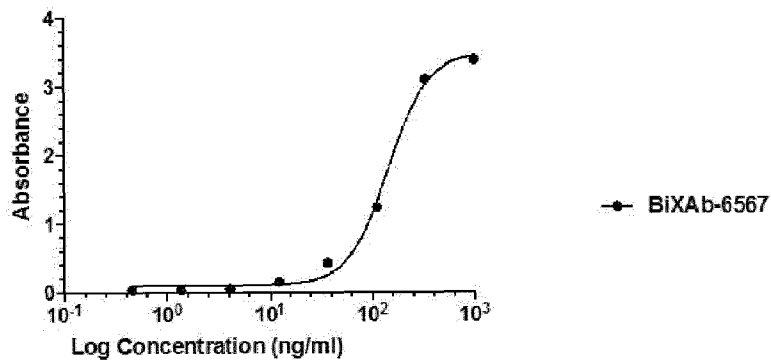


Figure 8C

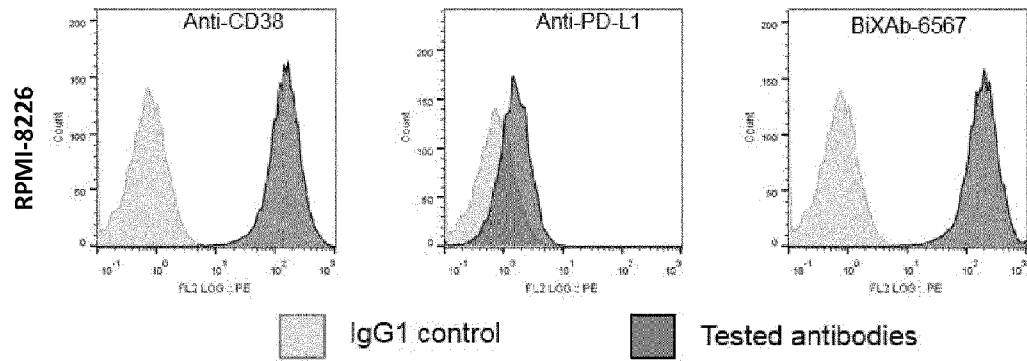


Figure 9A

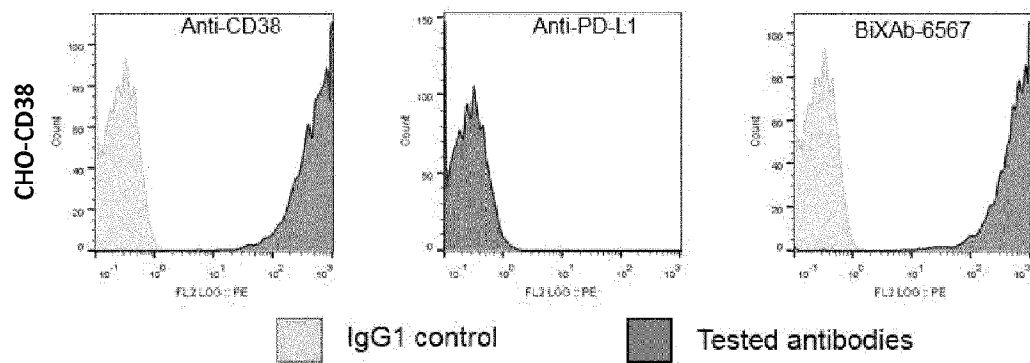


Figure 9B

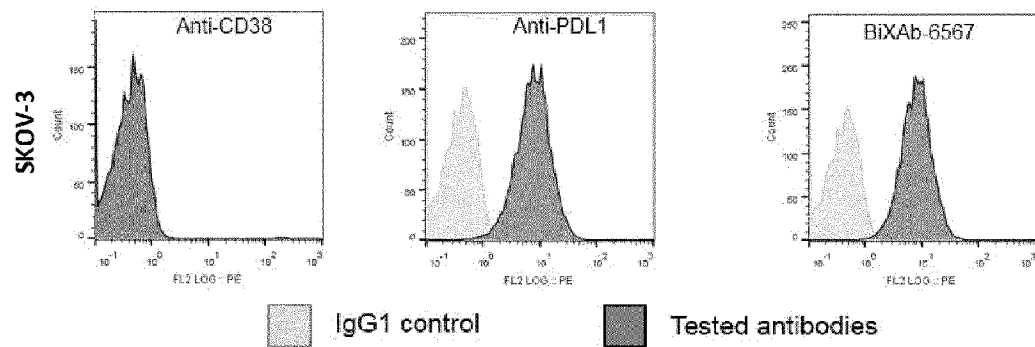


Figure 9C

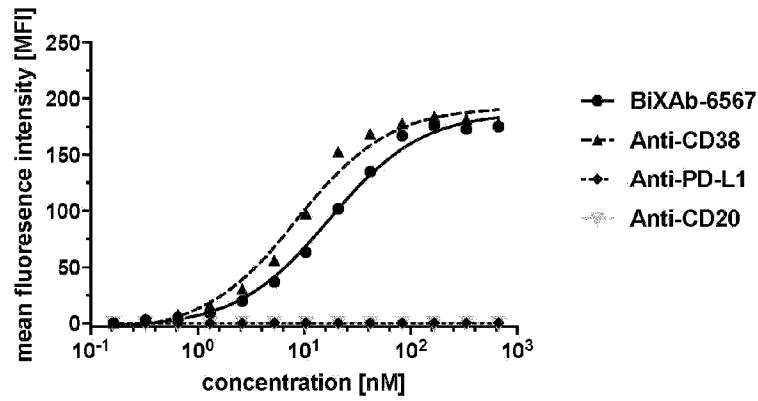


