

METHOD OF MANUFACTURING BISPECIFIC ANTIBODIES, BISPECIFIC ANTIBODIES AND THERAPEUTIC USE OF SUCH ANTIBODIES

SEQUENCE LISTING

- 5 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 30, 2018, is named PAT057716-WO-PCT_SL.txt and is 68,498 bytes in size.

TECHNICAL FIELD

- 10 The invention relates to bivalent bispecific monoclonal antibodies (bbmAb) or variants thereof, and methods of manufacturing such antibodies by co-expressing so called knob-into-hole modified FC-mutated derivatives of two different monoclonal antibodies in mammalian cell lines.

15 **BACKGROUND OF THE DISCLOSURE**

- Bispecific antibodies, i.e. antibodies binding to two distinct epitopes, are well known in the art. One approach for generating bispecific antibodies is the so called knobs-into-holes (KiH) approach described e.g. by Merchant et al., Nat. Biotechnol., 16:677-681 (1998), where a first heavy chain IgG is modified to display a hole like
20 structure by introducing point mutations like Y349C, T366S, L368A, Y407V ; and where a second heavy chain IgG is modified to display a knob like structure, by introducing point mutations S354C, T366W ((Merchant et al., Nat. Biotechnol., 16:677-681 (1998), page 678, table 1). The two different IgG structures then interact to form a bivalent bispecific antibody (bbmAb), i.e. a hetero-tetrameric protein, consisting of two different light and
25 two different heavy chains.

 When expressing two KiH modified mAbs in the same host cell line, the desired bbmAb statistically makes up only 25% of the expressed protein, but 75% are so called product related impurities (Klein, Ch. et al., 2012).

- Some approaches to overcome this are known in the art, such as facilitating
30 correct formation of bbmAbs by apply further sequence modifications to enforce the correct H-L-binding (for overview see Klein, Ch. et al., 2012; Kontermann, R. and Brinkmann, U., 2015). However, such additional modifications might increase the risk of anti-drug antibodies.

Another approach to generate bbmAbs is disclosed in WO12023053A2 or WO04009618A2, utilizing a shared heavy or light chain in combination with different variable chains. However, keeping either the heavy chain constant significantly reduces the diversity of the antibody repertoire where the binders can be screened.

5 Yet another approach to generation of bbmAbs is disclosed in US9212230, and entails individual expression and purification of the mAbs carrying different modifications. The resulting mAbs are finally shuffled *in vitro* to form the intended bbmAb. Such in vitro shuffling is a complex additional process step which required careful validation and analytical assessment, and could significantly increase the costs.

10 Thus, existing methods for generating bbmAbs might either limit diversity of the antibody repertoire available to screen for binders or might not provide sufficient overall yield, purity and product quality at a sufficiently cost effective way to enable manufacturing in a scale feasible for clinical development and commercialization. In addition, any modification of protein chains inherently increases the risk to induce anti-
15 drug antibodies. Therefore, approaches which only require minimal protein engineering might be clinically favorable.

SUMMARY OF THE DISCLOSURE

There is a need to provide an improved method for manufacturing of bivalent bispecific antibodies. Particularly, there is a need for a method for manufacturing of
20 bivalent bispecific monoclonal antibodies (bbmAb), ensuring a sufficient overall yield, purity and product quality to proceed with clinical development and commercial manufacturing, at a reasonable cost.

The present invention provides *inter alia* a method for the generation of bbmAbs with one or more of the following advantages: it enables the use of a large antibody
25 repertoire to identify binders as no shared light or heavy chains are required, it does not require any extensive protein engineering, beside the mutation driving the H-chain dimerization, and therefore limits the risk for anti-drug antibodies, it is cost effective as expression is done in a common cell line, therefore the bbmAb can be produced in one cell culture process without the need for a specific in vitro shuffling and it produces high
30 quality material suitable for human use as product related impurities can be efficiently removed.

The present invention is useful for identifying antibodies of kappa and lambda type, where the light chains do not show a strong promiscuous binding towards the heavy chain of the counterpart. This makes the antibodies suitable for use in methods of
35 the invention. An advantage of the method may be that antibody combinations where both light chains exchange the original heavy chain binding partner, which results in a

product related impurity of the H1L2-H2L1 type, can be deselected. This is advantageous, because such product related impurities are not easy to deplete using state of the art purification processes.

As will be shown below, embodiments of the invention enable manufacturing of
5 bbmAb by the use of a CHO co-expression at a yield and quality suitable for clinical development and commercialization of biologics.

In a first aspect of the invention a bispecific antibody suitable for co-expression in a common host cell is provided, wherein the antibody comprises a) a first part which is an immunoglobulin with a variable light chain of lambda wild type (VL1) and a variable
10 heavy chain of wild type (VH1), that binds specifically to a first target, and a first constant heavy chain (CH1) with a hetero-dimerization modification, and b) a second part which is an immunoglobulin with a variable light chain of kappa wild type (L2) and a variable heavy chain of wild type (H2), that binds specifically to a second target, different from the first target, and a second constant heavy chain (CH2) with a hetero-dimerization
15 modification which is complementary to the hetero-dimerization modification of the first constant heavy chain, wherein the first part and the second part, when co-expressed in a common host cell, form a bispecific antibody.

In a further embodiment of the first aspect, the bispecific antibody suitable for co-expression in a common host cell results, after purifying the bispecific antibody by
20 removing mismatched fragments from the correctly matched bispecific antibody, in a bispecific antibody which is at least 60% (mass), 70% (mass), 80% (mass), 85% (mass) pure, such as at least 90% (mass) pure, 95% (mass), 96% (mass), 97% (mass), 98% (mass), or 99% (mass) pure.

The first and second constant heavy chain of the bispecific antibody may be
25 human IgA, IgD, IgE, IgG, or IgM, preferably IgD, IgE or IgG. In a preferred embodiment, the first and second constant heavy chains are human IgG1, IgG2, IgG3, or IgG4, most preferably IgG1. In one embodiment, the first variable light chain is of lambda type, and the second variable light chain is of kappa type.

In a specifically preferred embodiment, the first variable light chain is of lambda
30 type, and the second variable light chain is of kappa 6 type.

The first and second constant heavy chain may be IgG1, wherein the first constant heavy chain has point mutations generating a knob structure and the second constant heavy has point mutations generating a hole structure, or the first constant heavy chain has point mutations generating a hole structure and the second constant
35 heavy has point mutations generating a knob structure. Optionally, the first and second constant heavy chains can additionally have mutations resulting in a disulfide bridge.

In one embodiment, the bispecific antibody comprises a first immunoglobulin VH1 domain, a first immunoglobulin VL1 domain, a second immunoglobulin VH2 domain and a second immunoglobulin VL2 domain, wherein the first immunoglobulin VH1 domain comprises (e.g. in sequence): hypervariable regions CDR1, CDR2 and CDR3, said
5 CDR1 having the amino acid sequence SEQ ID NO:76, said CDR2 having the amino acid sequence SEQ ID NO:77, and said CDR3 having the amino acid sequence SEQ ID NO:78; or hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:79, said CDR2 having the amino acid sequence SEQ ID NO:80, and said CDR3 having the amino acid sequence SEQ ID NO:81; and the first
10 immunoglobulin VL1 domain comprises (e.g. in sequence): hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:92, said CDR2 having the amino acid sequence SEQ ID NO:93, and said CDR3 having the amino acid sequence SEQ ID NO:94 or hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:95, said CDR2 having the amino
15 acid sequence SEQ ID NO:96, and said CDR3 having the amino acid sequence SEQ ID NO:97; the second immunoglobulin VH2 domain comprises (e.g. in sequence): hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:44, said CDR2 having the amino acid sequence SEQ ID NO:45, and said CDR3 having the amino acid sequence SEQ ID NO:46; or hypervariable
20 regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:47, said CDR2 having the amino acid sequence SEQ ID NO:48, and said CDR3 having the amino acid sequence SEQ ID NO:49; and the second immunoglobulin VL2 domain comprises (e.g. in sequence): hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:60, said CDR2 having the
25 amino acid sequence SEQ ID NO:61, and said CDR3 having the amino acid sequence SEQ ID NO:62 or hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:63, said CDR2 having the amino acid sequence SEQ ID NO:64, and said CDR3 having the amino acid sequence SEQ ID NO:65.

In one embodiment, the bispecific antibody comprises a first immunoglobulin VH1 domain, a first immunoglobulin VL1 domain, a second immunoglobulin VH2 domain and a second immunoglobulin VL2 domain, wherein: the first immunoglobulin VH1 domain comprises the amino acid sequence SEQ ID NO: 85, the first immunoglobulin VL1 domain comprises the amino acid sequence SEQ ID NO: 101, the second immunoglobulin VH2 domain comprises the amino acid sequence SEQ ID NO: 53, the
30 second immunoglobulin VL2 domain comprises the amino acid sequence SEQ ID NO: 69.

In one embodiment, the bispecific antibody comprises a first immunoglobulin heavy chain, a first immunoglobulin light chain, a second immunoglobulin heavy chain and a second immunoglobulin light chain, wherein: the first immunoglobulin heavy chain comprises the amino acid sequence SEQ ID NO: 87, the first immunoglobulin light chain
5 comprises the amino acid sequence SEQ ID NO: 103, the second immunoglobulin heavy chain comprises the amino acid sequence SEQ ID NO: 55, the second immunoglobulin light chain comprises the amino acid sequence SEQ ID NO: 71.

According to a second aspect, a method for selecting a bispecific antibody according to the first aspect is provided, said method comprising; a first step of selecting
10 the first part, and the second part; a second step of co-expressing the first part and the second part in a common host cell, resulting in a bispecific antibody comprising the first part and the second part; a third step of purifying the bispecific antibody by removing mismatched fragments from the correctly matched bispecific antibody. In an embodiment, the third step of purification results in a bispecific antibody which is at least
15 60% (mass), 70% (mass), 80% (mass), 85% (mass) pure, such as at least 90% (mass) pure, 95% (mass), 96% (mass), 97% (mass), 98% (mass), or 99% (mass) pure.

According to a third aspect, a method for manufacturing a bispecific antibody according to the first aspect by co-expression in a common host cell is provided, said method comprising; a first step of generating at least one vector encoding the first part
20 and the second part; a second step of introducing the at least one vector into the common host cell; a third step of selecting cells specifically expressing the bispecific antibody; a forth step of culturing the selected cells under conditions wherein the cells express the bispecific antibody; and a fifth step of purifying the bispecific antibody which is at least 60% (mass), 70% (mass), 80% (mass), 85% (mass) pure, such as at least
25 90% (mass) pure, 95% (mass), 96% (mass), 97% (mass), 98% (mass), or 99% (mass) pure.

In an embodiment, the first step comprises generating a first vector encoding the first part and a second vector encoding the second part.

According to a fourth aspect, an expression system comprising at least one
30 vector comprising a polynucleotide encoding the first part or the second part of the bispecific antibody according to the first aspect, and a selectable marker.

In an embodiment, the expression system comprises a polynucleotide encoding a first selectable marker (sm I); and a polynucleotide encoding a second selectable marker (sm II), which differs from the first selectable marker (sm I).

35 In an embodiment, the first selectable marker (sm I) is a folate transporter or a polynucleotide encoding a mutated folate receptor, wherein the mutated folate receptor

has a decreased folate binding affinity compared to the wildtype folate receptor and the second selectable marker (sm II) is DHFR.

In an embodiment, the first selectable marker (sm I) is Hygromycine and the second selectable marker (sm II) is Neo/G418.

5 In an embodiment, the expression system comprises two expression vectors wherein: a first vector comprising polynucleotide encoding at least a first selectable marker (sm I) and at least polynucleotides encoding the first part; and a second vector comprising polynucleotide encoding at least a second selectable marker (sm II) and at least polynucleotides encoding the second part.

10 The expression system may comprise a stop codon downstream of the polynucleotides encoding the heavy chain and a polynucleotide encoding an immunoglobulin membrane anchor located downstream of the stop codon.

According to a fifth aspect, a method for selecting a common host cell for use in a method according to previous aspects is provided, comprising a first step of providing a plurality of host cells, comprising the expression system according to previous aspects; and culturing said plurality of host cells under conditions selective for the selectable marker, thereby obtaining a host cell expressing the product of interest.

In an embodiment, the selective culture medium is selected from the group comprising a medium comprising a limiting concentration of folate; and/or comprising a folic acid in a concentration of 500nM or less; and/or comprising a folic acid in a concentration selected from: 1000 nM – 100 pM; 100 nM – 1 nM; 15nM – 1nM; 10nM – 1nM; and 10 nM – 2.5 nM; and/or comprising a DHFR inhibitor; and/or comprising an antifolate; and/or comprising an antifolate in a concentration of 500 nM or less; and/or comprising MTX in a concentration selected from: 500 nM – 3 nM; 100 nM – 10 nM; 50nM – 10nM; and 50 nM; and/or comprising a concentration of antifolate up to 20-fold of the folate concentration; and/or comprising a concentration of antifolate 10 – 20 fold of the folate concentration; and/or comprising a folic acid in a concentration up to 15 nM and an equimolar concentration up to 20 - fold of MTX.

In an embodiment, the host cell comprises the expression system wherein at least a portion of the first or second part is expressed as a fusion polypeptide comprising the immunoglobulin transmembrane anchor or fragment thereof, wherein said fusion polypeptide is being displayed on the surface of said host cell, further comprising a step of: contacting the plurality of host cells with a detection compound binding the fusion polypeptide; selecting at least one host cell based upon the presence or amount of the detection compound bound to the cell surface.

In an embodiment, the detection compound comprises the first or second target, or derivatives thereof, and a detection label.

In an embodiment, the fifth step of purifying the bispecific antibody comprises affinity chromatography and/or ion exchange chromatography.

In an embodiment, the chromatography comprises a first step of capturing; a second step of polishing; and optionally a third step of polishing.

5 In an embodiment, the first step of capturing is performed with a principle selected the group consisting of Fc-binding affinity chromatography, such as Protein A or Protein G, lambda light chain specific affinity chromatography, well known in the art, and readily available commercially, for example LambdaFabSelect™, kappa light chain specific affinity chromatography, well known in the art, and readily available
10 commercially, for example KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, well known in the art, and readily available commercially, for example Capto™ adhere, or Fractogel™ EMD SO₃, and hydrophobic interaction chromatography.

15 In an embodiment, the second step of polishing is performed with a principle selected the group consisting of Fc-binding affinity chromatography, such as Protein A or Protein G, lambda light chain specific affinity chromatography, such as LambdaFabSelect™, kappa light chain specific affinity chromatography, such as KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second
20 part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, such as Capto™ adhere, or Fractogel™ EMD SO₃, hydrophobic interaction chromatography, and virus inactivation.

In an embodiment, the third step of polishing is performed with a principle selected the group consisting of Fc-binding affinity chromatography, such as Protein A or
25 Protein G, lambda light chain specific affinity chromatography, such as LambdaFabSelect™, kappa light chain specific affinity chromatography, such as KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, such as Capto™ adhere, or
30 Fractogel™ EMD SO₃, hydrophobic interaction chromatography, and virus inactivation.

In an embodiment, the method comprises a first step of Protein A capturing, such as MabSelect™ SuRe™, a second step of lambda light chain affinity chromatography, such as LambdaFabSelect™, and a third step of kappa light chain affinity chromatography such as KappaSelect™; or a first step of Protein A, such as
35 MabSelect™ SuRe™, a second step of kappa light chain affinity chromatography such as KappaSelect™, and a third step of lambda light chain affinity chromatography, such as LambdaFabSelect™; or a first step of kappa light chain affinity chromatography such

as KappaSelect™ and a second step of lambda light chain affinity chromatography, such as LambdaFabSelect™; or a first step of lambda light chain affinity chromatography, such as LambdaFabSelect™ and a second step of kappa light chain affinity chromatography such as KappaSelect™.

5 In an embodiment, the cell line is selected from the group consisting of a CHO cell, a non-producing hybridoma, such as Sp 2/0 or NS0, a human derived cell line, such as HEK or PER.C6, a baby hamster kidney (BHK) derivative, a yeast or filamentous fungi, a prokaryotic bacteria, such as E. coli or Pseudomonas fluorescence, a plant derivative, an algae and a ciliate.

10 According to a sixth aspect, a pharmaceutical composition is provided, comprising the antibody according to the first aspect and a pharmaceutically acceptable carrier.

According to a seventh aspect, an antibody according to a first aspect, or the pharmaceutical composition according to the sixth aspect, for use as a medicament is
15 provided.

According to a seventh aspect, an antibody according to a first aspect, or the pharmaceutical composition according to the sixth aspect, for use as in the treatment of an inflammasome related disease is provided.

According to an eight aspect, an antibody according to a first aspect, or the
20 pharmaceutical composition according to the sixth aspect, for use as in the treatment of an inflammasome related disease is provided, wherein the inflammasome related disease is selected from the group consisting of sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease, atherosclerosis, vascular dysfunction, skeletal muscle ischemia, pulmonary sarcoidosis, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease and Crohn's disease.
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According to a ninth aspect, a method of treating an inflammasome related disorder comprising administering to a subject afflicted with a inflammasome related disorder an effective amount of an antibody according to the first aspect or a pharmaceutical composition according to the sixth aspect is provided.

30 The inflammasome related disorder may be sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease, atherosclerosis, vascular dysfunction, skeletal muscle ischemia, pulmonary sarcoidosis, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease or Crohn's disease.

BRIEF DESCRIPTION OF THE FIGURES

35 Figure 1 is a schematic overview of the vector setup according to an embodiment.

Figure 2A - 2E shows chromatograms according to an embodiment. Figure 2A is a RP-UV chromatogram of a de-glycosylated intact bbmAb according to an embodiment. Figure 2B is a de-convoluted mass spectrum of the intact de-glycosylated bbmAb1 according to an embodiment. Figure 2C is a RP-UV chromatogram showing papain digested fragments of a bbmAb according to an embodiment. Figure 2D is a RP-UV chromatogram showing IdeS digested fragments of a bbmAb according to an embodiment. Figure 2E is a RP-UV chromatogram showing de-glycosylated and DTT reduced fragments of a bbmAb according to an embodiment.

Figure 3A - 3D shows RP-UV chromatograms according to an embodiment. Figure 3A is a chromatogram showing the expression purity profile of a bbmAb according to an embodiment after cultivation. Figure 3B is a chromatogram of a bbmAb according to an embodiment after capturing by LambdaFabSelect™. Figure 3C is a chromatogram of a bbmAb according to an embodiment after capturing with MabSelect™ SuRe™. Figure 3D is a chromatogram of a bbmAb according to an embodiment after capturing with LambdaFabSelect™, polish by Fractogel™ EMD SO₃ and ultrafiltration.

Figure 4A - 4M is a schematic overview of different options for bispecific mismatching. Figure 4A is a schematic representation of a mAb1 knob (lambda) monomer, where number 1 represents the variable heavy domain, number 2 represents the first constant heavy domain, number 3 represents the second constant heavy domain and number 4 represents the third constant heavy domain. Number 5 represents the variable light domain and number 6 represents the variable heavy domain. Figure 4B is a schematic representation of a mAb1 knob (lambda) homodimer. Figure 4C is a schematic representation of a mAb2 hole (kappa) monomer, where number 7 represents the variable heavy domain, number 8 represents the first constant heavy domain, number 9 represents the second constant heavy domain, and number 10 represents the third constant heavy domain. Number 11 represents the variable light domain and number 12 represents the constant heavy domain. Figure 4D is a schematic representation of a mAb2 hole (kappa) homodimer. Figure 4E is a schematic representation of a mAb1 knob homodimer with one CH/LC mispairing. Figure 4F is a schematic representation of a mAb1 knob homodimer with two CH/LC mispairings. Figure 4G is a schematic representation of a mAb1 knob homodimer with one CH/LC mispairing. Figure 4H is a schematic representation of a mAb2 hole homodimer with one CH/LC mispairing. Figure 4I is a schematic representation of a mAb2 hole homodimer with two CH/LC mispairings. Figure 4J is a schematic representation of a mAb2 hole homodimer with one CH/LC mispairing. Figure 4K is a schematic representation of bbmAb1 with one kappa (CH/LC) mispairing. Figure 4L is a schematic representation of

bbmAb1 with one lambda (CH/LC) mispairing. Figure 4M is a schematic representation of bbmAb1 with two CH/LC mispairings.

Figure 5 shows titration curves of ECL-based affinity determination according to an Example.

5 Figure 6A - 6B shows two graphs according to an Example.

Figure 7A - 7B shows two graphs according to an Example.

Figure 8A - 8B shows mRNA expression levels according to an Example.

Figure 9A - 9B shows mRNA expression levels according to an Example.

Figure 10A - 10B shows two graphs according to an Example.

10 Figure 11 is a graph showing statistical correlations according to an Example.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure is *inter alia* based on the unexpected finding that certain antibodies with a light chain of lambda (λ) type are possible to co-express with certain antibodies with a light chain of kappa (κ) type, to form the desired bbmAb.

15 Without wishing to be bound by theory, the CDRs of each light chain and/or heavy chain could significantly influence which light chain of lambda (λ) type are possible to co-express with certain antibodies with a light chain of kappa (κ) type to successfully obtain the bbmab formed.

The antibodies with a light chain of lambda (λ) type that are possible to co-express with certain antibodies with a light chain of kappa (κ) type, to form the desired bbmAb, in the following also called monospecific binders, can be generated either by use of technologies offering the opportunity to obtain both types of antibodies of either kappa or lambda type antibodies, such as Phage display libraries, e.g. HuUCAL GOLD® or HuCAL PLATINUM® (MorphoSys), or by use of transgenic mice, where the relevant human immunoglobulin sequences have been introduced into the genome of an animal by genetic engineering, e.g. OmniAb antibodies (OMT), Kymouse™ (Kymab), Trianni Mouse™ (Trianni) or AlivaMab Mouse (Ablexis) (reference) can generate kappa or lambda type antibodies. The methods to generate such monospecific binders are well known in the expert field, and are widely applied to generate diverse sets of either kappa or lambda type monospecific binders towards the relevant target of interest. The individual monospecific binders are characterized with respect to relevant biological parameters such as affinity or potency, and also screened for e.g. physicochemical characteristics relevant to judge the so called developability characteristics which are also very well known in the field (e.g. Lorenz et al., American Pharmaceutical Review, August 2014). Monospecific binders showing the best characteristics are finally co-expressed in e.g. CHO cells as described in more detail below. Only combinations are

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tested by co-expression where a kappa type antibody binding to the first target is combined with a lambda type antibody binding to the second target, and vice versa. The resulting co-expression product and the relevant product related impurities are characterized in detail with the intent to select a combination which results in the best profile, especially the ones which show only a low amount of promiscuous binding of one light chain (e.g. L1, light chain 1, e.g. lambda), towards the wrong heavy chain (e.g. H2, heavy chain 2). An advantage of the method may be that antibody combinations where both light chains exchange the original heavy chain binding partner, which results in a product related impurity of H1L2-H2L1 type, can be deselected. This is advantageous, because such product related impurities are not easy to deplete using state of the art purification processes. The procedure how to co-express the individual antibodies and how to analyze the co-expression product is outlined in more detail below.

Specific antibodies were used as examples, primarily mAb2 binding to IL-1 β , with light chain Vk6 and the mAb1 binding to IL-18, with light chain V λ 1.

In one preferred embodiment, a KiH modification of the Fc-portion of the two antibodies according to Ridgway et al., (1996) was used. Other antibodies were also tested.

As will be shown in the specific examples below, a preferred embodiment bbmAb1 is expressed with a single, common cell line ensuring a sufficient overall yield, purity and product quality required for biologic or diagnostics to proceed with clinical development and commercialization.

1. Definitions

For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. Additional definitions are set forth throughout the detailed description.

The term "IL-18" is synonym to IL-18 polypeptide, Interleukin-18 polypeptide, IFN-gamma inducing factor or Interferon-gamma-inducing-factor or INF- γ inducing factor. The term "IL-18" refers to human IL-18, unless another species is indicated. IL-18 is well known to a person skilled in the art, and for example obtainable from MBL® International Corporation under product reference #B001-5. Throughout this specification, the term IL-18 encompasses both pro-IL-18 (precursor of mature IL-18 prior protease cleavage) and mature IL-18 (post protease cleavage) interchangeably unless it is specified that the pro- or mature form is meant.

The term "IL-1 β " or "IL-1b" is synonym to IL-1 β polypeptide and Interleukin-1 β polypeptide. The term "IL-1 β " refers to human IL-1 β unless another species is indicated.

IL-1 β is well known to a person skilled in the art, and for example obtainable from Sino Biological under product reference #10139-HNAE-5.

The term "antibody" refers to an intact immunoglobulin or a functional fragment thereof. Naturally occurring antibodies typically comprise a tetramer which is usually
5 composed of at least two heavy (H) chains and at least two light (L) chains. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region, usually comprised of three domains (CH1, CH2 and CH3). Heavy chains can be of any isotype, including IgG (IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (IgA1 and IgA2 subtypes), IgM and IgE. Each light chain is comprised of a
10 light chain variable region (abbreviated herein as VL) and a light chain constant region (CL). Light chain includes kappa (κ) chains and lambda (λ) chains. The heavy and light chain variable region is typically responsible for antigen recognition, whilst the heavy and light chain constant region may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g. effector cells) and
15 the first component (C1q) of the classical complement system. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1,
20 FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen.

The term "antigen-binding portion" of an antibody (or simply "antigen portion"), as used herein, refers to full length or one or more fragments of an antibody that retain the ability to specifically bind to IL-18 or IL-1 β antigen. It has been shown that the antigen-
25 binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and
30 CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward et al., (1989) Nature; 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR).

Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a
35 flexible linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g. Bird et al., (1988) Science 242:423-426; and Huston et al., (1988) Proc Natl Acad

Sc.; 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

- 5 The term "isolated" means throughout this specification, that the immunoglobulin, antibody or polynucleotide, as the case may be, exists in a physical milieu distinct from that in which it may occur in nature.

Throughout this specification, complementarity determining regions ("CDR") are defined according to the Kabat definition unless specified that the CDR are defined
10 according to another definition. The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering
15 scheme) and ImMunoGenTics (IMGT) numbering (Lefranc, M.-P., The Immunologist, 7, 132-136 (1999); Lefranc, M.-P. et al., Dev. Comp. Immunol., 27, 55-77 (2003) ("IMGT" numbering scheme). For example, for classic formats, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain
20 variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (HCDR1), 50-
25 65 (HCDR2), and 95-102 (HCDR3) in human VH and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in human VL. Under IMGT the CDR amino acid residues in the VH are numbered approximately 26-35 (CDR1), 51-57 (CDR2) and 93-102 (CDR3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (CDR1), 50-52 (CDR2), and 89-97 (CDR3) (numbering according to
30 "Kabat"). Under IMGT, the CDR regions of an antibody can be determined using the program IMGT/DomainGap Align.

By convention, the CDR regions in the heavy chain are typically referred to as H-CDR1, H-CDR2 and H-CDR3 and in the light chain as L-CDR1, LCDR2 and L-CDR3. They are numbered sequentially in the direction from the amino terminus to the carboxy
35 terminus.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A

monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, e.g. human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik, et al., (2000) J Mol Biol; 296:57-86).

The human antibodies of the invention may include amino acid residues not encoded by human sequences (e.g. mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human sequences.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g. a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, e.g. from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

As used herein, a binding molecule that "specifically binds to IL-18" is intended to refer to a binding molecule that binds to human IL-18 with a K_D of a 100nM or less, 10nM or less, 1nM or less.

As used herein, a binding molecule that "specifically binds to IL-1 β " is intended to refer to a binding molecule that binds to human IL-1 β with a K_D of a 100nM or less, 10nM or less, 1nM or less.

A binding molecule that "cross-reacts with an antigen other than IL-18 is intended to refer to a binding molecule that binds that antigen with a K_D of a 100nM or less, 10nM or less, 1nM or less. A binding molecule that "cross-reacts with an antigen other than IL-1 β is intended to refer to a binding molecule that binds that antigen with a K_D of a 100nM or less, 10nM or less, 1nM or less.

A binding molecule that "does not cross-react with a particular antigen" is intended to refer to a binding molecule that exhibits essentially undetectable binding against these proteins in standard binding assays.

As used herein, the term "antagonist" is intended to refer to a binding molecule that inhibits the signalling activity in the presence of activating compound. For example, in the case of IL-18, an IL-18 antagonist would be a binding molecule inhibiting the signalling activity in the presence of IL-18 in a human cell assay such as IL-18 dependent Interferon-gamma (IFN- γ) production assay in human blood cells. Examples of an IL-18 dependent IFN- γ production assay in human blood cells are described in more details in the examples below.

The term bivalent bispecific antibody or bivalent bispecific antibodies refer to antibodies binding to two different targets, such as IL-18 and IL-1 β .

The bispecific antibodies are "hetero-dimers", which means that one part comes from first antibody, specific for a first target, and another part comes from a second antibody, specific for a second target. A "hetero-dimerization modification" is a modification to one or both parts of the antibodies forming the hetero-dimeric bispecific antibody, intended to facilitate such formation. An example of hetero-dimerization modifications of the Fc domains of two IgG1 parts of antibodies intended to form a bispecific is a "knob" with a bulky amino acid (aa) side chain (S354C, T366W) in the first heavy chain and a "hole" with small aa side chains (Y349C, T366S, L368A, Y407V) were introduced in the second heavy chain as well as an additional disulfide bridge in the CH3 region connecting both heavy chains (Merchant et al., Nat. Biotechnol., 16:677-681 (1998), page 678, table 1).

As used herein, an antibody with "no agonistic activity" is intended to refer to a binding molecule that does not significantly increase target dependent signalling activity in the absence and/or presence of the target in a cell-based assay, such as in case of IL-

18, does not significantly increase IL-18 dependent signalling activity in the absence and/or presence of IL-18 human blood cells IFN- γ production assay. Such assays are described in more details in the examples below.

The term " K_{assoc} " or " K_a ", as used herein, is intended to refer to the association rate of a particular binding molecule -antigen interaction, whereas the term " K_{dis} " or " K_d ," as used herein, is intended to refer to the dissociation rate of a particular binding molecule -antigen interaction. The term " K_D ", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A method for determining the K_D of an antibody is by using surface plasmon resonance, such as a Biacore® system.

As used herein, the term "affinity" refers to the strength of interaction between binding molecule and antigen at single antigenic sites.

As used herein, the term "high affinity" for an antibody refers to an antibody having a K_D of 1nM or less for a target antigen.

As used herein, the term "subject" includes any human or non-human animal.

The term "non-human animal" includes all vertebrates, e.g. mammals and non-mammals, such as non-human primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

As used herein, the term, "optimized nucleotide sequence" means that the nucleotide sequence has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a cell of *Pichia pastoris*, a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence. The optimized sequences herein have been engineered to have codons that are preferred in CHO mammalian cells; however optimized expression of these sequences in other eukaryotic cells is also envisioned herein.

The term "identity" refers to the similarity between at least two different sequences. This identity can be expressed as a percent identity and determined by standard alignment algorithms, for example, the Basic Local Alignment Tool (BLAST) (Altschul et al., (1990) *J Mol Biol*; 215:403-410); the algorithm of Needleman et al., (1970) *J Mol Biol*; 48:444-453 or the algorithm of Meyers et al., (1988) *Comput Appl Biosci*; 4:11-17). A set of parameters may be the Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller, (1989) *CABIOS*; 4(1):1-17) which has been

incorporated into the ALIGN program (version 2.0), using a PAM 120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity is usually calculated by comparing sequences of similar length.

The term "immune response" refers to the action of, for example, lymphocytes,
5 antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or
10 tissues.

A "signal transduction pathway" or "signaling activity" refers to a biochemical causal relationship generally initiated by a protein-protein interaction such as binding of a growth factor to a receptor, resulting in transmission of a signal from one portion of a cell to another portion of a cell. In general, the transmission involves specific phosphorylation
15 of one or more tyrosine, serine, or threonine residues on one or more proteins in the series of reactions causing signal transduction. Penultimate processes typically include nuclear events, resulting in a change in gene expression.

The term "neutralises" and grammatical variations thereof means throughout this specification, that the biological activity of the target is reduced either totally or partially in
20 the presence of the binding protein or antibody, as the case may be.

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known
25 analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be
30 achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994))

The nucleotide in the "polynucleotide" or "nucleic acid" may comprise
35 modifications including base modifications such as bromouridine and inosine derivatives, ribose modification such as phosphorothioate, phosphorodithioate, phosphoroselenoate,

phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate.

The term "vector" means any molecule or entity (e.g. nucleic acid, plasmid, bacteriophage or virus) that is suitable for transformation or transfection of a host cell and contains nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more heterologous coding regions operatively linked thereto.

The term "co-expression" means that different polypeptides are expressed together in a single host cell, common for all the polypeptides. Co-expression of a bispecific antibody means that the different parts forming the functional bispecific antibody are expressed in a single, common, host cell. Co-expression may be achieved by incorporating several expression vectors in the expression host cell, such as one for each of the halves of a bispecific antibody, or by incorporating one expression vector encoding all parts of the bispecific antibody.

The term "mismatched" means that different parts of an intended protein complex, such as a bispecific antibody, do not complex bind as intended, which means that the protein complex does not look or behave as intended. Examples of mismatching in the context of a bispecific antibody are shown in Figure 4.

A "conservative variant" of a sequence encoding a binding molecule, an antibody or a fragment thereof refers to a sequence comprising conservative amino acid modifications. "Conservative amino acid modifications" are intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). Modifications can be introduced into a binding protein of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitution can also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. Non-naturally occurring amino

acids include, but are not limited to, peptidomimetic, reversed or inverted forms of amino acid moieties.

The term "epitope" is the part of an antigen that is recognized by the immune system, such as an antibody or a fragment thereof. Within the present specification, the
5 term "epitope" is used interchangeably for both conformational epitopes and linear epitopes. A conformational epitope is composed of discontinuous sections of the antigen's amino acid sequence, whilst a linear epitope is formed by a continuous sequence of amino acids from the antigen.

The term "treat", "treating", "treatment", "prevent", "preventing" or "prevention"
10 includes therapeutic treatments, prophylactic treatments and applications in which one reduces the risk that a subject will develop a disorder or other risk factor. Treatment does not require the complete curing of a disorder and encompasses the reduction of the symptoms or underlying risk factors. As used herein, a human antibody or a fragment thereof comprises heavy or light chain variable regions or full length heavy or light chains
15 that are "the product of" or "derived from" a particular germline sequence if the variable regions or full length chains of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human
20 antibody or fragment thereof that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody. A
25 human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence
30 encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g. murine germline sequences). In certain cases, a human antibody may be at least 60%, 70%, 80%, 90%, or at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid
35 sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded

by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Human antibodies may be produced by a number of methods known to those of skill in the art. Human antibodies can be made by the hybridoma method using human myeloma or mouse-human heteromyeloma cells lines (Kozbor, J Immunol; (1984) 133:3001; Brodeur, Monoclonal Isolated Antibody Production Techniques and Applications, pp51-63, Marcel Dekker Inc, 1987). Alternative methods include the use of phage libraries or transgenic mice both of which utilize human variable region repertoires (Winter G; (1994) Annu Rev Immunol 12:433-455, Green LL, (1999) J Immunol Methods 231:11-23).

Several strains of transgenic mice are now available wherein their mouse immunoglobulin loci has been replaced with human immunoglobulin gene segments (Tomizuka K, (2000) Proc Natl Acad Sci , 97:722-727; Fishwild DM (1996) Nature Biotechnol 14:845-851; Mendez MJ, (1997) Nature Genetics 15:146-156). Upon antigen challenge such mice are capable of producing a repertoire of human antibodies from which antibodies of interest can be selected. Of particular note is the TrimerTM system (Eren R et al, (1988) Immunology 93:154-161) where human lymphocytes are transplanted into irradiated mice, the Selected Lymphocyte Isolated antibody System (SLAM, Babcook *et al*, Proc Natl Acad Sci (1996) 93:7843-7848) where human (or other species) lymphocytes are effectively put through a massive pooled *in vitro* isolated antibody generation procedure followed by deconvoluted, limiting dilution and selection procedure and the XenomouseTM (Abgenix Inc). An alternative approach is available from Morphotek Inc using the MorphodomaTM technology.

Phage display technology can be used to produce human antibodies and fragments thereof, (McCafferty; (1990) Nature, 348:552-553 and Griffiths AD et al (1994) EMBO 13:3245-3260). According to this technique, isolated antibody variable domain genes are cloned in frame into either a major or minor coat of protein gene of a filamentous bacteriophage such as M13 or fd and displayed (usually with the aid of a helper phage) as function isolated antibody fragments on the surface of the phage particle. Selections based on the function properties of the isolated antibody result in selection of the gene encoding the isolated antibody exhibiting these properties. The phage display technique can be used to select antigen specific antibodies from libraries made from human B cells taken from individuals afflicted with a disease or disorder or alternatively from unimmunized human donors (Marks; J Mol Bio (1991) 222:581-591,). Where an intact human isolated antibody is desired comprising an Fc domain it is necessary reclone the phage displayed derived fragment into a mammalian expression

vectors comprising the desired constant regions and establishing stable expressing cell lines.

The technique of affinity maturation (Marks; Biotechnol (1992) 10:779-783) may be used to provide binding affinity wherein the affinity of the primary human isolated antibody is improved by sequentially replacing the H and L chain variable regions with naturally occurring variants and selecting on the basis of improved binding affinities. Variants of this technique such as 'epitope imprinting' are now also available (WO 93/06213; Waterhouse; Nucl Acids Res (1993) 21:2265-2266).

- 10 The term "pure" when used in the context of purified bispecific antibody relates to purity and identity of different bispecific antibody combinations and constructs after co-expression in selected cells under conditions wherein the cells express the bispecific antibody and after protein-A purification using an intact UPLC-MS mass screening approach. Pure or purity refers to the relative quantify of the formed hetero-and
- 15 homodimer bbmAbs. Using the method of the invention correctly formed heterodimeric bbmAb1 and bbmAb2 could be observed with a relative purity of over 85% based on intact mass signal intensity.

2. IL-18 antibody

- Particularly preferred IL-18 antibodies or antigen-binding fragments thereof used in the disclosed methods are human antibodies.

For ease of reference, the amino acid sequences of the hypervariable regions of a specific IL-18 antibody, called mAb1, based on the Kabat definition and the Chothia definition, as well as the V_L and V_H domains and full heavy and light chains are provided in **Table 1**, below.

25

Table 1. Amino acid sequences of the hypervariable regions (CDRs), variable domains (V_H and V_L) and full chains of mAb1. The DNA encoding the V_L of mAb1 is set forth in SEQ ID NO:18. The DNA encoding the V_H of mAb1 is set forth in SEQ ID NO:8.

mAb1 heavy chain		
CDR1	Kabat	SEQ ID NO:1
	Chothia	SEQ ID NO:4
CDR2	Kabat	SEQ ID NO:2
	Chothia	SEQ ID NO:5
CDR3	Kabat	SEQ ID NO:3

	Chothia	SEQ ID NO:6
VH		SEQ ID NO:7
Heavy Chain		SEQ ID NO:9
mAb1 light chain		
CDR1	Kabat	SEQ ID NO:11
	Chothia	SEQ ID NO:14
CDR2	Kabat	SEQ ID NO:12
	Chothia	SEQ ID NO:15
CDR3	Kabat	SEQ ID NO:13
	Chothia	SEQ ID NO:16
VL		SEQ ID NO:17
Light Chain		SEQ ID NO:19

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises at least one immunoglobulin heavy chain variable domain (V_H) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:1, said CDR2 having the amino acid sequence SEQ ID NO:2, and said CDR3 having the amino acid sequence SEQ ID NO:3. In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises at least one immunoglobulin heavy chain variable domain (V_H) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:4, said CDR2 having the amino acid sequence SEQ ID NO:5, and said CDR3 having the amino acid sequence SEQ ID NO:6.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises at least one immunoglobulin light chain variable domain (V_L) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:11, said CDR2 having the amino acid sequence SEQ ID NO:12 and said CDR3 having the amino acid sequence SEQ ID NO:13. In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises at least one immunoglobulin light chain variable domain (V_L) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:14, said CDR2 having the amino acid sequence SEQ ID NO:15 and said CDR3 having the amino acid sequence SEQ ID NO:16.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises at least one immunoglobulin V_H domain and at least one immunoglobulin V_L domain, wherein: a) the immunoglobulin V_H domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid
5 sequence SEQ ID NO:1, said CDR2 having the amino acid sequence SEQ ID NO:2, and said CDR3 having the amino acid sequence SEQ ID NO:3; or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:4, said CDR2 having the amino acid sequence SEQ ID NO:5, and said CDR3 having the amino acid sequence SEQ ID NO:6; and b) the immunoglobulin V_L domain comprises
10 (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:11, said CDR2 having the amino acid sequence SEQ ID NO:12, and said CDR3 having the amino acid sequence SEQ ID NO:13 or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:14, said CDR2 having the amino acid sequence SEQ ID NO:15,
15 and said CDR3 having the amino acid sequence SEQ ID NO:16.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof comprises: a) an immunoglobulin heavy chain variable domain (V_H) comprising the amino acid sequence set forth as SEQ ID NO:7; b) an immunoglobulin light chain variable domain (V_L) comprising the amino acid sequence set forth as SEQ ID NO:17; c)
20 an immunoglobulin V_H domain comprising the amino acid sequence set forth as SEQ ID NO:7 and an immunoglobulin V_L domain comprising the amino acid sequence set forth as SEQ ID NO:17; d) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; e) an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID
25 NO:11, SEQ ID NO:12 and SEQ ID NO:13; f) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6; g) an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; h) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3
30 and an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13; i) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 and an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; j) a light chain comprising
35 SEQ ID NO:19; k) a heavy chain comprising SEQ ID NO:9; or l) a light chain comprising SEQ ID NO:19 and a heavy chain comprising SEQ ID NO:9.

In some embodiments, the IL-18 antibody or antigen-binding fragment thereof (e.g. mAb1) comprises the three CDRs of SEQ ID NO:7. In other embodiments, the IL-18 antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:17. In other embodiments, the IL-18 antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:7 and the three CDRs of SEQ ID NO:17. In some embodiments, the IL-18 antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:9. In other embodiments, IL-18 antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:19. In other embodiments, the IL-18 antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:9 and the three CDRs of SEQ ID NO:19.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof (e.g. mAb1) is selected from a human IL-18 antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:1, said CDR2 having the amino acid sequence SEQ ID NO:2, and said CDR3 having the amino acid sequence SEQ ID NO:3; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:11, said CDR2 having the amino acid sequence SEQ ID NO:12, and said CDR3 having the amino acid sequence SEQ ID NO:13.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof (e.g. mAb1) is selected from a human IL-18 antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:4, said CDR2 having the amino acid sequence SEQ ID NO:5 and said CDR3 having the amino acid sequence SEQ ID NO:6; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:14, said CDR2 having the amino acid sequence SEQ ID NO:15, and said CDR3 having the amino acid sequence SEQ ID NO:16.

In one embodiment, the IL-18 antibody or antigen-binding fragment thereof is selected from a single chain antibody or antigen-binding fragment thereof that comprises an antigen-binding site comprising: a) a first domain comprising, in sequence, the

hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:1, said CDR2 having the amino acid sequence SEQ ID NO:2, and said CDR3 having the amino acid sequence SEQ ID NO:3; and b) a second domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1
5 having the amino acid sequence SEQ ID NO:11, said CDR2 having the amino acid sequence SEQ ID NO:12, and said CDR3 having the amino acid sequence SEQ ID NO:13; and c) a peptide linker which is bound either to the N-terminal extremity of the first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of the second domain.

10 In one embodiment, the IL-18 antibody or antigen-binding fragment thereof (e.g. mAb1) is selected from a single chain antibody or antigen-binding fragment thereof that comprises an antigen-binding site comprising: a) a first domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:4, said CDR2 having the amino acid sequence SEQ ID NO:5, and
15 said CDR3 having the amino acid sequence SEQ ID NO:6; and b) a second domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:14, said CDR2 having the amino acid sequence SEQ ID NO:15, and said CDR3 having the amino acid sequence SEQ ID NO:16; and c) a peptide linker which is bound either to the N-terminal extremity of the
20 first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of the second domain.

The V_H or V_L domain of an IL-18 antibody or antigen-binding fragment thereof used in the disclosed methods may have V_H and/or V_L domains that are substantially identical to the V_H or V_L domains set forth in SEQ ID NO:7 and 17. A human IL-18
25 antibody disclosed herein may comprise a heavy chain that is substantially identical to that set forth as SEQ ID NO:9 and/or a light chain that is substantially identical to that set forth as SEQ ID NO:19. A human IL-18 antibody disclosed herein may comprise a heavy chain that comprises SEQ ID NO:9 and a light chain that comprises SEQ ID NO:19. A human IL-18 antibody disclosed herein may comprise: a) one heavy chain, comprising a
30 variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:7 and the constant part of a human heavy chain; and b) one light chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:17 and the constant part of a human light chain.

Other preferred IL-18 antagonists (e.g. antibodies) for use in the disclosed
35 methods, kits and regimens are those set forth in US Patent No: 9,376,489, which is incorporated by reference herein in its entirety.

3. IL-1 β antibody

Particularly preferred IL-1 β antibodies or antigen-binding fragments thereof used in the disclosed methods are human antibodies.

For ease of reference, the amino acid sequences of the hypervariable regions of a specific IL-1 β antibody, called mAb2, based on the Kabat definition and the Chothia definition, as well as the V_L and V_H domains and full heavy and light chains are provided in **Table 2**, below.

Table 2. Amino acid sequences of the hypervariable regions (CDRs), variable domains (VH and VL) and full chains of mAb2. The DNA encoding the VL of mAb2 is set forth in SEQ ID NO:38. The DNA encoding the VH of mAb2 is set forth in SEQ ID NO:27.

mAb2 heavy chain		
CDR1	Kabat	SEQ ID NO:21
	Chothia	SEQ ID NO:24
CDR2	Kabat	SEQ ID NO:22
	Chothia	SEQ ID NO:25
CDR3	Kabat	SEQ ID NO:23
	Chothia	SEQ ID NO:26
VH		SEQ ID NO:27
Heavy Chain		SEQ ID NO:29
mAb2 light chain		
CDR1	Kabat	SEQ ID NO:31
	Chothia	SEQ ID NO:34
CDR2	Kabat	SEQ ID NO:32
	Chothia	SEQ ID NO:35
CDR3	Kabat	SEQ ID NO:33
	Chothia	SEQ ID NO:36
VL		SEQ ID NO:37
Light Chain		SEQ ID NO:39

In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises at least one immunoglobulin heavy chain variable domain (V_H) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:21, said CDR2 having the amino acid sequence SEQ ID NO:22, and said CDR3 having the amino acid sequence SEQ ID NO:23. In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises at least one immunoglobulin heavy chain variable domain (V_H) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:24, said CDR2 having the amino acid sequence SEQ ID NO:25, and said CDR3 having the amino acid sequence SEQ ID NO:26.

In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises at least one immunoglobulin light chain variable domain (V_L) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:31, said CDR2 having the amino acid sequence SEQ ID NO:32 and said CDR3 having the amino acid sequence SEQ ID NO:33. In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises at least one immunoglobulin light chain variable domain (V_L) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:34, said CDR2 having the amino acid sequence SEQ ID NO:35 and said CDR3 having the amino acid sequence SEQ ID NO:36.

In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises at least one immunoglobulin V_H domain and at least one immunoglobulin V_L domain, wherein: a) the immunoglobulin V_H domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:21, said CDR2 having the amino acid sequence SEQ ID NO:22, and said CDR3 having the amino acid sequence SEQ ID NO:23; or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:24, said CDR2 having the amino acid sequence SEQ ID NO:25, and said CDR3 having the amino acid sequence SEQ ID NO:26; and b) the immunoglobulin V_L domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:31, said CDR2 having the amino acid sequence SEQ ID NO:32, and said CDR3 having the amino acid sequence SEQ ID NO:33 or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:34, said CDR2 having the amino acid sequence SEQ ID NO:35, and said CDR3 having the amino acid sequence SEQ ID NO:36.