Figure 10

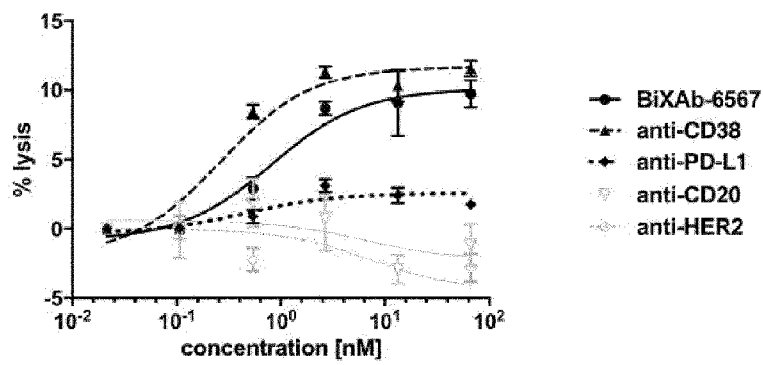


Figure 11

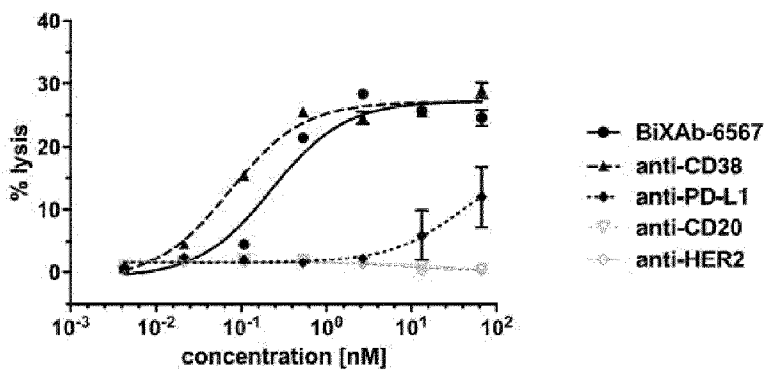


Figure 12

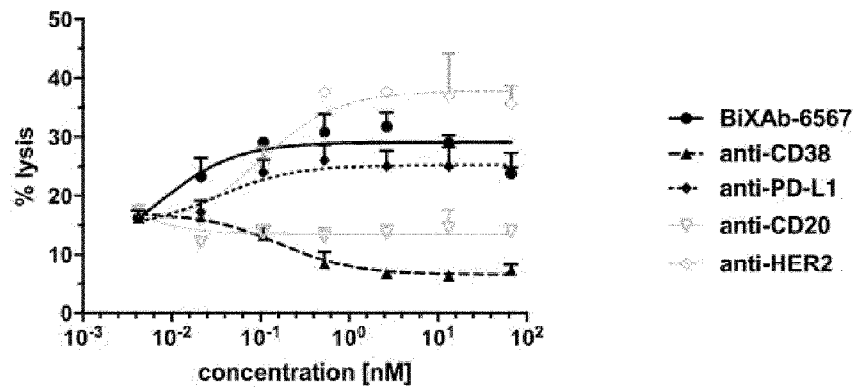


Figure 13

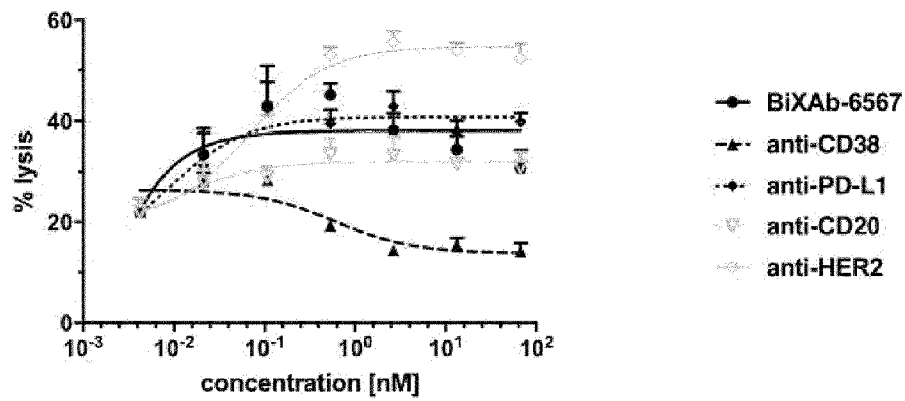


Figure 14