In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof comprises: a) an immunoglobulin heavy chain variable domain (V_H) comprising the

amino acid sequence set forth as SEQ ID NO:27; b) an immunoglobulin light chain variable domain (V_L) comprising the amino acid sequence set forth as SEQ ID NO:37; c) an immunoglobulin V_H domain comprising the amino acid sequence set forth as SEQ ID NO:27 and an immunoglobulin V_L domain comprising the amino acid sequence set forth as SEQ ID NO:37; d) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23; e) an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33; f) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26; g) an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; h) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23 and an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33; i) an immunoglobulin V_H domain comprising the hypervariable regions set forth as SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 and an immunoglobulin V_L domain comprising the hypervariable regions set forth as SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; j) a light chain comprising SEQ ID NO:37; k) a heavy chain comprising SEQ ID NO:29; or l) a light chain comprising SEQ ID NO:39 and a heavy chain comprising SEQ ID NO:29.

In some embodiments, the IL-1 β antibody or antigen-binding fragment thereof (e.g. mAb2) comprises the three CDRs of SEQ ID NO:37. In other embodiments, the IL-1 β antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:27. In other embodiments, the IL-1 β antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:37 and the three CDRs of SEQ ID NO:27. In some embodiments, the IL-1 β antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:39. In other embodiments, IL-1 β antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:29. In other embodiments, the IL-1 β antibody or antigen-binding fragment thereof comprises the three CDRs of SEQ ID NO:39 and the three CDRs of SEQ ID NO:29.

In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof (e.g. mAb2) is selected from a human IL-1 β antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:21, said CDR2 having the amino acid sequence SEQ ID NO:22, and said CDR3 having the amino acid sequence SEQ ID NO:23; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain

comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:31, said CDR2 having the amino acid sequence SEQ ID NO:32, and said CDR3 having the amino acid sequence SEQ ID NO:33.

5 In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof (e.g. mAb2) is selected from a human IL-1 β antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino
10 acid sequence SEQ ID NO:24, said CDR2 having the amino acid sequence SEQ ID NO:25 and said CDR3 having the amino acid sequence SEQ ID NO:26; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino
15 acid sequence SEQ ID NO:34, said CDR2 having the amino acid sequence SEQ ID NO:35, and said CDR3 having the amino acid sequence SEQ ID NO:36.

 In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof is selected from a single chain antibody or antigen-binding fragment thereof that comprises an antigen-binding site comprising: a) a first domain comprising, in sequence, the
20 hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:21, said CDR2 having the amino acid sequence SEQ ID NO:22, and said CDR3 having the amino acid sequence SEQ ID NO:23; and b) a second domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:31, said CDR2 having the
25 amino acid sequence SEQ ID NO:32, and said CDR3 having the amino acid sequence SEQ ID NO:33; and c) a peptide linker which is bound either to the N-terminal extremity of the first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of the second domain.

30 In one embodiment, the IL-1 β antibody or antigen-binding fragment thereof (e.g. mAb2) is selected from a single chain antibody or antigen-binding fragment thereof that comprises an antigen-binding site comprising: a) a first domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:24, said CDR2 having the amino acid sequence SEQ ID NO:25,
35 and said CDR3 having the amino acid sequence SEQ ID NO:26; and b) a second domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:34, said CDR2 having the

amino acid sequence SEQ ID NO:35, and said CDR3 having the amino acid sequence SEQ ID NO:36; and c) a peptide linker which is bound either to the N-terminal extremity of the first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of the second domain.

The V_H or V_L domain of an IL-1 β antibody or antigen-binding fragment thereof used in the disclosed methods may have V_H and/or V_L domains that are substantially identical to the V_H or V_L domains set forth in SEQ ID NO:27 and 37. A human IL-1 β antibody disclosed herein may comprise a heavy chain that is substantially identical to that set forth as SEQ ID NO:29 and/or a light chain that is substantially identical to that set forth as SEQ ID NO:39. A human IL-1 β antibody disclosed herein may comprise a heavy chain that comprises SEQ ID NO:29 and a light chain that comprises SEQ ID NO:39. A human IL-1 β antibody disclosed herein may comprise: a) one heavy chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:27 and the constant part of a human heavy chain; and b) one light chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:37 and the constant part of a human light chain.

Other preferred IL-1 β antagonists (e.g. antibodies) for use in the disclosed methods, kits and regimens are those set forth in US Patent Nos: 7,446,175 or 7,993,878 or 8,273,350, which are incorporated by reference herein in their entirety.

4. Fc modifications

In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (e.g. one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g. increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate

assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge
5 fragment such that the antibody has impaired Staphylococcal protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward et al.

In another embodiment, the antibody is modified to increase its biological half-life.
10 Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046
15 and 6,121,022 by Presta et al.

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but
20 retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter et al.

In another embodiment, one or more amino acids selected from amino acid
25 residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie et al.

In another embodiment, one or more amino acid residues are altered to thereby
30 alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer et al.

In yet another embodiment, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids.
35 This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have

been mapped and variants with improved binding have been described (see Shields, R.L. et al, (2001) J Biol Chem 276:6591-6604).

In certain embodiments, the Fc domain of IgG1 isotype is used. In some specific embodiments, a mutant variant of IgG1 Fc fragment is used, e.g. a silent IgG1 Fc which
5 reduces or eliminates the ability of the fusion polypeptide to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to bind to an Fcγ receptor. An example of an IgG1 isotype silent mutant wherein Leucine residue is replaced by Alanine residue at amino acid positions 234 and 235 as described by Hezareh et al, J. Virol (2001); 75(24):12161-8.

10 In certain embodiments, the Fc domain is a mutant preventing glycosylation at position 297 of Fc domain. For example, the Fc domain contains an amino acid substitution of asparagine residue at position 297. Example of such amino acid substitution is the replacement of N297 by a glycine or an alanine.

Silenced effector functions can be obtained by mutation in the Fc region of the
15 antibodies and have been described in the art: LALA and N297A (Strohl, W., 2009, Curr. Opin. Biotechnol. vol. 20(6):685-691); and D265A (Baudino et al., 2008, J. Immunol. 181:6664-69; Strohl, W., supra); and DAPA (D265A and P329A) (Shields RL., J Biol Chem. 2001;276(9):6591-604; U.S. Patent Publication US2015/0320880). Examples of silent Fc IgG1 antibodies comprise the so-called LALA mutant comprising L234A and
20 L235A mutation in the IgG1 Fc amino acid sequence. Another example of a silent IgG1 antibody comprises the D265A mutation. Another example of a silent IgG1 antibody is the so-called DAPA mutant, comprising D265A and P329A mutations to the IgG1 Fc amino acid sequence. Another silent IgG1 antibody comprises the N297A mutation, which results in aglycosylated/non-glycosylated antibodies. Additional Fc mutations for
25 providing silenced effector function are described in PCT publication no. WO2014/145806 (e.g., in Figure 7 of WO2014/145806), herein incorporated by reference in its entirety. One example from WO2014/145806 of a silent IgG1 antibody comprises a E233P, L234V, L235A, and S267K mutation, and a deletion of G236 (G236del). Another example from WO2014/145806 of a silent IgG1 antibody comprises a E233P, L234V,
30 and L235A mutation, and a deletion of G236 (G236del). Another example from WO2014/145806 of a silent IgG1 antibody comprises a S267K mutation.

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for the
35 antigen. Such carbohydrate modifications can be accomplished by; for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more

variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co et al.

5 Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, 10 expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, 15 such that antibodies expressed in such a cell line exhibit hypofucosylation. Therefore, in one embodiment, the antibodies of the invention are produced by recombinant expression in a cell line which exhibit hypofucosylation pattern, for example, a mammalian cell line with deficient expression of the FUT8 gene encoding fucosyltransferase. PCT Publication WO 03/035835 by Presta describes a variant CHO 20 cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. et al., 2002 J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g. beta(1,4)-N acetylglucosaminyltransferase III 25 (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al., 1999 Nat. Biotech. 17:176-180). Alternatively, the antibodies of the invention can be produced in a yeast or a filamentous fungi engineered for mammalian-like glycosylation pattern, and capable of producing antibodies lacking 30 fucose as glycosylation pattern (see for example EP1297172B1).

 Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (e.g. serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive 35 ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an

analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

Another modification of the antibodies that is contemplated by the invention is a conjugate or a protein fusion of at least the antigen-binding region of the antibody of the invention to serum protein, such as human serum albumin or a fragment thereof to increase half-life of the resulting molecule. Such approach is for example described in Ballance et al. EP0322094.

Another modification of the antibodies that is contemplated by the invention is one or more modifications to increase formation of a heterodimeric bispecific antibody. A variety of approaches available in the art can be used in for enhancing dimerization of the two heavy chain domains of bispecific antibodies, e.g., bbmAbs, as disclosed in, for example, EP 1870459A1; U.S. Pat. No. 5,582,996; U.S. Pat. No. 5,731,168; U.S. Pat. No. 5,910,573; U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441; U.S. Pat. No. 7,183,076; U.S. Patent Application Publication No. 2006204493A1; and PCT Publication No. WO2009/089004A1, the contents of which are incorporated herein in their entireties.

Generation of bispecific antibodies using knobs-into-holes is disclosed e.g. in PCT Publication No. WO1996/027011, Ridgway et al., (1996), and Merchant et al. (1998).

(1) Knob-in-Hole (KIH)

Multispecific molecules, e.g., multispecific antibody or antibody-like molecules, of the present invention may comprise one or more, e.g., a plurality, of mutations to one or more of the constant domains, e.g., to the CH3 domains. In one example, the multispecific molecule of the present invention comprises two polypeptides that each comprise a heavy chain constant domain of an antibody, e.g., a CH2 or CH3 domain. In an example, the two heavy chain constant domains, e.g., the CH2 or CH3 domains of the multispecific molecule comprise one or more mutations that allow for a heterodimeric association between the two chains. In one aspect, the one or more mutations are disposed on the CH2 domain of the two heavy chains of the multispecific, e.g., bispecific, antibody or antibody-like molecule. In one aspect, the one or more mutations are disposed on the CH3 domains of at least two polypeptides of the multispecific molecule. In one aspect, the one or more mutations to a first polypeptide of the multispecific

molecule comprising a heavy chain constant domain creates a “knob” and the one or more mutations to a second polypeptide of the multispecific molecule comprising a heavy chain constant domain creates a “hole,” such that heterodimerization of the polypeptide of the multispecific molecule comprising a heavy chain constant domain causes the

5 “knob” to interface (e.g., interact, e.g., a CH2 domain of a first polypeptide interacting with a CH2 domain of a second polypeptide, or a CH3 domain of a first polypeptide interacting with a CH3 domain of a second polypeptide) with the “hole.” As the term is used herein, a “knob” refers to at least one amino acid side chain which projects from the interface of a first polypeptide of the multispecific molecule comprising a heavy chain

10 constant domain and is therefore positionable in a compensatory “hole” in the interface with a second polypeptide of the multispecific molecule comprising a heavy chain constant domain so as to stabilize the heteromultimer, and thereby favor heteromultimer formation over homomultimer formation, for example. The knob may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the

15 interface). The preferred import residues for the formation of a knob are generally naturally occurring amino acid residues and are preferably selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). Most preferred are tryptophan and tyrosine. In the preferred embodiment, the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid,

20 glycine, serine, threonine or valine.

A “hole” refers to at least one amino acid side chain which is recessed from the interface of a second polypeptide of the multispecific molecule comprising a heavy chain constant domain and therefore accommodates a corresponding knob on the adjacent interfacing surface of a first polypeptide of the multispecific molecule comprising a heavy

25 chain constant domain. The hole may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the interface). The preferred import residues for the formation of a hole are usually naturally occurring amino acid residues and are preferably selected from alanine (A), serine (S), threonine (T) and valine (V). Most preferred are serine, alanine or threonine. In the preferred embodiment, the original

30 residue for the formation of the hole has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan.

In a preferred embodiment, a first CH3 domain is mutated at residue 366, 405 or 407 according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85 to create either a “knob” or a “hole” (as described above), and the second

35 CH3 domain that heterodimerizes with the first CH3 domain is mutated at: residue 407 if residue 366 is mutated in the first CH3 domain, residue 349 if residue 405 is mutated in the first CH3 domain, or residue 366 if residue 407 is mutated in the first CH3 domain,

according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85, to create a “hole” or “knob” complementary to the “knob” or “hole” of the first CH3 domain.

In another preferred embodiment, a first CH3 domain is mutated at residue 366 according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85 to create either a “knob” or a hole” (as described above), and the second CH3 domain that heterodimerizes with the first CH3 domain is mutated at residues 366, 368 and/or 407, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85, to create a “hole” or “knob” complementary to the “knob” or “hole” of the first CH3 domain. In one embodiment, the mutation to the first CH3 domain introduces a tyrosine (Y) residue at position 366. In an embodiment, the mutation to the first CH3 is T366Y. In one embodiment, the mutation to the first CH3 domain introduces a tryptophan (W) residue at position 366. In an embodiment, the mutation to the first CH3 is T366W. In embodiments, the mutation to the second CH3 domain that heterodimerizes with the first CH3 domain mutated at position 366 (e.g., has a tyrosine (Y) or tryptophan (W) introduced at position 366, e.g., comprises the mutation T366Y or T366W), comprises a mutation at position 366, a mutation at position 368 and a mutation at position 407, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85. In embodiments, the mutation at position 366 introduces a serine (S) residue, the mutation at position 368 introduces an alanine (A), and the mutation at position 407 introduces a valine (V). In embodiments, the mutations comprise T366S, L368A and Y407V. In one embodiment the first CH3 domain of the multispecific molecule comprises the mutation T366Y, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises the mutations T366S, L368A and Y407V, or vice versa. In one embodiment the first CH3 domain of the multispecific molecule comprises the mutation T366W, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises the mutations T366S, L368A and Y407V, or vice versa.

Additional steric or “skew” (e.g., knob in hole) mutations are described in PCT publication no. WO2014/145806 (for example, Figure 3, Figure 4 and Figure 12 of WO2014/145806), PCT publication no. WO2014/110601, and PCT publication no. WO 2016/086186, WO 2016/086189, WO 2016/086196 and WO 2016/182751 the contents of which are incorporated herein in their entireties. An example of a KIH variant comprises a first constant chain comprising a L368D and a K370S mutation, paired with a second constant chain comprising a S364K and E357Q mutation.

Additional knob in hole mutation pairs suitable for use in any of the multispecific molecules of the present invention are further described in, for example,

WO1996/027011, and Merchant et al., Nat. Biotechnol., 16:677-681 (1998), the contents of which are hereby incorporated by reference in their entirety.

In any of the embodiments described herein, the CH3 domains may be additionally mutated to introduce a pair of cysteine residues. Without being bound by theory, it is believed that the introduction of a pair of cysteine residues capable of forming a disulfide bond provide stability to the heterodimerized multispecific molecule. In embodiments, the first CH3 domain comprises a cysteine at position 354, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85. In embodiments, the first CH3 domain of the multispecific molecule comprises a cysteine at position 354 (e.g., comprises the mutation S354C) and a tyrosine (Y) at position 366 (e.g., comprises the mutation T366Y), and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349 (e.g., comprises the mutation Y349C), a serine at position 366 (e.g., comprises the mutation T366S), an alanine at position 368 (e.g., comprises the mutation L368A), and a valine at position 407 (e.g., comprises the mutation Y407V). In embodiments, the first CH3 domain of the multispecific molecule comprises a cysteine at position 354 (e.g., comprises the mutation S354C) and a tryptophan (W) at position 366 (e.g., comprises the mutation T366W), and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349 (e.g., comprises the mutation Y349C), a serine at position 366 (e.g., comprises the mutation T366S), an alanine at position 368 (e.g., comprises the mutation L368A), and a valine at position 407 (e.g., comprises the mutation Y407V).

(2) Alternative Knob and Hole: IgG Heterodimerization

In one aspect, heterodimerization of the polypeptide chains (e.g., of the half antibodies) of the multispecific molecule is increased by introducing one or more mutations in a CH3 domain which is derived from the IgG1 antibody class. In an embodiment, the mutations comprise a K409R mutation to one CH3 domain paired with F405L mutation in the second CH3 domain, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85. Additional mutations may also, or alternatively, be at positions 366, 368, 370, 399, 405, 407, and 409 according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85. Preferably, heterodimerization of polypeptides comprising such mutations is achieved under reducing conditions, e.g., 10-100 mM 2-MEA (e.g., 25, 50, or 100 mM 2-MEA) for 1-10, e.g., 1.5-5, e.g., 5, hours at 25-37C, e.g., 25C or 37C.

The amino acid replacements described herein are introduced into the CH3 domains using techniques which are well known in the art. Normally the DNA encoding the heavy chain(s) is genetically engineered using the techniques described in Mutagenesis: a Practical Approach. Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution variants of the DNA encoding the two hybrid heavy chains. This technique is well known in the art as described by Adelman et al., (1983) DNA, 2:183.

The IgG heterodimerization strategy is described in, for example, WO2008/119353, WO2011/131746, and WO2013/060867, the contents of which are hereby incorporated by reference in their entirety.

In any of the embodiments described herein, the CH3 domains may be additionally mutated to introduce a pair of cysteine residues. Without being bound by theory, it is believed that the introduction of a pair of cysteine residues capable of forming a disulfide bond provide stability to the heterodimerized multispecific molecule. In embodiments, the first CH3 domain comprises a cysteine at position 354, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85.

(3) Polar Bridge

In one aspect, heterodimerization of the polypeptide chains (e.g., of the half antibodies) of the multispecific molecule is increased by introducing mutations based on the "polar-bridging" rationale, which is to make residues at the binding interface of the two polypeptide chains to interact with residues of similar (or complimentary) physical property in the heterodimer configuration, while with residues of different physical property in the homodimer configuration. In particular, these mutations are designed so that, in the heterodimer formation, polar residues interact with polar residues, while hydrophobic residues interact with hydrophobic residues. In contrast, in the homodimer formation, residues are mutated so that polar residues interact with hydrophobic residues. The favorable interactions in the heterodimer configuration and the unfavorable interactions in the homodimer configuration work together to make it more likely for CH3 domains to form heterodimers than to form homodimers.

In an exemplary embodiment, the above mutations are generated at one or more positions of residues 364, 368, 399, 405, 409, and 411 of CH3 domain, amino acid numbering according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85.

In one aspect, one or more mutations selected from a group consisting of: Ser364Leu, Thr366Val, Leu368Gln, Asp399Lys, Phe405Ser, Lys409Phe and Thr411Lys are introduced into one of the two CH3 domains. (Ser364Leu: original residue of serine at position 364 is replaced by leucine; Thr366Val: original residue of threonine at position
5 366 is replaced by valine; Leu368Gln: original residue of leucine at position 368 is replaced by glutamine; Asp399Lys: original residue aspartic acid at position 399 is replaced by lysine; Phe405Ser: original residue phenylalanine at position 405 is replaced by serine; Lys409Phe: original residue lysine at position 409 is replaced by phenylalanine; Thr411Lys: original residue of threonine at position 411 is replaced by
10 lysine.).

In another aspect, the other CH3 can be introduced with one or more mutations selected from a group consisting of: Tyr407Phe, Lys409Gln and Thr411Asp (Tyr407Phe: original residue tyrosine at position 407 is replaced by phenylalanine; Lys409Glu: original residue lysine at position 409 is replaced by glutamic acid; Thr411Asp: original residue of
15 threonine at position 411 is replaced by aspartic acid).

In a further aspect, one CH3 domain has one or more mutations selected from a group consisting of: Ser364Leu, Thr366Val, Leu368Gln, Asp399Lys, Phe405Ser, Lys409Phe and Thr411Lys, while the other CH3 domain has one or more mutations selected from a group consisting of: Tyr407Phe, Lys409Gln and Thr411Asp.

20 In one exemplary embodiment, the original residue of threonine at position 366 of one CH3 domain is replaced by valine, while the original residue of tyrosine at position 407 of the other CH3 domain is replaced by phenylalanine.

In another exemplary embodiment, the original residue of serine at position 364 of one CH3 domain is replaced by leucine, while the original residue of leucine at
25 position 368 of the same CH3 domain is replaced by glutamine.

In yet another exemplary embodiment, the original residue of phenylalanine at position 405 of one CH3 domain is replaced by serine and the original residue of lysine at position 409 of this CH3 domain is replaced by phenylalanine, while the original residue of lysine at position 409 of the other CH3 domain is replaced by glutamine.

30 In yet another exemplary embodiment, the original residue of aspartic acid at position 399 of one CH3 domain is replaced by lysine, and the original residue of threonine at position 411 of the same CH3 domain is replaced by lysine, while the original residue of threonine at position 411 of the other CH3 domain is replaced by aspartic acid.

35 The amino acid replacements described herein are introduced into the CH3 domains using techniques which are well known in the art. Normally the DNA encoding the heavy chain(s) is genetically engineered using the techniques described in

Mutagenesis: a Practical Approach. Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution variants of the DNA encoding the two hybrid heavy chains. This technique is well known in the art as described by Adelman et al., (1983) DNA, 2:183.

5 The polar bridge strategy is described in, for example, WO2006/106905, WO2009/089004 and K.Gunasekaran, et al. (2010) The Journal of Biological Chemistry, 285:19637-19646, the contents of which are hereby incorporated by reference in their entirety.

10 Additional polar bridge mutations are described in, for example, PCT publication no. WO2014/145806 (for example, Figure 6 of WO2014/145806), PCT publication no. WO2014/110601, and PCT publication no. WO 2016/086186, WO 2016/086189, WO 2016/086196 and WO 2016/182751 the contents of which are incorporated herein in their entirety. An example of a polar bridge variant comprises a constant chain comprising a N208D, Q295E, N384D, Q418E and N421D mutation.

15 In any of the embodiments described herein, the CH3 domains may be additionally mutated to introduce a pair of cysteine residues. Without being bound by theory, it is believed that the introduction of a pair of cysteine residues capable of forming a disulfide bond provide stability to the heterodimerized multispecific molecule. In embodiments, the first CH3 domain comprises a cysteine at position 354, according to
20 the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85, and the second CH3 domain that heterodimerizes with the first CH3 domain comprises a cysteine at position 349, according to the EU numbering scheme of Edelman et al., PNAS, 1969 May, 63(1):78-85.

25 Additional strategies for enhancing heterodimerization are described in, for example, WO2016/105450, WO2016/086186, WO2016/086189, WO2016/086196, WO2016/141378, and WO2014/145806, and WO2014/110601, the entire contents of each of which is hereby incorporated by reference in its entirety. Any of said strategies may be employed in a multispecific molecule described herein.

30 In embodiments, two or more of the modifications discussed herein are combined in a single bispecific antibody, e.g., bbmAb.

5. Example 1: Generation of the bbmAb bbmAb1

By way of example, generation of a specific bbmAb is described below, to enable a person skilled in the art to practice the invention.

35 The resulting bbmAb, bbmAb1, is a bispecific IgG1, with LALA silencing mutations, simultaneously binding to two distinct targets, IL-1 β and IL-18. The antibody

combines two distinct antigen binding arms (Fab fragments), whereas the Fab directed against IL-1 β is based on mAb2 and contains a kappa light chain (Vk6). The Fab directed against IL-18 is based on mAb1 and is composed of a lambda light chain (V λ 1). In order to drive hetero-dimerization of the Fc domain during expression a “knob” with a bulky amino acid (aa) side chain (S354C and T366W) in the mAb1 heavy chain and a “hole” with small aa side chains (Y349C, T366S, L368A, Y407V) were introduced in the mAb2 heavy chain.

For ease of reference, the amino acid sequences of the hypervariable regions of bbmAb1, based on the Kabat definition and the Chothia definition, as well as the V_L and V_H domains and full heavy and light chains are provided in **Table 3**, below.

Table 3. Amino acid sequences of the hypervariable regions (CDRs), variable domains (VH and VL) and full chains of bbmAb1. The DNA encoding the first VL of is set forth in SEQ ID NO:102 and the DNA encoding the second VL is set forth in SEQ ID NO: 70. The DNA encoding the first VH is set forth in SEQ ID NO:86 and the DNA encoding the second VH is set forth in SEQ ID NO: 54.

bbmAb1 heavy chain 1 (from mAb1)		
CDR1-1	Kabat	SEQ ID NO:76
	Chothia	SEQ ID NO:79
	IMGT	SEQ ID NO:82
CDR2-1	Kabat	SEQ ID NO:77
	Chothia	SEQ ID NO:80
	IMGT	SEQ ID NO:83
CDR3-1	Kabat	SEQ ID NO:78
	Chothia	SEQ ID NO:81
	IMGT	SEQ ID NO:84
VH-1		SEQ ID NO:85
Heavy Chain-1		SEQ ID NO:87
bbmAb1 light chain 1 (from mAb1)		
CDR1-1	Kabat	SEQ ID NO:92

	Chothia	SEQ ID NO:95
	IMGT	SEQ ID NO:98
CDR2-1	Kabat	SEQ ID NO:93
	Chothia	SEQ ID NO:96
	IMGT	SEQ ID NO:99
CDR3-1	Kabat	SEQ ID NO:94
	Chothia	SEQ ID NO:97
	IMGT	SEQ ID NO:100
VL-1		SEQ ID NO:101
Light Chain-1		SEQ ID NO:103
bbmAb1 heavy chain 2 (from mAb2)		
CDR1-2	Kabat	SEQ ID NO:44
	Chothia	SEQ ID NO:47
	IMGT	SEQ ID NO:50
CDR2-2	Kabat	SEQ ID NO:45
	Chothia	SEQ ID NO:48
	IMGT	SEQ ID NO:51
CDR3-2	Kabat	SEQ ID NO:46
	Chothia	SEQ ID NO:49
	IMGT	SEQ ID NO:52
VH-2		SEQ ID NO:53
Heavy Chain-2		SEQ ID NO:55
bbmAb1 light chain 2 (from mAb2)		
CDR1-2	Kabat	SEQ ID NO:60
	Chothia	SEQ ID NO:63
	IMGT	SEQ ID NO:66

CDR2-2	Kabat	SEQ ID NO:61
	Chothia	SEQ ID NO:64
	IMGT	SEQ ID NO:67
CDR3-2	Kabat	SEQ ID NO:62
	Chothia	SEQ ID NO:65
	IMGT	SEQ ID NO:68
VL-2		SEQ ID NO:69
Light Chain-2		SEQ ID NO:71

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin heavy chain variable domain (V_{H1}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:76, said CDR2 having the amino acid sequence SEQ ID NO:77, and said CDR3 having the amino acid sequence SEQ ID NO:78. In one embodiment, IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin heavy chain variable domain (V_{H1}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:79, said CDR2 having the amino acid sequence SEQ ID NO:80, and said CDR3 having the amino acid sequence SEQ ID NO:81. In one embodiment, IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin heavy chain variable domain (V_{H1}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:82, said CDR2 having the amino acid sequence SEQ ID NO:83, and said CDR3 having the amino acid sequence SEQ ID NO:84.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin heavy chain variable domain (V_{H2}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:44, said CDR2 having the amino acid sequence SEQ ID NO:45, and said CDR3 having the amino acid sequence SEQ ID NO:46. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin heavy chain variable domain (V_{H2}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:47, said CDR2 having the amino acid sequence SEQ ID NO:48, and said CDR3 having the amino acid sequence SEQ ID NO:49. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin heavy chain variable domain (V_{H2}) comprising hypervariable regions CDR1, CDR2 and

CDR3, said CDR1 having the amino acid sequence SEQ ID NO:50, said CDR2 having the amino acid sequence SEQ ID NO:51, and said CDR3 having the amino acid sequence SEQ ID NO:52.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a first
5 immunoglobulin light chain variable domain (V_{L1}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:92, said CDR2 having the amino acid sequence SEQ ID NO:93 and said CDR3 having the amino acid sequence SEQ ID NO:94. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin light chain variable domain (V_{L1}) comprising
10 hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:95, said CDR2 having the amino acid sequence SEQ ID NO:96 and said CDR3 having the amino acid sequence SEQ ID NO:97. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin light chain variable domain (V_{L1}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1
15 having the amino acid sequence SEQ ID NO:98, said CDR2 having the amino acid sequence SEQ ID NO:99 and said CDR3 having the amino acid sequence SEQ ID NO:100.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin light chain variable domain (V_{L2}) comprising hypervariable regions
20 CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:60, said CDR2 having the amino acid sequence SEQ ID NO:61 and said CDR3 having the amino acid sequence SEQ ID NO:62. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin light chain variable domain (V_{L2}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino
25 acid sequence SEQ ID NO:63, said CDR2 having the amino acid sequence SEQ ID NO:64 and said CDR3 having the amino acid sequence SEQ ID NO:65. In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin light chain variable domain (V_{L2}) comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:66, said CDR2 having
30 the amino acid sequence SEQ ID NO:67 and said CDR3 having the amino acid sequence SEQ ID NO:68.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a first immunoglobulin V_{H1} domain and a first immunoglobulin V_{L1} domain, wherein: a) the first immunoglobulin V_{H1} domain comprises (e.g. in sequence): i) hypervariable regions
35 CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:76, said CDR2 having the amino acid sequence SEQ ID NO:77, and said CDR3 having the amino acid sequence SEQ ID NO:78; or ii) hypervariable regions CDR1, CDR2 and

CDR3, said CDR1 having the amino acid sequence SEQ ID NO:79, said CDR2 having the amino acid sequence SEQ ID NO:80, and said CDR3 having the amino acid sequence SEQ ID NO:81; or iii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:82, said CDR2 having the amino acid sequence SEQ ID NO:83, and said CDR3 having the amino acid sequence SEQ ID NO:84 and b) the first immunoglobulin V_{L1} domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:92, said CDR2 having the amino acid sequence SEQ ID NO:93, and said CDR3 having the amino acid sequence SEQ ID NO:94 or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:95, said CDR2 having the amino acid sequence SEQ ID NO:96, and said CDR3 having the amino acid sequence SEQ ID NO:97 or iii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:98, said CDR2 having the amino acid sequence SEQ ID NO:99, and said CDR3 having the amino acid sequence SEQ ID NO:100.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises a second immunoglobulin V_{H2} domain and a second immunoglobulin V_{L2} domain, wherein: a) the second immunoglobulin V_{H2} domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:44, said CDR2 having the amino acid sequence SEQ ID NO:45, and said CDR3 having the amino acid sequence SEQ ID NO:46; or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:47, said CDR2 having the amino acid sequence SEQ ID NO:48, and said CDR3 having the amino acid sequence SEQ ID NO:49; or iii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:50, said CDR2 having the amino acid sequence SEQ ID NO:51, and said CDR3 having the amino acid sequence SEQ ID NO:52 and b) the second immunoglobulin V_{L2} domain comprises (e.g. in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:60, said CDR2 having the amino acid sequence SEQ ID NO:61, and said CDR3 having the amino acid sequence SEQ ID NO:62 or ii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:63, said CDR2 having the amino acid sequence SEQ ID NO:64, and said CDR3 having the amino acid sequence SEQ ID NO:65 or iii) hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence SEQ ID NO:66, said CDR2 having the amino acid sequence SEQ ID NO:67, and said CDR3 having the amino acid sequence SEQ ID NO:68.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises: a) a first immunoglobulin heavy chain variable domain (V_{H1}) comprising the amino acid sequence set forth as SEQ ID NO:85; b) a first immunoglobulin light chain variable domain (V_{L1}) comprising the amino acid sequence set forth as SEQ ID NO:101; c) a first immunoglobulin V_{H1} domain comprising the amino acid sequence set forth as SEQ ID NO:85 and a first immunoglobulin V_{L1} domain comprising the amino acid sequence set forth as SEQ ID NO:101; d) a first immunoglobulin V_{H1} domain comprising the hypervariable regions set forth as SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78; e) a first immunoglobulin V_{L1} domain comprising the hypervariable regions set forth as SEQ ID NO:92, SEQ ID NO:93 and SEQ ID NO:94; f) a first immunoglobulin V_{H1} domain comprising the hypervariable regions set forth as SEQ ID NO:79, SEQ ID NO:80 and SEQ ID NO:81; g) a first immunoglobulin V_{L1} domain comprising the hypervariable regions set forth as SEQ ID NO:95, SEQ ID NO:96 and SEQ ID NO:97; h) a first immunoglobulin V_{H1} domain comprising the hypervariable regions set forth as SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78 and a first immunoglobulin V_{L1} domain comprising the hypervariable regions set forth as SEQ ID NO:92, SEQ ID NO:93 and SEQ ID NO:94; i) a first immunoglobulin V_{H1} domain comprising the hypervariable regions set forth as SEQ ID NO:79, SEQ ID NO:80, and SEQ ID NO:81 and a first immunoglobulin V_{L1} domain comprising the hypervariable regions set forth as SEQ ID NO:95, SEQ ID NO:96 and SEQ ID NO:97; j) a first light chain comprising SEQ ID NO:103; k) a first heavy chain comprising SEQ ID NO:87; or l) a first light chain comprising SEQ ID NO:103 and a first heavy chain comprising SEQ ID NO:87.

In one embodiment, the IL-18/IL-1 β bispecific antibody comprises: a) a second immunoglobulin heavy chain variable domain (V_{H2}) comprising the amino acid sequence set forth as SEQ ID NO:53; b) a second immunoglobulin light chain variable domain (V_{L2}) comprising the amino acid sequence set forth as SEQ ID NO:69; c) a second immunoglobulin V_{H2} domain comprising the amino acid sequence set forth as SEQ ID NO:53 and a second immunoglobulin V_{L2} domain comprising the amino acid sequence set forth as SEQ ID NO:69; d) a second immunoglobulin V_{H2} domain comprising the hypervariable regions set forth as SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; e) a second immunoglobulin V_{L2} domain comprising the hypervariable regions set forth as SEQ ID NO:60, SEQ ID NO:61 and SEQ ID NO:62; f) a second immunoglobulin V_{H2} domain comprising the hypervariable regions set forth as SEQ ID NO:47, SEQ ID NO:48 and SEQ ID NO:49; g) a second immunoglobulin V_{L2} domain comprising the hypervariable regions set forth as SEQ ID NO:63, SEQ ID NO:64 and SEQ ID NO:65; h) a second immunoglobulin V_{H2} domain comprising the hypervariable regions set forth as SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46 and a second immunoglobulin V_{L2}

domain comprising the hypervariable regions set forth as SEQ ID NO:60, SEQ ID NO:61 and SEQ ID NO:62; i) a second immunoglobulin V_{H2} domain comprising the hypervariable regions set forth as SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49 and a second immunoglobulin V_{L2} domain comprising the hypervariable regions set forth
5 as SEQ ID NO:63, SEQ ID NO:64 and SEQ ID NO:65; j) a second light chain comprising SEQ ID NO:81; k) a second heavy chain comprising SEQ ID NO:55; or l) a second light chain comprising SEQ ID NO:81 and a second heavy chain comprising SEQ ID NO:55.

In some embodiments, the IL-18/IL-1 β bispecific antibody comprises a the three CDRs of SEQ ID NO:53. In other embodiments, the IL-18/IL-1 β bispecific antibody
10 comprises the three CDRs of SEQ ID NO:69. In other embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:53 and the three CDRs of SEQ ID NO:69. In some embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:85. In other embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:101. In other embodiments, the IL-18/IL-1 β
15 bispecific antibody comprises the three CDRs of SEQ ID NO:85 and the three CDRs of SEQ ID NO:101.

In some embodiments, the IL-18/IL-1 β bispecific antibody comprises a the three CDRs of SEQ ID NO:85. In other embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:101. In other embodiments, the IL-18/IL-1 β
20 bispecific antibody comprises the three CDRs of SEQ ID NO:85 and the three CDRs of SEQ ID NO:101. In some embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:53. In other embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:69. In other embodiments, the IL-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:53 and the three
25 CDRs of SEQ ID NO:69. In an embodiment, the L-18/IL-1 β bispecific antibody comprises the three CDRs of SEQ ID NO:85, the three CDRs of SEQ ID NO:101, the three CDRs of SEQ ID NO:53 and the three CDRs of SEQ ID NO:69.

In one embodiment, the first part of the IL-18/IL-1 β bispecific antibody is selected from a human IL-18 antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof
30 of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:76, said CDR2 having the amino acid sequence SEQ ID NO:77, and said CDR3 having the amino acid sequence SEQ ID NO:78; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human
35 light chain, said CDR1 having the amino acid sequence SEQ ID NO:92, said CDR2

having the amino acid sequence SEQ ID NO:93, and said CDR3 having the amino acid sequence SEQ ID NO:94. Furthermore the second part of the IL-18/IL-1 β bispecific antibody is selected from a human IL-1 β antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:44, said CDR2 having the amino acid sequence SEQ ID NO:45, and said CDR3 having the amino acid sequence SEQ ID NO:46; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:60, said CDR2 having the amino acid sequence SEQ ID NO:61, and said CDR3 having the amino acid sequence SEQ ID NO:62.

In one embodiment, the first part of the IL-18/IL-1 β bispecific antibody is selected from a human IL-18 antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:76, said CDR2 having the amino acid sequence SEQ ID NO:77 and said CDR3 having the amino acid sequence SEQ ID NO:78; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:92, said CDR2 having the amino acid sequence SEQ ID NO:93, and said CDR3 having the amino acid sequence SEQ ID NO:94. Furthermore, the second part of the IL-18/IL-1 β bispecific antibody is selected from a human IL-1 β antibody that comprises at least: a) an immunoglobulin heavy chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2 and CDR3 and the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence SEQ ID NO:44, said CDR2 having the amino acid sequence SEQ ID NO:45 and said CDR3 having the amino acid sequence SEQ ID NO:46; and b) an immunoglobulin light chain or fragment thereof which comprises a variable domain comprising, in sequence, the hypervariable regions CDR1, CDR2, and CDR3 and the constant part or fragment thereof of a human light chain, said CDR1 having the amino acid sequence SEQ ID NO:60, said CDR2 having the amino acid sequence SEQ ID NO:61, and said CDR3 having the amino acid sequence SEQ ID NO:62.

The first V_{H1} or V_{L1} domain of an IL-18/IL-1 β bispecific antibody used in the disclosed methods may have a first V_{H1} and/or first V_{L1} domains that are substantially identical to the V_H or V_L domains set forth in SEQ ID NO:85 and 101. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise a first heavy chain that is substantially identical to that set forth as SEQ ID NO:87 and/or a first light chain that is substantially identical to that set forth as SEQ ID NO:103. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise a first heavy chain that comprises SEQ ID NO:87 and a first light chain that comprises SEQ ID NO:103. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise: a) a first heavy chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:85 and the constant part of a human heavy chain having a hetero-dimerization modification; and b) a first light chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:101 and the constant part of a human light chain. The constant part of the human heavy chain may be IgG1. In one embodiment, the IgG1 is a human IgG1 without effector mutations. In one embodiment, the human heavy chain IgG1 comprising a silencing mutation N297A, D265A or a combination of L234A and L235A. In one specific embodiment, the human heavy chain IgG1 comprises the silencing mutation which is a combination of L234A and L235A, according to SEQ ID NO:87.

The second V_{H2} or V_{L2} domain of an IL-18/IL-1 β bispecific antibody used in the disclosed methods may have a second V_{H2} and/or first V_{L2} domains that are substantially identical to the V_H or V_L domains set forth in SEQ ID NO:53 and 69. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise a second heavy chain that is substantially identical to that set forth as SEQ ID NO:55 and/or a second light chain that is substantially identical to that set forth as SEQ ID NO:71. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise a second heavy chain that comprises SEQ ID NO:53 and a second light chain that comprises SEQ ID NO:69. An IL-18/IL-1 β bispecific antibody disclosed herein may comprise: a) a second heavy chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:53 and the constant part of a human heavy chain having a hetero-dimerization modification, which is complementary to the hetero-dimerization of the first heavy chain; and b) a second light chain, comprising a variable domain having an amino acid sequence substantially identical to that shown in SEQ ID NO:69 and the constant part of a human light chain. The constant part of the human heavy chain may be IgG1. In one embodiment, the IgG1 is a human IgG1 without effector mutations. In one embodiment, the human heavy chain IgG1 comprising a silencing mutation N297A, D265A or a combination of L234A and L235A. In one specific embodiment, the human heavy chain

IgG1 comprises the silencing mutation which is a combination of L234A and L235A, according to SEQ ID NO:55.

Other preferred IL-18 antagonists (e.g. antibodies) for use as the first part of a bispecific antibody in the disclosed methods, kits and regimens are those set forth in US Patent No: 9,376,489, which is incorporated by reference herein in its entirety.

Other preferred IL-1 β antagonists (e.g. antibodies) for use as the second part of a bispecific in the disclosed methods, kits and regimens are those set forth in US Patent Nos: 7,446,175 or 7,993,878 or 8,273,350, which are incorporated by reference herein in their entirety.

10 (1) Vector design

Two vectors, vector A and vector B, were generated according to the following setup. Vector A was designed for the antibody portion mAb1 (anti-IL18 IgG1). The constant region of the heavy chain 1 was modified by two point mutations, T to W as seen in position 366 of SEQ ID NO: 87, and S to C as seen in position 354 of SEQ ID NO: 87, for generating a knob structure and enabling Cys-bridging. Furthermore, the constant region of the heavy chain 1 was modified by with point mutations, L to A, as seen in position 234 of SEQ ID NO: 87 and L to A as seen in position 235 of SEQ ID NO: 87 (so called LALA), for partial silencing of FC effector functions. The antibody has a variable light region, which is of the lambda 1 type, V λ 1.

Vector B was designed for the antibody portion mAb2 (anti IL-1 β IgG1). The constant region of heavy chain 2 was modified by four point mutations T to S as seen in position 366 of SEQ ID NO: 55, L to A as seen in position 368 of SEQ ID NO: 55, Y to V as seen in position 407 of SEQ ID NO: 55, and Y to C as seen in position 349 of SEQ ID NO: 55, for generating a hole structure and enabling an additional Cys-bridging. The hole structure interacts with the knob structure, to facilitate generation of a bispecific antibody. Furthermore, the constant region of heavy chain 2 was modified with the two LALA mutations, L to A as seen in position 234 of SEQ ID NO: 55, L to A as seen in position 235 of SEQ ID NO: 55, for silencing of FC effector functions. The antibody has a variable light region, which is of the kappa 6 type, V κ 6.

Vector A and B carry a combination of DHFR and neomycin selection markers as well as FOLR and hygromycin selection markers, respectively. Folic acid is a vitamin essential for purine and methionine synthesis and needs to be taken up by mammalian cells from the culture medium. The "folic acid receptor" (FolR) present on the expression plasmid A is a mutant FolR with altered affinity for folates, facilitating the transport of folic acid from the medium into mammalian cells. Given that high affinity folic acid receptors are only weakly expressed in cultured CHO cells, cells expressing recombinant FolR

have a clear growth advantage under low folate conditions (50 nM). The FolR selection marker was encoded at vector B

In addition to FolR, “dihydrofolate reductase” (DHFR) is present on vector A as selection marker. DHFR converts folic acid into vital precursors for purine and methionine synthesis. MTX is a chemical analogue of folic acid. It is competing for free binding sites on DHFR and thereby blocking the enzyme. Cells overexpressing exogenous DHFR can deal with elevated MTX concentrations, giving the cells a clear selective advantage growing in medium supplemented with MTX. Combined FolR and DHFR selection is well known to a person skilled in the art, and disclosed e.g. in the patent document WO2010/097240A1, incorporated here by reference in its entirety. MTX is well known to a person skilled in the art and disclosed e.g. in the patent document WO2010/097239A1, incorporated here by reference in its entirety. Expression vectors are well known to a person skilled in the art, and disclosed e.g. in the patent document WO2009/080720A1, incorporated here by reference in its entirety.

15

A schematic overview of the two vectors is seen in Figure 1.

(2) Host cell line and transfection

A parental CHO cell line was used as host cell line for the production of the bbmAb1 expressing cell line. The host cell line was derived from the CHO-K1 cell line, well known to a person skilled in the art, in a way described e.g. in the patent applications WO2015092737 and WO2015092735, both incorporated by reference in their entirety.

A single vial from the CHO line was used to prepare the bbmAb1 recombinant cell line. The CHO line was prepared in chemically defined medium.

25

The cells were grown in chemically defined cultivation medium.

One µg of Swal linearized plasmid DNA, expression vector A & B encoding for the bbmAb, was added per transfection. The transfection reaction was performed in chemically defined cultivation medium.

Transfections were performed by electroporation using an AMAXA Gene Pulser, according to the manufactures instructions. The parental CHO cells used for transfection were in exponential growth phase with cell viabilities higher than 95%. In total, three transfections were performed with 5×10^6 cells per transfection.

Immediately after transfection, cells were transferred into Shake Flasks, containing medium chemically defined cultivation medium.

35

Cell pools were incubated for 48 hours at 36.5°C and 10% CO₂ before starting the selection process.

(3) Cell selection and sorting

A selection procedure was carried out using the selection marker encoded by the individual expression vector A and B, as described above. Both proteins (FolR and DHFR) are participating in the same molecular pathway; the FolR is transporting folic acid as well as the folate analogue MTX into the cell, the DHFR is converting it into vital precursors for purine and methionine synthesis. Combining them as selective principle, a particular strong selective regime can be taken to enrich for recombinant cells expressing both recombinant protein.

48 h after transfection and growth under low folate conditions, additional selective pressure was applied by adding 10 nM MTX to the chemically defined cultivation medium. 22 days after the start of MTX selection, pool populations consisting predominantly of MTX resistant cells have emerged. After pool recovery cells were frozen and cell pellets prepared. Standard batches in chemically defined cultivation medium were set up for determination of concentration of the bbmAb.

Protein A HPLC methodology was used to determine complete all kind of product and related impurities carrying a Fc part, whereas a reversed phase chromatography (RPC) was use to obtain a fingerprint with respect to the distribution of the individual fractions – individual peaks have been assigned by MS methodology.

CHO cell pools producing bbmAb1 have been used for a FACS cloning procedure to obtain individualized clonal cell lines as starting material for all further evaluations. Cell selection using FACS analysis is described e.g. in the patent application US20110281751, incorporated here by reference in its entirety.

Individual clonal CHO cell lines expressing bbmAb1 were generated by Fluorescent-activated cell sorting (FACS). To enable the FACS sorting the cells were incubated with a FITC-labeled anti IgG1 Fab for 30 min and washed twice in PBS before being used in FACS assisted single cell sorting, a process well known to a person skilled in the art.

FACS cell sorting was performed with a FACS Aria (Becton Dickinson), equipped with an Automatic Cell Deposition Unit (ACDU) using the FACS Diva software.

In order to assure that only single cells were sorted by the FACS instrument, settings were adjusted to single cell precision mode using a nozzle of 130 µm as well as an appropriate flow rate insuring good sorting quality.

In Single Cell mode, the Purity Mask was set to the maximum, so only drops free of particles or further cells were sorted.

The Phase Mask was set to half the maximum, so only particles centered within the sorted drop were deflected. Drop trajectory and count accuracy were optimized at the

expense of yield to increase the probability that each droplet contained not more than 1 single cell.

In order to verify and document monoclonal origin, and to confirm the single cell status at day 0 after FACS cloning images of all wells of the 96well plates were taken
5 using an imaging system.

The image of day 0 referring to bbmAb1 production clone was visually inspected in duplicate, confirming that only one single cell could be identified in the image of the respective well taken by the imaging system.

This underlined the single cell origin of bbmAb1 production clone.

10 (4) Cell expansion

After FACS cloning, the clones were handled by a robotic system for the first weeks and handled manually later on, step wise expanded from 96-well, 24-well towards shaker, and finally bioreactor cultivations, well known to a person skilled in the art, to assess performance (productivity and quality of bbmAb expression).

15 During the expansion/cultivation recombinant CHO cells were cultivated in chemically defined cultivation medium supplemented with Methotrexate (MTX) at a final concentration of 10 nM.

Cells have been passaged 2-3 times per week into fresh medium and were maintained in logarithmic growth phase throughout the study

20 Productivity was assessed by Protein A HPLC, an initial product quality profile was determined by reversed phase chromatography (RPC).

All frozen stocks were generated in culture medium, supplemented with 7.5% DMSO.

(5) Clone stability

25 Isolated bbmAb from pools and clones were carefully evaluated by different analytical methodologies to judge product characteristics and quality parameters to ensure the selection of the best suited production clone.

In addition an excessive analysis of the production stability was performed for the production clones to ensure the selection of the most suitable production clones.

30 Different state of the art analytical methodologies were used to assess clone stability: affinity liquid chromatography, reverse phase chromatography, FACS and MS.

(6) Manufacturing

(a) Upstream processing

bbmAb material was produced either in shake flasks or wave fed batch cultures. Frozen stock of pools or clones such as PSLs were thawed and expanded over the required period of time in chemically defined medium to obtain the required number of cells to seed the production culture, typical with a seed cell density of 4.0×10^5 cells/ml.

- 5 The individual culture was cultivated over a period of 13-14 day. During the cultivation in-process controls were performed to monitor the concentration of the bbmAb and the quality profile in the supernatant. At the end of the cultivation process cells were separated from the culture supernatant either by centrifugation (e.g. shaker) or deep filtration followed by sterile filtration before further DSP processing.

10 (b) *Downstream processing*

- Based on the format design and the co-expression approach not only the intact product bbmAb1 and common impurities such as aggregates, DNA and host cell proteins, but also mAb1 and mAb2 derived monomers, homodimers and miss-paired light/heavy chain bbmAb1 variants, as shown in Figure 4, were expected in the
15 supernatant after cell cultivation and cell debris removal. Miss-paired light/heavy chain bbmAb1 variants (Figure 4E to 4M) are suspected to have the same biophysical properties as the intact bbmAb1 which cannot easily be removed at preparative scale.

Approach I: Purification by capturing on MabSelect™ SuRe™, polish on
20 *LambdaFabSelect™ and KappaSelect™*

- bbmAb1 and bbmAb1 variants carrying Fc-part were captured from cell free supernatant by a first affinity liquid chromatography (ALC) step on MabSelect™ SuRe™. bbmAb1 variants containing kappa light chains only (mAb2 hole, Figure 4C and 4D) and HCP were removed by a first polish on LambdaFabSelect™ and bbmAb1 variants
25 containing lambda light chains only (mAb1 knob, Figure 4A and 4B) and HCP were removed by a second polish step on KappaSelect™.

- Chromatography was performed at room temperature using 4 minutes residence time (RT) throughout the method. All columns were equilibrated with 4 column volumes (CV) 20 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 7.0 before loading. To deplete unspecific bound
30 impurities from product, such as host cell proteins (HCP), media components and DNA the chromatography column was washed with 4 CV 250 mM Arginine-HCl, 1 M NaCl, 88 mM NaOH, pH 9.0 and 3 CV equilibration buffer after loading cell-free bbmAb1 supernatant from shakeflask onto the ALC column. bbmAb1 and potential bbmAb1 variants were eluted from the chromatographic column by using 50 mM acetic acid, pH
35 3.0 respectively 50 mM acetic acid/HCl, pH 2.0 for elution from KappaSelect™ and LambdaFabSelect™. Product peak collection started and ended at 0.5 AU/cm or

0.25 AU/cm (280 nm). The pH of the bbmAb1 eluates were adjusted to ~pH 5.0 with 0.1 or 1 M Tris prior to storage at 2-8°C respectively for analytical assessment.

5 *Approach II: Purification by capturing on LambdaFabSelect™, polish on Capto™ adhere and Fractogel™ EMD SO₃*

In a second approach intact bbmAb1 and bbmAb1 variants containing lambda light chains only (mAb1 knob monomer and homodimer, Figure 4A and 4B) were captured from cell-free supernatant by affinity liquid chromatography on LambdaFabSelect™. In order to inactivate potentially present enveloped viruses a low pH treatment of the ALC eluate was performed followed by two chromatographic polish steps on Capto adhere and Fractogel™ EMD SO₃ to remove product related impurities, DNA and HCP. Potentially present viruses were subsequently removed by nanofiltration prior to a final concentration and buffer exchange step using tangential-flow filtration.

15 a) Affinity Liquid Chromatography (ALC) on LambdaFabSelect™

ALC was performed at 18-28°C using 3.6-4.4 minutes residence time (RT) throughout the method. First, the ALC column was equilibrated with 4-6 CV 20 mM Na₂HPO₄/NaH₂PO₄, pH 7.0. Then clarified cell-free bbmAb1 supernatant from waves or bioreactors was loaded onto the LambdaFabSelect™ column with a loading density of 7-23 g/L. A column wash with 4-6 CV 250 mM Arginine-HCl, 1 M NaCl, 88 mM NaOH, pH 9.0 and a second wash with 3-5 CV equilibration buffer was performed prior to product elution with 4-6 CV 50 mM acetic acid. The product peak was collected from 0.5-2.0 AU/cm (280 nm) ascending and 0.5-2.0 AU/cm (280 nm) descending. The LambdaFabSelect™ column was cleaned using 3-5 CV 120 mM phosphoric acid, 167 mM acetic acid, pH 1.5 prior to re-equilibration with 3-5 CV 20 mM Na₂HPO₄/NaH₂PO₄, pH 7.0 and storage in 4-6 CV 20 % ethanol.

b) Virus inactivation

The pH of the ALC eluate was adjusted to pH 3.4-3.6 using 0.3 M phosphoric acid. Then the protein solution was subsequently incubated for 60-90 min at this low pH before pH adjustment to 7.3-7.7 with 1M Tris. A depth filtration step using Millipore B1HC Pod filters was used applying a flow rate of 100-300LMH before sterile filtration with 0.45/0.2 µm Sartopore™ sterile filter.

35 c) Multimodal Anion Exchange Chromatography (MAC) on Capto™ adhere

MAC was performed in flow-through mode at 18-28°C using 4-6 minutes residence time throughout the method. First, the MAC column was equilibrated with 7-9

CV 20 mM Tris/Tris-HCl, pH 7.5. Then the low pH treated ALC eluate was loaded onto the Capto™ adhere column using a loading density of 175-350 g/L. Product peak collection started thereby at 0.5-2.0 AU/cm (280nm) ascending. Then the MAC column was washed with 5-7 CV equilibration buffer and product peak collection ended at 0.5-
5 2.0 AU/cm (280nm) descending. The Capto™ adhere column was subsequently stripped with 6-8 CV 100 mM acetic acid, followed by a cleaning-in place step with 3-5 CV 0.5 M NaOH and storage in 3-5 CV 0.1 M NaOH.

d) Cation Exchange Chromatography on Fractogel™ EMD SO₃.

10 CEC on Fractogel™ EMD SO₃ was performed in bind-elute mode at 18-28°C. A residence time of 6-8 minutes was used during equilibration, strip, CIP and storage and 8-10 min residence time during load, wash and elution. The CEC column was equilibrated with 6-8 CV 20 mM succinic acid, 35.1 mM NaOH, pH 6.0. Then the MAC percolate was loaded onto the column with a loading density of 35-70 g/L. Subsequently,
15 the CEC column was washed with 5-7 CV equilibration buffer. Elution was performed using a linear salt gradient from 10 to 90 % 20 mM succinic acid, 500 mM NaCl, 37.4 mM NaOH, pH 6.0 over 15 CV. The bbmAb1 product peak was collected starting at 0.1-0.4 AU/cm ascending to 20 %-40% maximum peak height at 300 nm. The Fractogel™ EMD SO₃ column was stripped with 3-5 CV 1 M NaCl, followed by a cleaning-in place step
20 with 3-5 CV 0.5 M NaOH and storage in 3-5 CV 0.1 M NaOH.

e) Nanofiltration

Potentially present viruses were removed by nanofiltration using Planova™ 20N nanofilter and a 0.5/0.1 µm Millipore SHR-P pre-filter. Pre- and nanofiltration was
25 performed by applying a differential pressure of 0.7-0.9 bar.

f) Tangential-flow filtration and Formulation

In order to concentrate and diafiltrate bbmAb1 a tangential-flow filtration step on Millipore™ Pellicon™ 3 RC 30 kDa membranes was performed at 18-28°C. First, the
30 nanofiltered bbmAb1 protein solution was concentrated with a maximum loading density of 1000g/m² to 60-80 g/L using a feed flow pressure of 0.5 to 1.2 bar and a transmembrane pressure (TMP) of 0.3-0.6 bar. Then, bbmAb1 was diafiltered against 7-9 diafiltration volumes 20 mM histidine/histidine-HCl, pH 6.0 at a feed flow pressure of 0.8 to 1.8 bar and a TMP of 0.4-0.9 bar. A second concentration step to 134±10 g/L at a
35 feed flow pressure of 1.4-3.0 bar and a TMP of 0.7 to 1.5 bar was performed. The ultrafiltered bbmAb1 protein solution was finally formulated to a concentration of 100±10

g/L and 0.04 % (w/v) Polysorbate 20. The final drug substance (DS) is filtered through a 0.2µm filter and stored frozen at ≤-60°C.

5

(7) Analytical characterization and purity assessment

(a) *LC-MS screening of intact bbmAbs and variants*

100 µg of protein-A purified bbmAb samples were lyophilized in a 96-well plate and de-glycosylated by PNGaseF (New England Biolabs) for 18 hours at 37°C in 100 µl of 50 mM Tris-HCl pH 7.5) buffer. Samples were measured by LC-ESI-MS on an H-Class UPLC (Waters) connected to a Synapt G2 Q-TOF mass spectrometer (Waters). MassPREP Micro Desalting column 2.1x 5 mm (Waters) was used at 80°C column temperature. A linear gradient was applied at 0.3 ml/min with mobile phase A: 0.1 % formic acid in water, mobile phase B: 0.1% FA in acetonitrile: 0-2 min 5% B, 2-12 min 5-90% B, followed by several wash steps at 0.5 ml/min. MS parameters: ESI + Resolution mode, Capillary voltage 3 kV, sampling cone 40 V, source temperature 150°C, desolvation temperature 400°C. System was calibrated with NaCl calibration solution, lock mass was Leucine Enkephalin. Data was processed by automatic MaxEnt1 deconvolution (mass range 60-150 kDa, harmonic suppression) with UNIFI 1.6 software (Waters). Identification and relative quantification of bbmAb species and miss paired variants is based on the match to the theoretically expected mass and the relative mass signal intensity of the de-convoluted mass spectrum.

(b) *LC-MS characterization of bbmAb1*

Intact de-glycosylated bbmAb: Purified bbmAb1 antibody was diluted in 20 mM Tris-HCl pH 7.5 to 1 mg/ml and was de-glycosylated for 4 h at 37°C using 2 µl PNGaseF enzyme (New England Biolabs). The digestion was stopped by adding trifluoroacetic acid (TFA) to 2%.

Reduced de-glycosylated bbmAb: bbmAb1 was diluted to 5 mg/ml in 20 µl 0.1 M Tris-HCl pH 7.5. 2 µl of PNGase F was added and incubated for 4 h at 37 °C, then 80 µl of denaturation buffer (50 mM Tris-HCl pH 8.0, 6 M guanidine hydrochloride) and 1 µl of 1 M DTT was added to the mixture. After incubation for 1 hour at 37°C, the sample was acidified with 1 µl TFA.

Papain digestion of bbmAb into Fab and Fc. bbmAb1 was mixed with digestion buffer (20 mM succinic acid, 35.1 mM NaOH, pH 6.0, 1 mM Cys-HCl, 1 mM EDTA) to 5 mg/ml, then papain protease (Roche, Germany) was added to final

concentration of 5 µg/ml (protease/ protein ratio 1:1000) and incubated for 2 hours at 37°C under shaking. After incubation, the solution was stopped by adding iodoacetamide solution to 1.2 mM final concentration.

IdeS digestion of bbmAb to F(ab')₂ and Fc. 100 µg bbmAb1 were mixed with
5 cleavage buffer (50 mM sodium phosphate, 150 mM NaCl, pH 6.6) and **digested** with 100 U IdeS protease (Fabricator, Genovis) overnight at 37°C. After incubation, the solution was stopped by adding TFA to 2% final concentration.

Reduced LysC digestion - Peptide mapping. (According to Rombach-Riegraf et al, PlosOne 2014) 200 µg protein were denatured using 150 µl of denaturing solution
10 (6 M guanidine hydrochloride, 50 mM Tris-HCl, 5 mM Na₂EDTA, pH 8.0) and reduced by adding 1.5 µl of 1 M DTT and incubation at 37°C for one hour. The alkylation was performed by adding 3 µl of 1 M iodoacetamide followed by incubation at 37°C in the dark. The reaction was quenched with 1 µl of 1 M DTT. Following reduction/alkylation, 750 µl digestion buffer (50 mM Tris-HCl, pH 8) were added to the sample. The sample
15 was then digested by two additions of 4 µl of a 1 µg/µl endoproteinase LysC solution (Wako (Osaka, Japan)) and incubation at 37°C for 1 hour and 3 hours respectively. 5 µl TFA was added to quench the digestion.

LC-MS measurements. Protein samples were subjected to LC-MS system using Waters UPLC H-Class equipped with a BEH C4 RP column (1.7 µm, 2.1 x 100 mm, 300
20 Å, Waters) and a Xevo G2 TOF mass spectrometer (Waters, Milford). Eluents were A: 0.1 % TFA in water and B: 0.09 % TFA in acetonitrile. The column was set to 80°C. Flowrate was 0.2 ml/min. Proteins were eluted with a 40 min gradient as follows: 0-5 min 10% B, 5-10 min 10-30 % B, 10-25 min 30-40% B, 25-26 min 40-95%B, 26-28 min 95%, 28-40 min 10 % B.

25 MS settings: ESI (+) TOF mode, Resolution mode, Mass Range 400-4000 Da, Scan Time 1s, capillary voltage 3 kV, sampling cone 25 V-40 V. System was calibrated using NaCsl solution.

Peptide digests were analyzed by RP-LC-MS on H-Class UPLC (Waters) coupled to a Synapt G2 Q-TOF mass spectrometer (Waters) using an CSH130 C18 2.1 mm x
30 150, 1.7 µm (Waters, Milford). Mobile phase A: 0.1% TFA in water and mobile phase B: 0.09 % TFA in acetonitrile. Peptides were eluted from the column with a following gradient: 0-5 min 0%B, 5-10 min 0-2%B, 10-40 min 2-20%B, 40-120 min 20-40%B, 120-135 min 40-70% at 40°C column temperature. UV chromatograms were recorded at 214 nm and MS data acquisition was performed in positive ES(+) resolution mode as an MS^E
35 experiment using low energy (4 eV) and high energy fragmentation (30-55 V). Lock mass was Leucin Enkephalin (Waters).

Data processing and evaluation was performed by MassLynx 4.2 or UNIFI 1.6 software (Waters). MaxEnt1 algorithm was used for deconvolution of protein mass spectra. Theoretical mass calculations were performed with GPMW 9.2 software (Lighthouse data).

5

(c) *Reversed phase chromatography*

bbmAb samples were analyzed on Agilent 1260 HPLC using Poroshell 300 SB-C8 RP column, 2.1 mm x 75 mm, 5 μ m (Agilent). Column temperature was set to 80°C, flow rate was 2 ml/min, mobile phase A: 90% water, 10 % acetonitrile, 0.1 % TFA, 0.3 % PEG-300, mobile phase B: 10 % water, 90 % acetonitrile, 0.1% TFA, 0.3 % PEG-300. Gradient used was: 0-5 min 22-37% B, 5.0-5.1 min 100% B, 5.1-6 min 100% B, 6.1.-8.5 min 22 % B. UV signal was recorded at 210 nm. Data acquisition and evaluation was controlled and performed using Chromeleon™ 6.8 software (Thermo Scientific).

10

(d) *Size exclusion chromatography*

bbmAb1 sample were subjected to TSK gel G3000SWXL (Tosoh #808541, 5 μ m, 7.8 mm x 300 mm) SEC column, pore size 250 A using an Agilent 1260 system. Mobile phase was 150 mM potassium phosphate solution, pH 6.5, Flow rate was 0.4 ml/min, column temperature was 30 °C. UV was recorded at 210 nm. Data acquisition and peak integration was performed using Chromeleon™ 6.8 (Thermo Scientific).

20

(e) *Capillary Electrophoresis CE-SDS*

For non-reducing CE-SDS, bbmAb sample was mixed with sample buffer (0.1 sodium phosphate/ 1.0 % SDS, pH 6.6) and then mixed with iodoacetamide solution. For reducing CE-SDS, the protein was mixed with 0.1 M Tris/ 1% SDS sample buffer, pH 8.0 and reduced with 5 % (v/v) mercaptoethanol. Both samples underwent a heat denaturation step at 70°C for 10 minutes.

25

Samples were analyzed on a Beckman PA 800 system equipped with a bare fused silica capillary (50 μ m, 375 OD, 67 cm, Beckman) with of 30 cm total capillary length and filled with Beckman SDS MW sieving gel buffer. Separation was done from negative to positive polarity at 15 kV and 25°C capillary temperature, detection by UV at 214 nm. Electropherograms were processed and integrated using Chromeleon™ 6.8 software.

30

(f) *Capillary Zone Electrophoresis CZE*

Separations were performed on a Beckman Coulter PA 800 Pharmaceutical Analysis System equipped with 214 nm UV detector. Protein was separated on fused silica capillary (50 μ m ID) of a total length of 40 cm with a capillary voltage of 20 kV at 25°C and positive polarity. Running buffer: 400 mM 6-aminocaproic acid /acetic acid pH 5.7 with 2 mM TETA and 0.03% Tween 20. Peak integration was performed with Chromeleon™ 6.8 software.

(8) Analytical Results

The purity and identity of different bispecific antibody combinations and constructs after co-expression was analyzed after protein-A purification using an intact UPLC-MS mass screening approach. This approach was used to confirm and relatively quantify the formed hetero- and homodimers from cell supernatant. Correctly formed heterodimeric bbmAb1 and bbmAb2 could be observed with a relative purity of over 85% based on intact mass signal intensity. Main impurities observed in the screen were the miss paired antibody with two kappa light chains, two lambda light chains and HC hole-dimer molecules.

Table 4. Summary of analytical results obtained for bbmAb1-bbmAb11.

Candidate	Knob/ Hole	L1/L2	Bispecific Ab variants identity by MS		Ranking
			ID	Rel. Quantification	
bbmAb1	mAb1/ mAb2	mAb1 (l)/ mAb2 (k)	bsAb KH:L2L2 KH:L1L1	85% 5% 10%	1
bbmAb2	mAb2/ mAb1	mAb1 (l)/ mAb2 (k)	bsAb KH:L1L1 KH:L2L2	84% 6% 10%	1
bbmAb3	mAb3/ mAb4	mAb3 (k)/ mAb4 (k)	bsAb KH:L1L1 HH:L1L1 (H dimer)	54% 35% 11%	2
bbmAb4	mAb5/ mAb2	mAb2 (k)/ mAb5 (l)	bsAb KH:L2L2 HH:L1L1 (H dimer) KH:L2L2	63% 9% 11% 17%	2
bbmAb5	mAb1/ mAb6	mAb6(k)/ mAb1	bsAb HH:L2L2 (H dimer)	53% 47%	2

bbmAb6	mAb7/ mAb1	mAb1(l)/ mAb7 (k)	bsAb KH:L2L2 KH:L1L1 HH:L1L1 (H dimer)	51% 21% 20% 9%	3
bbmAb7	mAb8/ mAb1	mAb8 (l)/ mAb1(l)	bsAb HH:L2L2 (H dimer) Partial Ab: H:L2, H:L2+Cys	80% 20%	2
bbmAb8	mAb9/ mAb1	mAb9 (k)/ mAb1 (l)	bsAb KH:L2L2 KH:L1L1 HH:L2L2 (H dimer)	35% 34% 17% 14%	3
bbmAb9	mAb10/ mAb1	mAb10 (k) /mAb1 (l)	bsAb KH:L1L1 HH:L1L1 (H dimer) KH:L2L2	58% 23% 12% 7%	3
bbmAb10	mAb11/ mAb1	mAb11 (k) /mAb1 (l)	bsAb KH:L1L1 HH:L1L1 (H dimer) bsAb HC clip Partial Ab: H:L1, H:L1+Cys	45% 22% 23% 10%	2
bbmAb11	mAb12/ mAb1	mAb12 (k) /mAb1 (l)	bsAb HH:L1L1 (H dimer) KH:L1L1 Partial Ab: H:L1, H:L1+Cys	49% 41% 10%	2

Designation l is lambda chain, k is kappa chain.

mAb3 is an antibody of type VH3, Vk1.

mAb4 is an antibody of type VH3, Vk1.

5 mAb5 is a graft version of mAb1 (VH1, VI1).

mAb6 is a graft version of mAb2 (VH1_46, Vk3).

mAb7 is an antibody of type VH3, Vk1.

mAb8 is an antibody of type VH1_2, Vk2.

mAb9 is an antibody of type VH5, Vk6.

10 mAb10 is an antibody of type VH1_46, Vk6.

mAb11 is an antibody of type VH3, Vk3.

mAb12 is an antibody of type VH3, Vk2.

bbmAb1 was characterized in more detail to evaluate the all formed product
5 variants and impurities after the different purification steps by LC-MS using several
sample preparation methods, and with other separation techniques. The mass of the
intact 2-step (lambda/CEC) purified product was determined after de-glycosylation with
PNGaseF enzyme and subsequent injection into the RP-LC-MS setup. The de-
convoluted mass spectrum of intact bbmAb1 confirmed the correct formation of the knob-
10 in-hole heterodimer after co-expression and lambda-select purification. No major
impurities like homodimers or partial antibodies could be detected after lambda-select
purification. Identity of the four different antibody chains could be confirmed after
reduction and de-glycosylation of the sample.

To check for chain mispairing and other low level impurities, the purified sample
15 was digested by papain protease to analyze the individual Fab and Fc fragments. The
measured masses of the fragments confirmed again the correct formation of the different
Fab arms (Knob-lambda, Hole-kappa), as well as the correct formation of the knob-in-
hole Fc fragment. A mispaired Fab fragment (Fab4, Knob-kappa) could be observed in
levels <1 %. An alternative approach the generate Fab fragments with a limited LysC
20 digestion was tested which allows faster sample prep during pool and clone selection.

Another digestion strategy using IdeS (Fabricator) enzyme was tested to
generate Fc and F(ab')₂ fragments. In this experiment, the mass of the Fc heterodimer
was observed and the correctly formed heterodimeric F(ab')₂. The presence of a Fc
heterodimer is also proof of the correct formation of the additional di-sulfide bridge in Fc
25 part of bbmAb1, as otherwise only a Fc/2 fragment of smaller mass would be generated.

LysC peptide mapping with LC-MS could confirm identity of the molecule with an
overall sequence coverage of the peptides of 99%.

Specific results of the purified samples are shown in Figure 2. Particularly, RP-UV
chromatogram of de-glycosylated intact bispecific mAb is shown in Figure 2A. Papain
30 digested bbmAb1 fragments are shown in Figure 2C. IdeS digested bbmAb1 fragments
are shown in Figure 2D. De-glycosylated and DTT reduced bbmAb1 fragments are
shown in Figure 2E. De-convoluted mass spectrum of the intact deglycosylated bispecific
mAb bbmAb1 is shown in Figure 2B.

35 Results are shown in **Table 5**.

Table 5. Assignment of measured bbmAb1 masses by RP-LC-MS

bbmAb1	Identity	Observed mass (Da)	Theoretical mass (Da)	Mass deviation (Δ Da)
Intact de-glycosylated bsAB	L1H1:L2H2 mAb	144104	144101	+ 4
Papain digest	Fc (H1-H2)+ bG0/bG0/0K	52570	52570	0
	Fab1 (L1H1 lambda /knob)	46952	46952	0
	Fab2 (L2H2 kappa/hole)	47503	47504	- 1
	Fab4	47329	47330	+ 1
IdeS digest	F(ab') ₂	96644	96643	+1
	Fc (H1-H2) dimer +bG0/bG0 / 0K	50383	50383	0
Reduced de-glycosylated bsAb	L1 (λ -LC)	22980	22980	0
	L2 (κ -LC) pyro-E	23358	23358	0
	H1 (knob-CH) 0K	48953	48952	1
	H2 (hole-CH) pE,0K	50290	50289	1

The increased purity obtained after applying different steps described above is seen in Figure 3. Figure 3A is a chromatogram showing the expression profile after cultivation, Figure 3B is a chromatogram after capturing by LambdaFabSelect™, Figure 3C is a chromatogram after capturing with MabSelect™ SuRe™, and Figure 3D is a chromatogram after capturing with LambdaFabSelect™, polish by Fractogel™ EMD SO₃ and ultrafiltration.

The final 2-step purified (lambda/CEC) bispecific bbmAb1 was further analyzed by methods listed in **Table 6**. Overall the material shows high purity with low level of aggregates or degradation products as detected by several separation methods such as size exclusion chromatography (SEC), CE-SDS and capillary zone electrophoresis (CZE).

Table 6. Purity analytics of purified bbmAb1

Method	Parameter	Value
Purity by SEC	Aggregation Products	1.3 %
	Degradation Products	1.2 %
CZE	Acidic Species	24.7 %
	Basic Species	16.6 %
Purity by CE-SDS	Reducing	98.2 %
	Non-reducing	94.6 %

Additional combinations of other antibodies have been tested as well. **Table 4**
5 shows a summary of analytical results obtained.

6. Example 2: In vitro activity of bbmAb1

Binding activity of bbmAb1 was tested in a variety of different cell assays.

(1) Materials and methods

10 (a) For solution equilibrium titration (SET) assays

The following material was used:

Recombinant human IL-18, biotinylated (BTP25828)

Recombinant Cynomolgus monkey IL-1 β (Novartis)

Anti-human IgG antibody, SULFO-TAG labeled (Meso Scale discovery (MSD) # R32AJ-

15 5)

Goat anti-human Fab specific, conjugated with MSD SULFO-TAG NHS Ester (Jackson

Immuno Research # 109-005-097, MSD #R91AN-1) BSA (Sigma # A-9647)

MSD read buffer T with surfactant (MSD #R92TC-1)

Phosphate-buffered saline (PBS) 10x (Teknova #P0195) Tris-buffered saline, pH 7.5

20 (TBS) 10x (Teknova #T1680) Tween-20 (Fluka #93773)

Polypropylene microtiter plate (MTP) (Greiner #781280)

384-well plates, standard (MSD #L21XA)

(b) For cellular assays and SET assays

mAb2 as described in section IL-1 β antibody.

25 mAb1 as described in section IL-18 antibody.

bbmAb1 as described in Example 1.

- Recombinant human IL-18 (BTP 25829) purchased from MBL Int. Corp. (#B001-5)
 Recombinant marmoset IL-1 β (Novartis)
 Recombinant marmoset IL-18 (Novartis)
 Recombinant human IL-12 (#573008) was purchased from Biolegend KG-1 cell line
 5 (ATCC #CCL-246)
 Normal human dermal fibroblasts (#CC-2509) were purchased from Lonza
 Marmoset skin fibroblasts (#42637F (510))
 HEK-Blue™ IL-18/IL-1 β cells (#hkb-il18) were purchased from InvivoGen
 PBMC were isolated from buffy coats were obtained from the Blutspendezentrum Bern
 10 Marmoset blood was obtained from SILABE, Niederhausbergen
 IL-6 ELISA: Human (BioLegend, #430503); Marmoset (U-CyTech biosciences, CT974-5)
 IFN γ ELISA: Human (BD555142) and marmoset (U-CyTech biosciences #CT340A)
 QUANTI-Blue™ assays (#rep-qb1) for the detection of SEAP was purchased from
 InvivoGen
 15 Cell medium: RPMI 1640 (Invitrogen #31870) supplemented with 10% Foetal
 Bovine Serum (Invitrogen #10108-157), 1% L-Glutamine (Invitrogen #25030-03), 1%
 penicillin/ streptomycin (Invitrogen #15140-148), 10 μ M 2-Mercaptoethanol (Gibco
 #31350-010), 5mM Hepes (Gibco #15630-080)
 Round-bottomed, tissue-culture treated 96-well plates (Costar #3799)
 20 Flat-bottomed, tissue-culture treated 96-well plates (Costar #3596)
 Ficoll-Paque™ Plus (GE Healthcare Life Sciences #17-1440-02) PBS 1X, without
 Calcium & Magnesium (Gibco #14190094)
 Leucosep tubes with porous barrier, 50ml, polypropylene (Greiner bio-one #227290)
 Falcon 15ml polypropylene conical tubes (BD#352096)
 25 Falcon 50ml polypropylene conical tubes (BD #352070)

(c) *Affinity measurements by SET*

SET individual target binding assay

- 22 serial 1.6n dilutions of the antigens (highest conc.: huIL-18, 5 nM; marIL-18,
 10 nM; huIL-1 β , 0.5 nM; marIL-1 β , 0.5 nM) were prepared in sample buffer (PBS
 30 containing 0.5 % Bovine serum albumin (BSA) and 0.02 % Tween-20) and a constant
 concentration of antibody was added (for IL-18 readout 10 pM, for IL-1 β readout 1 pM). A
 volume of 60 μ l/well of each antigen-antibody mix was distributed in duplicates to a 384-
 well polypropylene microtiter plate (MTP). Sample buffer served as negative control and
 a sample containing only antibody as positive control (Maximal
 35 electrochemiluminescence signal without antigen, B_{max}). The plate was sealed and
 incubated overnight (o/n, at least 16 h) at room temperature (RT) on a shaker.

IL-18 readout: A streptavidin coated 384-well MSD array MTP was coated with 30 µl/well biotinylated hull-18 (0.1 µg/ml, PBS) and incubated for 1h at RT on a shaker.

IL-1β readout: A standard 384-well MSD array MTP was coated with 30 µl/well of hull-1 (3 µg/ml, PBS) diluted in PBS as capture agent and incubated overnight at 4°C.

- 5 The plate was blocked with 50 µl/well blocking buffer (PBS containing 5 % BSA) for 1 hour (h), at room temperature (RT). After washing (TBST, TBS containing 0.05 % Tween 20), a volume of 30 µl/well of the equilibrated antigen- antibody mix was transferred from the polypropylene MTP to the coated MSD plate and incubated for 20 min at RT. After an additional wash step, 30 µl sulfo tag-labeled anti-IgG detection
10 antibody (0.5 µg/ml) diluted in sample buffer were added to each well and incubated for 30 min at RT on a shaker. The MSD plate was washed and 35µl/well MSD read buffer were added and incubated for 5 min at RT. Electrochemiluminescence (ECL) signals were generated and measured by the MSD Sector Imager 6000.

15 SET simultaneous target binding assays

- The SET assay was performed as described above, except for Assay A: The equilibration process (antibody/antigen mix) was performed in presence of an excess of one target (500 pM of either IL18 or IL-1β) while assessing the K_D of the other target. Assay B: The equilibration process (antibody/antigen mix) was performed with both
20 targets in serial dilutions in one mix simultaneously (constant concentration of antibody 10 pM, highest antigen conc. see above). The same mix was then analyzed for its free antibody concentration on IL18 and IL-1β coated plates as described above.

- The SET Data were exported to Xlfit, an MS Excel add-in software. Average ECL- signals were calculated from duplicate measurements within each assay. Data were
25 baseline adjusted by subtracting the lowest value from all data points and plotted against the corresponding antigen concentration to generate titration curves. K_D values were determined by fitting the plot with the following:

1:2 binding model for the monospecific Ab

$$y = \frac{2B_{\max}}{[IgG]} \left[\frac{[IgG]}{2} - \frac{\left(\frac{x + [IgG] + K_D}{2} - \sqrt{\left(\frac{x + [IgG] + K_D}{4} \right)^2 - x[IgG]} \right)}{2[IgG]} \right]$$

30

1:1 binding model for the knob in hole bispecific Ab

$$y = B_{\max} - \left(\frac{B_{\max}}{2[Fab]} \left(([Fab] + x + K_D - \sqrt{([Fab] + x + K_D)^2 - 4x[Fab]}) \right) \right)$$

wherein

y: blank subtracted ECL signal

$B_{\max}$: maximal ECL signal at zero antigen concentration

[IgG]: applied antibody concentration

[Fab]: applied total Fab concentration

K_D : Dissociation equilibrium constant

5 x: applied antigen concentration

(d) Cell culture

KG-1 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 1% L-Glutamine and 1% penicillin/streptomycin at a density of 2×10^5 to 1×10^6 viable cells/mL.

10 Normal human fibroblasts and marmoset fibroblasts were grown in FBM (Clonetics, CC-3131) including bFGF (1 ng/ml, CC-4065), insulin (5 μ g/ml, CC-4021), and 2% FCS (CC-4101). As starving medium, Fibroblast Basal Medium (LONZA # CC-3131) was used.

HEK-Blue™ IL-18/IL-1 β cells were grown in Growth Medium (DMEM, 4.5 g/l glucose, 15 10% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 mg/ml streptomycin, 100 mg/ml Normocin™, 2 mM L-glutamine supplemented with 30 μ g/ml of Blastidin, 200 μ g/ml of HygroGold™ and 100 μ g/ml of Zeocin™.

Human peripheral blood mononuclear cells (PBMC) were freshly isolated from buffy coats using LeucoSep tubes according to the instructions of the manufacturer. In 20 brief, 13 ml of Ficoll-Paque was preloaded in a 14 ml LeucoSep tube by centrifugation for 30 s at $1,000 \times g$. The heparinized whole- blood samples were diluted with equal volumes of PBS, and 25 ml of the diluted blood was added to a LeucoSep tube. The cell separation tubes were centrifuged for 15 min at $800 \times g$ without break at room temperature. The cell suspension layer was collected, and the cells were washed twice 25 in PBS (for 10 min at 640 and $470 \times g$, respectively, for the two successive washes) and re-suspended in culture medium before counting.

Marmoset blood was collected in heparinized tubes and filtered using a 70 μ m cell strainer (BD Biosciences #352350)

(e) IL-1 β neutralization assays

30 The IL-1 β induced IL-6 production assay in fibroblasts was conducted essentially as described (Gram 2000) with only minor modifications. Briefly, fibroblasts were seeded at a density of 5×10^3 cells per well (in 100 μ l) in a 96 well flat bottom tissue culture plate. The following day, cells were starved for 5h in starving medium before addition of the recombinant IL-1 β /compound solution mix (IL-1 β concentration indicated in the table).

35 The IL-1 β /compound solution mix was prepared beforehand by incubating recombinant

IL-1 β with a concentration range of compound for 30min at 37°C. The cell supernatants were collected after o/n incubation at 37°C and the amount of released IL-6 determined by ELISA. The IL-1 β induced IL-6 production assay in PBMC was performed according to the following. PBMC were seeded at 3x10⁵ cells per well (in 100 μ l) in a 96 well tissue culture plate and incubated with a recombinant IL-1 β /compound solution mix for 24h at 37°C (IL-1 β concentration indicated in the table). The IL-1 β /compound solution mix was prepared beforehand by incubating recombinant IL-1 β with a concentration range of compound for 30min at 37°C. The cell supernatants were collected after 24h of stimulation and the amount of released IL-6 determined by ELISA.

10 (f) *IL-18 neutralization assays*

The assay was conducted essentially according to the following. KG-1 cells (starved for 1h in PBS + 1% FCS beforehand) or PBMC at a density of 3 x10⁵ per well were seeded into round bottom 96-well cell culture plates and incubated with a solution mix of recombinant IL-18/IL-12 together with a concentration range of compounds (IL-18/IL-12 concentrations indicated in the table). After an incubation of 24h at 37°C, supernatants were collected and the amount of released IFN γ determined by ELISA. For the assays with marmoset blood 85 μ l of blood per well were used.

(g) *Dual IL1 β /IL-18 neutralization in HEK-Blue™ cells*

The assay was conducted essentially as described in the manufacturer's handling procedures. Briefly, the HEK-Blue™ cells were seeded at a density of 4 x10⁴ per well into 96-well cell culture plates and incubated with a solution mix of recombinant IL-1 β and IL-18 (to produce a 1:1 SEAP signal) together with a concentration range of compounds. After an incubation of 24h at 37°C, supernatants were collected and the amount of released SEAP determined by using the QUANTI-Blue™ method according to the manufacturer's instructions.

All Data were exported to EXCEL software and IC50 values calculated by plotting dose-response curves for the logistic curve fitting functions using either EXCEL/XLfit4 or GraphPad Prism software.

(2) Results

30 (a) *Affinities to recombinant human and marmoset IL1 β and IL-18*

Binding affinities of bbmAb1 to human and marmoset recombinant IL-1 β and IL-18 proteins were measured by solution equilibrium titration (SET) titration (Figure 5) and the K_D values generated were compared to those of mAb2 for IL-1 β and mAb1 for IL-18

binding. Figure 5 shows titration curves of ECL-based affinity determination in solution, constant concentration of antibody: for IL-18 readout 10 pM, for IL-1 β readout 1 pM; antigen dilutions: highest conc.: huIL-18, 5 nM; marIL-18, 10 nM; huIL-1 β , 0.5 nM; marIL-1 β , 0.5 nM. Solid lines represent a fit of the data using the model described above.

5 Dotted lines indicate the 95 % confidence interval, n=3.

Comparing binding affinities in the individual target binding assay, bbmAb1 showed a similar mean K_D compared to mAb1 for human and marmoset IL-18 (**Table 7**). For human IL-1 β binding the mean K_D value was slightly higher for bbmAb1 (2.6 pM) compared to mAb2 (0.6 pM) but still in the same low pM range. Subsequent

10 measurements in the simultaneous dual target binding assay (**Table 8**) confirmed that bbmAb1 binding K_D values for IL-1 β were similar to values of mAb2 with the pre-clinical as well as with the clinical grade material. Thus, bbmAb1 possesses binding affinities for both targets in humans and marmosets that are in similar to mAb2 and mAb1, respectively.

15

Table 7. Affinities to recombinant human (hu) and marmoset (mar) IL-1 β and IL-18 measured by SET (individual target binding determination)

	Independent IL-18/IL-1 β affinity determination			
Samples	huIL-18 K_D [pM]	marIL-18 K_D [pM]	huIL-1 β K_D [pM]	marIL-1 β K_D [pM]
mAb1	9 $\pm$ 2	21 $\pm$ 3	n/a	n/a
mAb2	n/a	n/a	0.6 $\pm$ 0.1	1.0 $\pm$ 0.7
bbmAb1	12 $\pm$ 4	33 $\pm$ 7	2.6 $\pm$ 0.1	3.0 $\pm$ 2.4

In addition to the individual target binding results, simultaneous dual target

20 binding affinities of bbmAb1 were investigated (**Table 8**) by applying either excess of one target during the assessment of the binding the K_D values of the other target (Assay A) or by applying a mixture of both targets in serial dilutions (Assay B). Simultaneous IL-1 β /IL-18 affinity determination showed no significant difference between Assay A (excess of one antigen) and Assay B (mixture of both antigens in serial dilutions) which proved

25 that both targets are bound simultaneously without affecting the binding of the other target. Furthermore, the K_D values obtained with the simultaneous dual binding assays were similar to the K_D values obtained with the standard assay (**Table 7**; in the absence of the second antigen) which proved that bbmAb1 can bind both antigens independently. Thus, bbmAb1 binds simultaneously and independently both human IL-1 β and IL-18 and

30 fully cross-reacts with the corresponding marmoset proteins.

Table 8. Affinities to recombinant human (hu) and marmoset (mar) IL-1 β and IL-18 measured by SET (simultaneous target binding determination)

Simultaneous IL-18/IL-1 β affinity determination								
	huIL-18 K _D [pM]		marIL-18 K _D [pM]		huIL-1 β K _D [pM]		marIL-1 β K _D [pM]	
Samples	Assay A	Assay B	Assay A	Assay B	Assay A	Assay B	Assay A	Assay B
mAb1	13.5	11.4	27.1	26.3	n/a	No binding	n/a	No binding
mAb2	n/a	No binding	n/a	No binding	1.1	3.2	0.8	4.8
bbmAb1	14.8	19.5	47.9	44.2	3	0.5	2	0.6

(b) *Neutralizing activity of bbmAb1 in human and marmoset cell assays*

The neutralizing activity of bbmAb1 for both cytokines (IL1 β and IL-18) was assessed (mAb2/mAb1). In addition, the potency of bbmAb1 for the neutralization of marmoset IL-1 β and IL-18 using marmoset cell assay systems was assessed (see section d).

(c) *Individual and simultaneous IL-1 β and IL-18 neutralization in human cells*

The neutralizing activity of bbmAb1 on IL-1 β was assessed by the inhibition of recombinant IL-1 β -induced IL-6 production in human dermal fibroblasts (IL-1 β used at 6pM) and in human PBMC (IL-1 β used at 60pM). The neutralizing activity of bbmAb1 on IL-18 was measured by the inhibition of recombinant IL-18-induced IFN- γ production in KG-1 cells and human PBMC (both cells activated with 3nM recombinant human IL-18 together with 1ng/ml of recombinant human IL-12). The inhibitory potency of bbmAb1 on IL-1 β and IL-18 was always compared to that of either mAb2 or mAb1, respectively. Depending on the assays, the mean IC₅₀ values of bbmAb1 were in sub-nM or single digit nM ranges and up to 2-to 4-fold higher in direct comparison mAb2 (for IL-1 β) and mAb1 (for IL-18), respectively (**Table 9** and **Table 10**). The monovalent format of bbmAb1 as compared to the bivalent format of mAb2/mAb1 but also potentially the KiH mutations may be reasons for this slight difference in potency of bbmAb1.

Table 9. Mean IC₅₀ values for IL-1 β neutralization by bbmAb1 in comparison to mAb2 in human dermal fibroblasts and human PBMC. *Inhibition of IL-6 production in human dermal fibroblasts or PBMC stimulated with recombinant human IL-1 β (6pM for dermal fibroblasts and 60pM for PBMC). Shown are mean values $\pm$ SEM (n=3 PBMC and n=6 human dermal fibroblasts)

IL-1 β inhibition	IL-6 prod.* derm. fibrobl. IC ₅₀ [nM]	IL-6 prod.* PBMC IC ₅₀ [nM]
mAb2	0.031 $\pm$ 0.006	0.29 $\pm$ 0.67
bbmAb1	0.136 $\pm$ 0.045	1.35 $\pm$ 0.59

Table 10. Mean IC₅₀ values for IL-18 neutralization by bbmAb1 in comparison to mAb1 in KG-1 cells and human PBMC. **Inhibition of IFN γ production in KG-1 cells or PBMC stimulated with recombinant human IL-18 (3nM) and human IL-12 (1ng/ml). Shown are mean values $\pm$ SEM (n=3 KG-1 and n=4 PBMC)

IL-18 inhibition	IFN γ prod.** KG-1 cells IC ₅₀ [nM]	IFN γ prod.** PBMC IC ₅₀ [nM]
mAb1	0.035 $\pm$ 0.011	0.78 $\pm$ 0.49
bbmAb1	0.071 $\pm$ 0.046	0.87 $\pm$ 0.51

bbmAb1 was able to neutralize simultaneously the bioactivity of both IL-1 β and IL-18 as demonstrated with the HEK Blue™ reporter cells producing SEAP in response to a 1+1 stimulation with recombinant IL-1 β and IL-18 (**Table 11**). A similar inhibition of SEAP in this assay system was only achievable by the combination of mAb2 and mAb1 but not by the use of the individual antibodies.

Table 11. Mean IC₅₀ values for simultaneous neutralization of IL-1 β and IL-18 on SEAP reporter activity in HEK Blue™ cells. Shown are means $\pm$ SEM of n=5 experiments.

Inhibition of SEAP in HEK reporter cells stimulated simultaneously with IL-1 β and IL-18	IC ₅₀ [nM]
mAb2 or mAb1 alone	>30
mAb2 and mAb1 combined	0.24 $\pm$ 0.09
bbmAb1	0.71 $\pm$ 0.28

(d) Neutralizing activity of bbmAb1 on marmoset IL-1 β and marmoset IL-18 in marmoset cell assays

In order to demonstrate the inhibitory activity of bbmAb1 in marmoset, similar in vitro assays were performed with marmoset cells as with human cells however using recombinant marmoset IL-1 β and IL-18 for stimulation. When assessing the inhibition of recombinant marmoset IL-1 β -induced IL-6 production in marmoset dermal fibroblasts, bbmAb1 displayed sub-nM potency with 2-to 3-fold higher IC₅₀ values compared to mAb2 (**Table 12**). Testing bbmAb1 with human dermal fibroblasts stimulated with marmoset IL-1 β generated a similar inhibition profile as with human IL-6.

Table 12. Inhibition of recombinant marmoset IL-1 β induced IL-6 production in marmoset and human fibroblasts by bbmAb1. * Inhibition of IL-6 production in marmoset or human dermal fibroblasts stimulated with recombinant marmoset IL-1 β (18pM). Results of 3 individual experiments (A, B and C) are shown.

Marmoset IL-1 β	IL-6 prod.* marmoset dermal fibroblasts IC ₅₀ [nM]		IL-6 prod.* human derm. fibroblasts IC ₅₀ [nM]
	Exp. A	Exp. B	Exp. C
bbmAb1	0.174	0.364	0.220
mAb2	0.095	0.138	0.114

Single to double digit nM IC₅₀ values of bbmAb1 confirmed the neutralizing activity of bbmAb1 for marmoset IL-18 tested in the IFN γ production assay with marmoset blood cells (**Table 3 -7**). Testing bbmAb1 with human PBMC stimulated with marmoset IL-18 generated a similar inhibition profile when measuring the production of human IFN γ .

Thus, bbmAb1 was shown to be fully cross -reactive to marmoset IL-1 β and marmoset IL-18 in functional assays using marmoset responder cells.

Table 13. Mean IC₅₀ values for inhibition of recombinant marmoset IL-18 induced IFN γ production in marmoset whole blood or human PBMC. ** Inhibition of IFN γ production in marmoset whole blood (n=3 each compound/condition) or human PBMC (n=6) stimulated with recombinant marmoset IL-18 (concentration indicated) & human IL-12 (10ng/ml). Shown are mean values $\pm$ SEM

Marmoset IL-18	IFN γ prod.** Marmoset blood IC ₅₀ [nM]	IFN γ prod.** Human PBMC IC ₅₀ [nM]	Marmoset IL-18 conc. used
bbmAb1	10.0 $\pm$ 4.1		1 nM

mAb1	4.7±2.6		0.3 nM
mAb1	181±108		3 nM
mAb1		6.6±5.0	1 nM

It was demonstrated that bbmAb1, a KiH format IL-1 β /IL-18 bi-specific mAb retains the high affinity binding as well as the cytokine neutralizing potency to the two individual targets IL-1 β and IL-18 when compared to the original mAbs, mAb2 and mAb1, in a variety of different cell assays. The dual IL-1 β and IL-18 neutralizing properties of bbmAb1 were not only demonstrated for the human cytokines/cells but also for the corresponding marmoset cytokines/cells, facilitating appropriate toxicology studies. The up to 2-to 4-fold higher IC₅₀ values that were generated in some of the cellular assays for IL-1 β and IL-18 neutralization may be the consequence of the monovalent binding of bbmAb1 as opposed to bi-valent binding of mAb2 and mAb1, respectively. Nevertheless, the dual cytokine neutralization by bbmAb1 may result in additive or synergistic inhibitory activities *in vivo* that may not be adequately represented in our *in vitro* cellular systems.

7. Example 3: Effects of combined IL-1 β and IL-18 stimulation and blockade in PBMC

Inflammasome activation-dependent cleavage of the effector cytokines IL-1 β and IL-18 leads to the induction of secondary pro-inflammatory mediators and promotes immune cell recruitment/activation not only systemically but also at the site of inflammation. In two different mouse models for lethal systemic inflammation (a) LPS injection model and (b) FCAS mice (activating missense mutations in NLRP3), the simultaneous absence/inhibition of both IL-1 β and IL-18 was more protective from lethality compared to the single IL-1 β or single IL-18 absence/inhibition, demonstrating additive or synergistic mechanisms for immune activation (Brydges 2013, van den Berghe 2014). bbmAb1 is a human/marmoset IL-1 β /IL-18 reactive bi-specific mAb with no rodent cross-reactivity and thus cannot be tested in mouse models. Therefore, we used LPS/IL-12 to mimic inflammasome-dependent pathway activation *in vitro* for the stimulation of human PBMC to reveal additive or synergistic inhibitory effects of combined IL-1 β /IL-18 neutralization by bbmAb1 and performed a non-biased gene expression analysis using microarrays. As a complementary activity we also compared the gene expression profiles of PBMCs from different donors stimulated with either the combination of recombinant IL-1 β and recombinant IL-18 or the single cytokines alone.

(1) Materials and methods

(a) Cell culture and ELISA

- RPML 1640 (Invitrogen #31870 or Gibco #61870-010) supplemented with 10% Foetal Bovine Serum (Invitrogen #10108-157), 1% L-Glutamine (Invitrogen #25030-03), 1% penicillin/ streptomycin (Invitrogen #15140-148), 10µM 2-Mercaptoethanol (Gibco
- 5 #31350-010), 5mM Hepes (Gibco #15630-080)
- Recombinant Human IL-1β was purchased from Sino Biological Inc. (#10139-HNAE-5)
- Recombinant human IL-18 was purchased from MBL (#B001-5)
- Recombinant human IL-12 was purchased from Biolegend (#573008)
- IFNγ ELISA: MAX Standard Set, BioLegend, #430103 or BD OptEIA human IFNγ ELISA
- 10 Set, BD #555142
- IL-6 ELISA: MAX Standard Set, BioLegend, #430503
- IL-26 ELISA: Cloud Clone Corp #SEB695Hu
- mAb2 as described in section IL-1β antibody.
- mAb1 as described in section IL-18 antibody.
- 15 bbmAb1 as described in Example 1.
- LPS from Salmonella enterica serotype enteritidis, Sigma #L7770
- PBMC were isolated from buffy coats that were obtained from the Blutspendezentrum Bern
- Round-bottomed, tissue-culture treated 96-well plates (Costar #3799) Flat-bottomed,
- 20 tissue-culture treated 96-well plates (Costar #3596) Ficoll-Pacque TMPlus (GE Healthcare Life Sciences #17-1440-02) PBS 1X, without Calcium & Magnesium (Gibco #14190094)
- Falcon 15ml polypropylene conical tubes (BD#352096) Falcon 50ml polypropylene conical tubes (BD #352070)
- LeucosepTM tubes with porous barrier, 50ml, Greiner bio-one #227290
- 25 Cell strainer 70µM, BD Biosciences #352350
- Trypanblue, Sigma # T8154
- RNA isolation, quantity and quality measurements and qPCR:
- Nuclease-free water, Ambion #AM9938
- Rnase Zap, Ambion #AM9780
- 30 1.5ml Eppendorf tubes, sterile, Rnase & Dnase free
- RLT buffer, Qiagen #1015762
- Rneasy Mini Kit, Qiagen #74104
- RNase-Free DNase Set, Qiagen #79254
- Agilent RNA 6000 Nano Kit, Agilent #5067-1511
- 35 Chip priming station, Agilent #5065- 4401
- IKA vortex mixer
- RNaseZAP®, Ambion #9780
- Agilent 2100 Bioanalyzer

High Capacity cDNA reverse transcription kit, Applied Biosystems, # PN4374966

Nase-free, Thin-Walled, forsted Lid 0.2ml PCR tubes, Ambion #AM12225

MicroAmp Optical 384 well reaction plate, Applied Biosystems #4309849

TaqMan GenEx Master Mix, Applied Biosystems #4369514

5 PCR primer (Applied Biosystems)

Target	Assay ID Taqman	color/quencher
IFN γ	Hs00989291_m1	FAM-MGB
IL-26	Hs00218189_m1	FAM-MGB
RPL27	Hs03044961_g1	FAM-MGB
HPRT1	Hs02800695_m1	FAM-MGB

PBMC preparation: PBMCs were isolated from buffy coat by means of Ficoll - Paque gradient centrifugations in Leucosep tubes according to the manufacturer's instructions. Briefly, 15mL of Histopaque was put in 50mL LeucosepTM tubes and centrifuged for 30 sec at 1300rpm at RT. With a pipette, 30mL of a diluted suspension of the buffy coat was added on the top of the Histopaque solution and centrifuged during 15min at RT at 1000g without break. Plasma was discarded (approx. 20ml) and the interface ring collected (=human PBMC) and transferred in a 50 ml falcon tube. The tube was filled with 50mL of sterile PBS and centrifuged once at 1200rpm during 5min at RT. This centrifugation was repeated 2 times. The supernatant was gently discarded and cells re-suspended in 50mL of PBS with 2% FCS and 2mM EDTA. The cell suspension was filtered using a 70 μ m cell strainer and cells counted using trypan blue staining (500 μ L of trypan blue + 200 μ L of cells + 300 μ L of PBS).

20 LPS/IL-12 stimulation of PBMC: Cytokine production in supernatants was prepared according to the following. 250'000 cells/well in 100ul final volume were distributed in 96-well round bottom plates. LPS was used at concentrations between 0.3ug/ml and 3000ug/ml together with recombinant IL-12 at 10ng/ml. Supernatants were harvested after 24h at 37°C and 10% CO₂.

25 RNA extraction from cell pellets was performed according to the following. 3x10⁶ cells/well in 1000ul final volume were distributed in flat bottom 24-well plates. LPS was used at 3ug/ml together with recombinant IL-12 at 10ng/ml. Cells were harvested after 24h at 37°C and 10% CO₂.

30 Stimulation of PBMC with recombinant cytokines: 7x10⁶ PBMC per well of a 12-well plate were used in 1.5ml final of complete RPMI medium. Recombinant cytokines were added at the following final concentrations: 10ng/ml of recombinant IL-1 β , 3nM of

recombinant IL-18, 1ng/ml of recombinant IL-12. Both, supernatants as well as cells were collected after 4h and 24h at 37°C and 10% CO₂.

RNA isolation, quantity and quality assessments: Cells were pelleted and the pellet lysed in 350µl of Qiagen RTL buffer with 2% β-mercaptoethanol and frozen at -20°C or -80°C until all samples of the study have been collected. The RNA isolation was performed using the Qiagen standard protocol. Briefly, 350µl of 70% Ethanol was added in all samples prior to the transfer to the RNeasy spin column and centrifuged for 15s at 8000g. After discarding the flow-through, 350µl of buffer RW1 was added and the column centrifuged for 15s at 8000g to wash the spin column membrane. DNase I incubation mix solution was prepared according to the manufacturer's instructions and added to the RNeasy spin column and incubated for 15min at RT. After washes with 350µl and 500µl of buffer RW1, the RNeasy spin column was placed in new 2 ml collection tube and centrifuged at full speed for 1min. RNA was finally collected by adding 35µl RNase-free water directly to the spin column membrane and a centrifugation for 1min at 8000g to elute the RNA. The amount of RNA was measured using Nanodrop ND-1000 and the RNA was stored at -20°C. RIN measurements were performed for the RNA quality assessment according to manufacturer's instructions. Briefly 1µl of RNA or ladder were pipetted into an Agilent RNA 6000 Nano chip and measured by using the Agilent 2100 Bioanalyser.

20

Cytokine gene expression analysis by qPCR:

The method was performed corresponding to the manufacturer's instructions. Briefly, 400ng of RNA was reverse transcribed according to the instructions using the High-Capacity cDNA Reverse Transcription Kit. The cDNA solutions were diluted 1/10 in RNA/DNA free water and 1µl cDNA was transferred into a 384-well reaction plate and then mixed with 1µl of 20X TaqMan® Gene Expression Assay target FAM gene and 10µl of 2x TaqMan® Gene Expression Master Mix and 10µl RNA/DNA free water. The plate was loaded onto the Applied Biosystems ViiA™ 7 Real-Time PCR System and the following instrument settings were used:

25

Plate document/experiment parameters	Thermal cycling conditions		
	Stage	Temp (°C)	Time (mm:ss)
Rxn. Volume: 20µL Ramp rate: Fast	Hold	50	2:00
	Hold	95	0:20
	Cycle (40 cycles)	95	0:01
		60	0:20

30

The house keeping genes used for this study were HPRT1 and RLP27. The following formula was used to calculate the relative expression levels of target genes:

- 1) $Ct [Ref] = (Ct [HPRT1] + Ct [RLP27]) / 2$
- 2) $dCt [Ref] = 40 - Ct [Ref]$
- 5 3) $dCt [Target] = Ct [Target] - Ct [Ref]$
- 4) $ddCt = dCt [Ref] - dCt [Target]$
- 5) Relative target gene expression = 2^{ddCt}

Microarrays was performed according to the following. Samples were processed
10 by CiToxLAB France on Affymetrix HG_U133_Plus2 microarrays. They were RMA normalized and analyzed in GeneSpring 11.5.1 (Agilent Technologies, Santa Clara, CA). Pathway analysis was done using Ingenuity Pathway Analysis (IPA) and Nextbio (Illumina). The two datasets were treated independently.

Initially, the data were subject to standard quality control (QC) by CiToxLAB, in-
15 house QC by using an R script (MA_AffyQC.R) in Rstudio suite and in GeneSpring (PCA, hybridization controls). Subsequently, it was filtered to eliminate unreliable expression levels: Entities (probesets) were kept where at least 100 percent of samples in any 1 of the experimental conditions have values above the 20th percentile.

Differentially expressed genes (DEG) were identified using the “filter on volcano
20 plot” feature in GeneSpring. Using the filtered genes (expression between 20.0 - 100.0th percentiles) with an unpaired T-test, probesets with a corrected p-value below 0.05 and a fold change above 2.0 were considered differentially expressed. Where possible, i.e. in the study with LPS (NUID-0000-0202-4150) a Benjamini-Hochberg Multiple Testing Correction was used.

25 For cytokine stimulation experiments, synergism was calculated using the following formula: $Signal A+B / (Signal A + Signal B - Control) \geq 1.5$

The respective signatures (or DEG lists) were used to calculate p-values with a Fisher's exact test which represent the statistical significance of observing an overlap
30 between the signature and the 'disease gene list' (lesional vs non-lesional) of public datasets. To do so, the lists were uploaded into Illumina Base Space Correlation Engine (former Nextbio) and compared using the Meta-Analysis feature and keyword search for diseases.

All Data were exported to EXCEL software and IC₅₀ values calculated by plotting
35 dose- response curves for the logistic curve fitting functions using either EXCEL/XLfit4 or GraphPad Prism software. Differences between treatment groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison using GraphPad Prism software and results were considered statistically significant at $p < 0.05$.

5 (2) Results

(a) *bbmAb1 is highly efficacious in inhibiting LPS/IL-12 induced IFN γ production in whole blood*

Exposure of human whole blood to LP S supplemented with 10ng/ml IL-12 results in an IFN γ response that is largely but not exclusively dependent on the “native” IL -18
10 produced by the blood cells. The addition of IL-12 enhances the LPS induced IFN γ responses, likely by up-regulating IL-18 receptors on responder cells.

In the experimental conditions used, IL-18 neutralization with mAb1 lead only to an incomplete inhibition of IFN γ production whereas IL-1 β blockade (using mAb2) had only small effects on the IFN γ response. Interestingly, the combined inhibition of IL-1 β
15 and IL-18 either by bbmAb1 or the combination of mAb2 and mAb1 was more profoundly and completely inhibiting IFN γ production compared to the single cytokine neutralization (Figure 6). Figure 6 shows inhibition of LPS (0.3 μ g/ml) / IL-12 induced IFN γ in whole blood by bbmAb1, mAb2, mAb1 or combined mAb2 & mAb1 (Combo) (typical inhibition curve shown in Figure 6A). Percent IFN γ inhibition of n=4 individual donors in whole
20 blood by bbmAb1, mAb1 or mAb2 used at 100nM (mean and SEM shown in Figure 6B).

Apart from IFN γ , none of the other cytokines tested (IL-2,-4,-6,-8,-10,-13 and TNF α) were additively inhibited by the combined neutralization of IL-1 β and IL-18 in our cell assay (data not shown). The potency of bbmAb1 was in the same range as the combination (combo) of mAb2 and mAb1, considering the monovalent format of the
25 bispecific molecule.

(b) *IFN γ is additively inhibited by bbmAb1 (i.e. combined IL-1 β /IL-18 inhibition) compared to single IL-1 β or IL-18 inhibition in LPS/IL-12 activated human PBMC*

An unbiased transcriptomics evaluation was required in order to reveal further
30 additive effects (apart of IFN γ) by combined IL-1 β /IL-18 inhibition using bbmAb1. Since whole blood is not optimal for transcriptomics analysis we adapted the LPS/IL-12 stimulation assay conditions, described in the materials and method section above, to human PBMC samples. By using PBMCs from a total of 9 donors, we could confirm that bbmAb1 additively inhibited IFN γ protein secretion into the supernatants of the PBMCs
35 (Figure 7). Compared to whole blood experiments, IFN γ production was inhibited at

approximately 10-fold lower concentrations of the respective mAbs used. Importantly, a similar inhibition pattern was demonstrated at the mRNA level for IFN γ (Figure 7) which confirmed the suitability of the samples for a non -biased microarray based gene expression analysis. Figure 7 shows the inhibition of LPS (0.3 μ g/ml) /IL-12 induced IFN γ protein production (Figure 7A) and IFN γ gene expression (Figure 7B) by bbmAb1, mAb2 and mAb1 (at 10nM conc. each) in human PBMC. Shown is the percent inhibition in n=9 donors $\pm$ SEM. ***p < 0.05 (one way ANOVA)

The Affymetrix microarray was conducted with n=5 individual donors from PBMCs that were sampled from the LPS/IL-12 stimulation experiments described in the materials and method section above. Unfortunately, the overall assessment of the gene expression profiles evidenced a strong LPS/IL-12 stimulation effect and the PCA showed clustering per donor rather than compound within the stimulated or unstimulated groups. Nevertheless, comparing the LPS/IL-12 stimulated samples with the stimulated plus bbmAb1 for differentially expressed genes revealed a shortlist of genes that are downregulated by the combined IL-1 β /IL-18 blockade with bbmAb1 (**Table 14**). Apart from the strong downregulation of the IFN γ gene that re-validated our microarray data, also the IL-26 gene was a further cytokine gene additively inhibited by bbmAb1 compared to the single IL-1 β inhibition (by mAb2) or IL-18 inhibition (by mAb1) (see Figure 8). Figure 8 and Figure 9 show microarray data derived gene expression levels for IFN γ and IL-26 and the inhibition by bbmAb1, mAb2 and mAb1 (10nM each) in LPS (0.3 μ g/ml) / IL-12 stimulated PBMC at 24h. Values of individual donors are shown in Figure 8A (IFN γ) and Figure 8B (IL-26) and percent inhibition (mean $\pm$ SEM) from the n=5 donors are shown in Figure 9A (IFN γ) and Figure 9B (IL-26).

Table 14. Differentially expressed genes (downregulated genes only between the bbmAb1 and control group in LPS/IL-12 stimulated samples). FC= fold change.

Probe Set ID	Gene Symbol	Entrez Gene	p-value	FC
222974_at	IL22	50616	0.03188	6.6
221111_at	IL26	55801	0.00224	5.2
223939_at	SUCNR1	56670	0.00234	4.0
1560791_at	OTTHUMG0000010886		0.03660	3.7
211122_s_at	CXCL11	6373	0.02954	3.5
203915_at	CXCL9	4283	0.02211	3.4
235229_at			0.02400	3.3
210163_at	CXCL11	6373	0.02707	3.2
210354_at	IFNG	3458	0.00007	2.9
243541_at	IL31RA	133396	0.01200	2.5
236003_x_at	OR2I1P		0.04942	2.4
203131_at	PDGFRA	5156	0.00161	2.4

219991_at	SLC2A9	56606	0.00191	2.4
201860_s_at	PLAT	5327	0.00139	2.3
205692_s_at	CD38	952	0.04855	2.3
1555600_s_at	APOL4	80832	0.02610	2.3
215305_at	PDGFRA	5156	0.01180	2.2
236191_at			0.04037	2.1
204533_at	CXCL10	3627	0.04847	2.1
229915_at	FAM26F	441168	0.02912	2.0
210072_at	CCL19	6363	0.02827	2.0
236101_at			0.03246	2.0

(c) *IL-26 is another pro-inflammatory cytokine additively inhibited by by bbmAb1 in LPS/IL-12 stimulated PBMC*

To further confirm that LPS/IL-12 driven IL-26 gene expression and protein
5 production is most efficiently inhibited by combined IL-1 β /IL-18 blockade using bbmAb1, the study was extended to a total of n=9 PBMC donors and investigated IL-26 gene expression by qPCR and IL-26 protein production by ELISA. As shown in Figure 10, it largely confirmed the inhibition of IL-26 gene expression obtained with the microarray approach (Figure 10A). Interestingly, IL-26 protein levels in supernatants were only partly
10 reduced at 24h by the addition of the mAbs (Figure 10B). The reasons for this differences are unknown, could however be related to kinetic differences between IL-26 gene expression and protein production as well as differences in the consumption of IL-26 compared to IFN γ . Nevertheless, bbmAb1 was superior in reducing IL-26 protein levels in the PBMC supernatants compared to mAb2 and mAb1. Figure 10 shows the
15 inhibition of LPS (0.3ug/ml) /IL-12 induced IL-26 gene expression (by qPCR) (Figure 10A) and IL-26 protein levels (Figure 10B) by bbmAb1, mAb2 and mAb1 (10nM each) in human PBMC. Percent inhibition of n=9 individual PBMC donors (mean and SEM). ***p < 0.05 (one way ANOVA).

(d) *IL1 β /IL18 signaling signatures correlate with disease*

20

Previously established PBMC culture conditions where recombinant IL-1 β stimulation resulted in either IL-6 production or recombinant IL-18/IL-12 stimulation resulted in IFN γ production was combined to reveal additive or synergistic downstream target genes or signatures (data not shown). With PBMCs from n=4 donors sampled at
25 two different time points (6h and 24h) an Affymetrix microarray evaluation for unbiased assessment of the gene expression profiles was conducted. Genes that were synergistically upregulated at 6h and at 24h with the combined stimulation by IL-1 β and

IL-18 were revealed (data not shown). The addition of IL-12 to the IL-1 β /IL-18 combination largely increased the synergy for a series of upregulated genes. The generated signalling signatures of single or combined IL-1 β /IL-18 pathway stimulation (UP-regulated genes only) were used to interrogate dataset from patients across several autoimmune diseases. For example, correlation to public sarcoidosis datasets is shown as an example in Figure 11. P-values (calculated with a Fisher's exact test) show a significant correlation to several public studies comparing healthy to diseased tissues from sarcoidosis patients. Tissues include skin as well as lung, lacrimal glands and anterior orbit. Across all datasets, the combination of IL-1 β /IL-18 signaling shows the best correlation to disease, followed by IL-1 β and IL-18. IL-1 β /IL-18 differentially up-regulated genes (DEG) in PBMC (x-axis) compared to 5 different sarcoidosis tissue 'diseased vs healthy' DEG. P-values (y-axis) represent the statistical significance of observing an overlap between the signature and the 'disease gene list'. Black bar is skin from cutaneous sarcoidosis lesion vs skin from healthy patients. Light grey bar is skin from cutaneous sarcoidosis lesion vs non-lesioned skin. White bar is lacrimal glands from sarcoidosis patients vs normal. Dark grey bar is anterior orbit tissues from sarcoidosis patients vs normal. Striped bar is lung samples with progressive fibrotic, pulmonary sarcoidosis vs nodular self-limiting pulmonary sarcoidosis.

(e) *Conclusion*

LPS and recombinant IL-12 was used to mimic pathogen associated molecular pattern (PAMP)-dependent NLRP3 inflammasome activation within the first 24h of in vitro culture. It was demonstrated that combined inhibition of IL-1 β and IL-18, by using bbmAb1, acts additively to decrease/inhibit IFN γ production in PBMC stimulated with LPS/IL-12. IL-12 was previously described to act synergistically with IL-18 to induce IFN γ production in T, B, NK cells, macrophages and dendritic cells (as reviewed by Nakanishi, 2001) but now an additional stimulatory effect of IL-1 β on IFN γ production could be demonstrated under the experimental conditions used. Thus, the co-incubation of PBMC with LPS/IL-12 drives efficiently the production of "native" IL-1 β and IL-18 which contribute both to a strong IFN γ response. By using unbiased microarray transcriptomics, additional genes were identified that were additively down-regulated by combined IL-1 β /IL-18 neutralization vs. single IL-1 β or IL-18 blockade. Amongst those was IL-26, a member of the IL-20 cytokine subfamily (IL-19, IL-20, IL-22, IL-24, and IL-26), which is conserved in most vertebrate species but absent in most rodent strains (including mice and rats) (Donnelly 2010). It signals through a heterodimeric receptor complex composed of the IL-20R1 and IL-10R2 chains. IL-26 receptors are primarily expressed on non-hematopoietic cell types, particularly epithelial cells. Increased levels of IL-26

were reported in serum and particularly in the synovial fluid of RA patients (Corvaisier 2012) where it could act as factor to promote Th17 cell growth and differentiation. Unfortunately, the discovery of further genes/pathways induced by the combined blockade of IL-1 β and IL-18 was hampered by the strong effect of the LPS/IL-12 stimulation of the PBMC samples. Nevertheless, both IFN γ and IL-26 and to some extend also IL-22 were also among the genes that were synergistically upregulated by the combined stimulation with recombinant IL-1 β and IL-18 in PBMC, confirming that these two factors are downstream effectors in this activation pathway. Thus, the IL-20 subfamily of cytokines (including IL-26 and IL-22) seems to be strongly dependent on the simultaneous signals from IL-1 β and IL-18. With all due caution about selectivity of the individual signalling signatures as well as potential efficacy of blocking, these comparisons are useful to show that the respective pathways are active in diseases like sarcoidosis.

8. Example 4: Therapeutic use

Combined targeting of IL-1 β and IL-18 may represent a more effective treatment strategy than single cytokine blockade in inflammasome-driven inflammatory conditions where both innate and adaptive immunity components are involved. Simultaneous neutralization of IL-1 β and IL-18 targets both innate and adaptive immunity components, including neutrophils, Th1/Tc1 and NK cells, adhesion molecules on immune and endothelial cells and pro-inflammatory cytokines (e.g. IL-6, IFN γ and IL-17). The advantage of blocking both IL-1 β and IL-18 is supported by data obtained in a pre-clinical mouse model of Familial Cold Autoimmune Syndrome (FCAS) which is driven by constitutive Nlrp3 inflammasome activation and overproduction of IL-1 β and IL-18 (Brydges 2013). There, a partial rescue from the FCAS condition was achieved in mice when either IL-1 β or IL-18 signalling was genetically ablated demonstrating involvement of both cytokines in disease pathogenesis. Importantly, FCAS mice lacking both IL-18 and IL-1 β signalling developed even less disease compared to mice in which only one of the two cytokines was inactivated demonstrating additive effects of dual IL-1 β /IL-18 neutralization. Additive effects of IL-1 β and IL-18 neutralization were also demonstrated in another mouse model, where high doses of LPS were injected in mice to cause septic shock (van den Berghe 2014). In this model either genetic deficiency for both IL-1 β and IL-18 or the combined neutralization of both cytokines by neutralizing antibodies completely prevented LPS lethality whereas single cytokine deficiency/neutralization was only partly protective.

The overall clinical strategy for a bispecific, which targets both IL-1 β and IL-18 simultaneously, may represent a more effective means of treatment than currently

available options. To identify candidate diseases, preclinical and translational research was utilized to demonstrate active involvement of both IL-1 β and IL-18 downstream pathways in the underlying pathophysiology of candidate diseases. There is novel evidence that chronic pulmonary sarcoidosis is an inflammasome-driven disease with both innate and adaptive immunity involvement. Furthermore, initial findings indicate important roles for both IL-1 β and IL-18 effector cytokines in this disease. Thus, sarcoidosis represents an ideal opportunity to demonstrate the dual specificities of bbmAb1 in a disease with established, chronic tissue inflammation. Efficacy of bbmAb1 in sarcoidosis may lead to development in other interstitial lung diseases such as hypersensitivity (occupational) lung diseases due to silica or beryllium. Other candidate diseases are granulomatous inflammation involving other organ tissues, such as Crohn's disease.

Vascular inflammation with tissue injury and endothelial dysfunction also represents a potential target for bbmAb1. Dysfunctional endothelial cells can respond to effective anti-inflammatory treatment, resulting in improved vascular flow even in the presence of fixed intravascular defects. Recent literature evidence has identified sickle cell disease (SCD) to have a strong inflammasome-driven component via high rates of constitutive intravascular hemolysis. Inflammasome activation due to release of danger signals (uric acid, heme/Fe³⁺, other intracellular components) from chronic RBC lysis triggers inflammasome receptors, activation of same and leads to intravascular inflammatory cascade, giving upregulation of endothelial cell adhesion molecules, activation of neutrophils and platelets, resulting in chronic activation of endothelial cells. This unremitting vascular inflammation in SCD patients leads to recurrent, painful vaso-occlusive crises and acute on chronic tissue damage. Preliminary internal evidence provides support for involvement of IL-18 as well as IL-1 β in the underlying disease process of SCD. Thus, reducing the basal inflammation in SCD patients by bbmAb1 treatment could attenuate chronic background inflammation and prevent acute crises with associated end-organ damage, prevent acute sickle cell crises and associated end organ damage, along with improving patients' general quality of life by reducing associated chronic pain and fatigue. Demonstration of bbmAb1 therapeutic efficacy in SCD patients may lead to development in other chronic or acute on chronic inflammatory conditions involving high rates of hemolysis, such as malaria and hemodialysis-dependent, chronic kidney disease. Further indications that might benefit from both IL-1 β and IL-18 modulation are indications associated with ischemia-reperfusion tissue injury, such as cardiovascular diseases or improved healing of wounds of all types, but in particular the most severe soft tissue injury of burn.

Thus, in an embodiment of the invention, a method of treating an inflammasome related disorder comprising administering to a subject, afflicted with an inflammasome related disorder, an effective amount of a bbmAb disclosed herein, such as bbmAb1. Potential inflammasome related disorders are cryopyrin-associated autoinflammatory syndrome (CAPS), familial Mediterranean fever (FMF), systemic juvenile idiopathic arthritis (SJIA), lupus nephritis, diabetic nephropathy, acute kidney injury, renal hypertension, IgA nephropathy, glomerulonephritis (GN), frontotemporal dementia (FTD), Alzheimer's disease (AD), epilepsy, stroke, Parkinson's disease (PD), depression, sarcoidosis, such as pulmonary sarcoidosis, pancreatitis, idiopathic pulmonary fibrosis (IPF), non-alcoholic steatohepatitis (NASH), atherosclerosis, giant-cell arteritis, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, age-related macular degeneration (AMD), graft-versus-host disease, type 2 diabetes, acne, sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease (PAD), atherosclerosis, vascular dysfunction, skeletal muscle ischemia, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease or Crohn's disease.

In one embodiment, a method of treating sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease, atherosclerosis, vascular dysfunction, skeletal muscle ischemia, pulmonary sarcoidosis, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease or Crohn's disease in a subject is provided, by administering an effective amount of a bbmAb disclosed herein, such as bbmAb1, to the subject.

9. Example 5: Pharmaceutical compositions

Provided herein are pharmaceutical compositions comprising the bbmAb antibodies, such as bbmAb1, formulated together with a pharmaceutically acceptable carrier. The compositions can additionally contain one or more other therapeutic agents that are suitable for treating a medical condition. Pharmaceutically acceptable carriers enhance or stabilize the composition, or can be used to facilitate preparation of the composition. Pharmaceutically acceptable carriers include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

A pharmaceutical composition described herein can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. It is preferred that administration be intravitreal, intravenous, intramuscular, intraperitoneal, or subcutaneous, or administered proximal to the site of the target. The pharmaceutically acceptable carrier should be suitable for intravitreal, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration

(e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e. bbmAb, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The composition should be sterile and fluid. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

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

Actual dosage levels of the active ingredients in the pharmaceutical compositions described herein can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions described herein employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions

employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors.

A physician or veterinarian can start doses of the antibodies described herein employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, effective doses of the compositions described herein, for the treatment of a wasting disorders described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Treatment dosages need to be titrated to optimize safety and efficacy. For systemic administration with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 15 mg/kg, of the host body weight. For intravitreal administration with an antibody, the dosage may range from 0.1 mg/eye to 5 mg/eye. An exemplary treatment regime entails systemic administration once per every two weeks or once a month or once every 3 to 6 months. An exemplary treatment regime entails systemic administration once per every two weeks or once a month or once every 3 to 6 months, or as needed (PRN).

Biological therapeutics, such as bbmAb1, are usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of bbmAb1 in the patient. In addition alternative dosing intervals can be determined by a physician and administered monthly or as necessary to be efficacious. In some methods of systemic administration, dosage is adjusted to achieve a plasma antibody concentration of 1–1000 µg/ml and in some methods 25–500 µg/ml. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show longer half-life than that of chimeric antibodies and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

SEQUENCE TABLE

Useful amino acid and nucleotide sequences for practicing the invention are disclosed in **Table 15**.

5 **Table 15.** Sequences according to embodiments of the invention

SEQ ID NUMBER	Ab region	Sequence
mAb1		
SEQ ID NO: 1 (Kabat)	HCDR1	SYAIS
SEQ ID NO: 2 (Kabat)	HCDR2	NIIPMTGQTYYAQKFQG
SEQ ID NO: 3 (Kabat)	HCDR3	AAYHPLVFDN
SEQ ID NO: 4 (Chothia)	HCDR1	GGTFKSY
SEQ ID NO: 5 (Chothia)	HCDR2	IPMTGQ
SEQ ID NO: 6 (Chothia)	HCDR3	AAYHPLVFDN
SEQ ID NO: 7	VH	EVQLVQSGAEVKKPGSSVKVSKASG GTFKSYAISWVRQAPGQGLEWMGNIIP MTGQTYYAQKFQGRVTITADESTSTAY MELSSLRSEDTAVYYCARAAHYHPLVFD NWGQGTTLTVSS
SEQ ID NO: 8	DNA VH	GAGGTGCAGCTGGTGCAGAGCGGCG CCGAGGTGAAGAAGCCCGGCAGCAG CGTGAAGGTGAGCTGCAAGGCCAGC GGCGGCACCTTCAAGAGCTACGCCA TCAGCTGGGTGAGGCAGGCCCCCGG CCAGGGCCTGGAGTGGATGGGCAAC ATCATCCCCATGACCGGCCAGACCTA CTACGCCCCAGAAGTTCCAGGGCAGG GTGACCATCACCGCCGACGAGAGCA CCAGCACCGCCTACATGGAGCTGAG CAGCCTGAGGAGCGAGGACACCGCC GTGTACTACTGCGCCAGGGCCGCCT ACCACCCCCTGGTGTTCGACAACCTG GGCCAGGGCACCCCTGGTGACCGTGA GCAGC
SEQ ID NO: 9	Heavy Chain	EVQLVQSGAEVKKPGSSVKVSKASG GTFKSYAISWVRQAPGQGLEWMGNIIP MTGQTYYAQKFQGRVTITADESTSTAY

		<p> MELSSLRSED TAVYYCARAAYHPLVFD NWGQGLTVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKHTCTPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK </p>
SEQ ID NO: 10	DNA Heavy Chain	<p> GAGGTGCAGCTGGTGCAGAGCGGCCGAGG TGAAGAAGCCCGGCAGCAGCGTGAAGGTG AGCTGCAAGGCCAGCGCGGCACCTCAAGA GCTACGCCATCAGCTGGGTGAGGCAGGCC CCCGGCCAGGGCCTGGAGTGGATGGGCAACA TCATCCCCATGACCGGCCAGACCTACTAC GCCCAGAAGTTCCAGGGCAGGGTGACCATCAC CGCCGACGAGAGCACCAGCACC GCCTAC ATGGAGCTGAGCAGCCTGAGGAGCGAGGACA CCGCCGTGTACTACTGCGCCAGGGCCGCC TACCACCCCTGGTGTTGACAACCTGGGGCCA GGGCACCCTGGTGACCGTGAGCAGCGCC AGCACCAAGGGCCCCAGCGTGTTCCCCCTGGC CCCCAGCAGCAAGAGCACCAGCGGCGGC ACCGCCGCCCTGGGCTGCCTGGTGAAGGACTA CTCCCCGAGCCCGTGACCGTGAGCTGG AACAGCGGCGCCCTGACCAGCGGCGTGACA CCTTCCCCGCCGTGCTGCAGAGCAGCGGC CTGTACAGCCTGAGCAGCGTGGTGACCGTGCC CAGCAGCAGCCTGGGCACCCAGACCTAC </p>

		ATCTGCAACGTGAACCACAAGCCCAGCAACAC CAAGGTGGACAAGAGGGTGGAGCCCAAG AGCTGCGACAAGACCCACACCTGCCCCCCTG CCCC GCCCCGAGGCCGCCGGCGGCCCC AGCGTGTTCTGTTCCTCCCAAGCCCAAGGA CACCCTGATGATCAGCAGGACCCCCGAG GTGACCTGCGTGGTGGTGGACGTGAGCCACG AGGACCCCGAGGTGAAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCACAACGCCAAGA CCAAGCCCAGGGAGGAGCAGTACAACAGC ACCTACAGGGTGGTGGAGCGTGCTGACCGTGCT GCACCAGGACTGGCTGAACGGCAAGGAG TACAAGTGCAAGGTGAGCAACAAGGCCCTGCC CGCCCCATCGAGAAGACCATCAGCAAG GCCAAGGGCCAGCCCAGGGAGCCCCAGGTGT ACACCCTGCCCCCAGCAGGGAGGAGATG ACCAAGAACCAGGTGAGCCTGACCTGCCTGGT GAAGGGCTTCTACCCAGCGACATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCCGAGA ACAACTACAAGACCACCCCCCGTGCTG GACAGCGACGGCAGCTTCTTCTGTACAGCAA GCTGACCGTGGACAAGAGCAGGTGGCAG CAGGGCAACGTGTTTCAGCTGCAGCGTGATGCA CGAGGCCCTGCACAACCACTACACCCAG AAGAGCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 11 (Kabat)	LCDR1	SGSSSNIGNHYVN
SEQ ID NO: 12 (Kabat)	LCDR2	RNNHRPS
SEQ ID NO: 13 (Kabat)	LCDR3	QSWDYSGFSTV
SEQ ID NO: 14 (Chothia)	LCDR1	SSSNIGNHY
SEQ ID NO: 15 (Chothia)	LCDR2	RNN
SEQ ID NO: 16 (Chothia)	LCDR3	WDYSGFST
SEQ ID NO: 17	VL	DIVLTQPPSVSGAPGQRVTISCSGSSS NIGNHYVNWYQQLPGTAPKLLIYRNNH RPSGVPDRFSGSKSGTSASLAITGLQS

		EDEADYYCQSWDYSGFSTVFGGGTKL TVL
SEQ ID NO: 18	DNA VL	GATATCGTCCTGACTCAGCCCCCTAG CGTCAGCGGCGCTCCCGGTCAGAGA GTGACTATTAGCTGTAGCGGCTCTAG CTCTAATATCGGTAATCACTACGTGA ACTGGTATCAGCAGCTGCCCCGGCAC CGCCCCTAAGCTGCTGATCTATAGAA ACAATCACCGGCCTAGCGGCGTGCC CGATAGGTTTACGCGGATCTAAGTCAG GCACTAGCGCTAGTCTGGCTATCACC GGACTGCAGTCAGAGGACGAGGCCG ACTACTACTGTCAGTCCTGGGACTAT AGCGGCTTTAGCACCGTGTTTCGGCG GAGGCACTAAGCTGACCGTGCTG
SEQ ID NO: 19	Light Chain	DIVLTQPPSVSGAPGQRVTISCSGSSS NIGNHYVNWYQQLPGTAPKLLIYRNNH RPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSWDYSGFSTVFGGGTKL TVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKA GVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTE CS
SEQ ID NO: 20	DNA Light Chain	GATATCGTCCTGACTCAGCCCCCTAG CGTCAGCGGCGCTCCCGGTCAGAGA GTGACTATTAGCTGTAGCGGCTCTAG CTCTAATATCGGTAATCACTACGTGA ACTGGTATCAGCAGCTGCCCCGGCAC CGCCCCTAAGCTGCTGATCTATAGAA ACAATCACCGGCCTAGCGGCGTGCC CGATAGGTTTACGCGGATCTAAGTCAG GCACTAGCGCTAGTCTGGCTATCACC GGACTGCAGTCAGAGGACGAGGCCG ACTACTACTGTCAGTCCTGGGACTAT AGCGGCTTTAGCACCGTGTTTCGGCG

		GAGGCACTAAGCTGACCGTGCTGGG TCAGCCTAAGGCTGCCCCCAGCGTG ACCCTGTTCCCCCCCAGCAGCGAGG AGCTGCAGGCCAACAAGGCCACCCT GGTGTGCCTGATCAGCGACTTCTACC CAGGCGCCGTGACCGTGGCCTGGAA GGCCGACAGCAGCCCCGTGAAGGCC GGCGTGGAGACCACCACCCCCAGCA AGCAGAGCAACAACAAGTACGCCGC CAGCAGCTACCTGAGCCTGACCCCC GAGCAGTGGAAGAGCCACAGGTCCT ACAGCTGCCAGGTGACCCACGAGGG CAGCACCGTGGAAAAGACCGTGGCC CCAACCGAGTGCAGC
mAb2		
SEQ ID NO: 21 (Kabat)	HCDR1	VYGMN
SEQ ID NO: 22 (Kabat)	HCDR2	IIWYDGDNQYYADSVKG
SEQ ID NO: 23 (Kabat)	HCDR3	DLRTGPFDY
SEQ ID NO: 24 (Chothia)	HCDR1	GFTFSVY
SEQ ID NO: 25 (Chothia)	HCDR2	WYDGDN
SEQ ID NO: 26 (Chothia)	HCDR3	DLRTGPFDY
SEQ ID NO: 27	VH	QVQLVESGGGVVQPGRSLRLSCAASG FTFSVYGMNWVRQAPGKGLEWVAIIW YDGDNQYYADSVKGRFTISRDN SKNTL YLQMNGLR AEDTAVYYCARDLRTGPF DYWGQGTLVTVSS
SEQ ID NO: 28	DNA VH	CAGGTGCAGCTGGTGGAGAGCGGCG GCGGCGTGGTGCAGCCCGGCAGGA GCCTGAGGCTGAGCTGCGCCGCCAG CGGCTTCACCTTCAGCGTGTACGGC ATGAACTGGGTGAGGCAGGCCCCCG GCAAGGGCCTGGAGTGGGTGGCCAT CATCTGGTACGACGGCGACAACCAG TACTACGCCGACAGCGTGAAGGGCA GGTTCACCATCAGCAGGGACAACAG CAAGAACACCCTGTACCTGCAGATGA

		ACGGCCTGAGGGCCGAGGACACCGC CGTGTACTIONGCGCCAGGGACCTG AGGACCGGCCCTTCGACTACTGGG GCCAGGGCACCTGGTGACCGTGAG CAGC
SEQ ID NO: 29	Heavy Chain	QVQLVESGGGVVQPGRSLRLSCAASG FTFSVYGMNWRQAPGKGLEWVAIIW YDGDNQYYADSVKGRFTISRDNKNTL YLQMNGLR AEDTAVYYCARDLRTGPF DYWGQGLTVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 30	DNA Heavy Chain	CAGGTGCAGCTGGTGGAGAGCGGCGGCGCG TGGTGCAGCCCGGCAGGAGCCTGAGGCTG AGCTGCGCCGCCAGCGGCTTCACCTCAGCGT GTACGGCATGAACTGGGTGAGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGGCCATCA TCTGGTACGACGGCGACAACCACTACTAC GCCGACAGCGTGAAGGGCAGGTTACCATCA GCAGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACGGCCTGAGGGCCGAGGACA CCGCCGTGTACTACTGCGCCAGGGACCTG AGGACCGGCCCTTCGACTACTGGGGCCAGG GCACCCTGGTGACCGTGAGCAGCGCCAGC ACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCC CAGCAGCAAGAGCACCAGCGGCGGCACC

		GCCGCCCTGGGCTGCCTGGTGAAGGACTACTT CCCCAGCCCGTGACCGTGAGCTGGAAC AGCGGCGCCCTGACCAGCGGCGTGACACCTT CCCCGCCGTGCTGCAGAGCAGCGGCCTG TACAGCCTGAGCAGCGTGGTGACCGTGCCCAG CAGCAGCCTGGGCACCCAGACCTACATC TGCAACGTGAACCACAAGCCCAGCAACACCAA GGTGGACAAGAGGGTGGAGCCCAAGAGC TGCACAAGACCCACACCTGCCCCCCTGCCCC GCCCCGAGCTGCTGGGCGGCCCCAGC GTGTTCTGTTCCTCCCCCAAGCCCAAGGACACC CTGATGATCAGCAGGACCCCGAGGTG ACCTGCGTGGTGGTGGACGTGAGCCACGAGG ACCCCGAGGTGAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCACAACGCCAAGACCA AGCCCAGGGAGGAGCAGTACAACAGCACC TACAGGGTGGTGAAGCGTGCTGACCGTGCTGCA CCAGGACTGGCTGAACGGCAAGGAGTAC AAGTGCAAGGTGAGCAACAAGGCCCTGCCCCG CCCCCATCGAGAAGACCATCAGCAAGGCC AAGGGCCAGCCCAGGGAGCCCCAGGTGTACA CCCTGCCCCCAGCAGGGAGGAGATGACC AAGAACCAGGTGAGCCTGACCTGCCTGGTGAA GGGCTTCTACCCAGCGACATCGCCGTG GAGTGGGAGAGCAACGGCCAGCCCAGGAACA ACTACAAGACCACCCCCCGTCTGGAC AGCGACGGCAGCTTCTCCTGTACAGCAAGCT GACCGTGGACAAGAGCAGGTGGCAGCAG GGCAACGTGTTGAGCTGCAGCGTGATGCACGA GGCCCTGCACAACCACTACACCCAGAAG AGCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 31 (Kabat)	LCDR1	RASQSIGSSLH
SEQ ID NO: 32 (Kabat)	LCDR2	YASQSFS
SEQ ID NO: 33 (Kabat)	LCDR3	HQSSSLPFT

SEQ ID NO: 34 (Chothia)	LCDR1	SQSIGSS
SEQ ID NO: 35 (Chothia)	LCDR2	YAS
SEQ ID NO: 36 (Chothia)	LCDR3	SSSLPF
SEQ ID NO: 37	VL	EIVLTQSPDFQSVTPKEKVTITCRASQS IGSSLHWYQQKPDQSPKLLIKYASQSF SGVPSRFSGSGSGTDFTLTINSLEAED AAAYYCHQSSSLPFTFGPGTKVDIK
SEQ ID NO: 38	DNA VL	GAGATCGTGCTGACCCAGTCACCCG ACTTTCAGTCAGTGACCCCTAAAGAA AAAGTGACTATCACCTGTAGGGCCTC CCAGTCTATCGGCTCTAGCCTGCACT GGTATCAGCAGAAGCCCGATCAGTC ACCTAAGCTGCTGATTAAGTACGCCT CTCAGTCCTTTAGCGGCGTGCCCTCT AGGTTTAGCGGCTCAGGCTCAGGCA CCGACTTCACCCTGACTATCAATAGC CTGGAAGCCGAGGACGCCGCTGCCT ACTACTGTCATCAGTCAAGTAGCCTG CCCTTCACCTTCGGCCCTGGCACTAA AGTGGATATTAAG
SEQ ID NO: 39	Light Chain	EIVLTQSPDFQSVTPKEKVTITCRASQS IGSSLHWYQQKPDQSPKLLIKYASQSF SGVPSRFSGSGSGTDFTLTINSLEAED AAAYYCHQSSSLPFTFGPGTKVDIKRT VAAPSVFIFPPSDEQLKSGTASVCLLN NFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 40	DNA Light Chain	GAGATCGTGCTGACCCAGTCACCCG ACTTTCAGTCAGTGACCCCTAAAGAA AAAGTGACTATCACCTGTAGGGCCTC CCAGTCTATCGGCTCTAGCCTGCACT GGTATCAGCAGAAGCCCGATCAGTC ACCTAAGCTGCTGATTAAGTACGCCT CTCAGTCCTTTAGCGGCGTGCCCTCT AGGTTTAGCGGCTCAGGCTCAGGCA

		CCGACTTCACCCTGACTATCAATAGC CTGGAAGCCGAGGACGCCGCTGCCT ACTACTGTCATCAGTCAAGTAGCCTG CCCTTCACCTTCGGCCCTGGCACTAA AGTGGATATTAAGCGTACGGTGGCC GCTCCCAGCGTGTTTCATCTTCCCCC CAGCGACGAGCAGCTGAAGAGCGGC ACCGCCAGCGTGGTGTGCCTGCTGA ACAACTTCTACCCCCGGGAGGCCAA GGTGCAGTGGAAGGTGGACAACGCC CTGCAGAGCGGCAACAGCCAGGAGA GCGTCACCGAGCAGGACAGCAAGGA CTCCACCTACAGCCTGAGCAGCACC CTGACCCTGAGCAAGGCCGACTACG AGAAGCATAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGG GCGAGTGC
Second part from mAb2		
SEQ ID NO: 41 (Combined)	HCDR1	GFTFSVYGMN
SEQ ID NO: 42 (Combined)	HCDR2	IIWYDGDNQYYADSVKG
SEQ ID NO: 43 (Combined)	HCDR3	DLRTGPFDY
SEQ ID NO: 44 (Kabat)	HCDR1	VYGMN
SEQ ID NO: 45 (Kabat)	HCDR2	IIWYDGDNQYYADSVKG
SEQ ID NO: 46 (Kabat)	HCDR3	DLRTGPFDY
SEQ ID NO: 47 (Chothia)	HCDR1	GFTFSVY
SEQ ID NO: 48 (Chothia)	HCDR2	WYDGDN
SEQ ID NO: 49 (Chothia)	HCDR3	DLRTGPFDY
SEQ ID NO: 50 (IMGT)	HCDR1	GFTFSVYG
SEQ ID NO: 51 (IMGT)	HCDR2	IWYDGDNQ
SEQ ID NO: 52 (IMGT)	HCDR3	ARDLRTGPFDY
SEQ ID NO: 53	VH	QVQLVESGGGVVQPGRSLRLSCAASG FTFSVYGMNWVRQAPGKGLEWVAIIW

		YDGDNQYYADSVKGRFTISRDNSKNTL YLQMNGLRAEDTAVYYCARDLRTGPF DYWGQGTTLTVSS
SEQ ID NO: 54	DNA VH	CAGGTGCAGCTGGTGGGAATCAGGCG GCGGAGTGGTGCAGCCTGGTAGATC ACTGAGACTGAGCTGCGCTGCTAGT GGCTTCACCTTTAGCGTCTACGGAAT GAACTGGGTCCGACAGGCCCTGGG AAAGGCCTGGAGTGGGTGGCAATTA TCTGGTACGACGGCGATAATCAGTAC TACGCCGATAGCGTGAAGGGACGGT TCACTATCTCTAGGGATAACTCTAAG AACACCCTGTACCTGCAGATGAACGG CCTGAGAGCCGAGGACACCGCCGTC TACTACTGCGCTAGGGACCTGAGAAC CGGCCCTTCGACTACTGGGGACAG GGCACCTGGTCACCGTGTCTAGC
SEQ ID NO: 55	Heavy Chain	QVQLVESGGGVVQPGRSRLSCAASG FTFSVYGMNWRQAPGKGLEWVAIIW YDGDNQYYADSVKGRFTISRDNSKNTL YLQMNGLRAEDTAVYYCARDLRTGPF DYWGQGTTLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKHTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPRE PQVCTLPPSREEMTKNQVSLSCAVKG FYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 56	DNA Heavy Chain	CAGGTGCAGCTGGTGGGAATCAGGCG GCGGAGTGGTGCAGCCTGGTAGATC

		<p>ACTGAGACTGAGCTGCGCTGCTAGT GGCTTCACCTTTAGCGTCTACGGAAT GAACTGGGTCCGACAGGCCCTGGG AAAGGCCTGGAGTGGGTGGCAATTA TCTGGTACGACGGCGATAATCAGTAC TACGCCGATAGCGTGAAGGGACGGT TCACTATCTCTAGGGATAACTCTAAG AACACCCTGTACCTGCAGATGAACGG CCTGAGAGCCGAGGACACCGCCGTC TACTACTGCGCTAGGGACCTGAGAAC CGGCCCCCTTCGACTACTGGGGACAG GGCACCTGGTCACCGTGTCTAGCG CCTCTACTAAGGGCCCAAGCGTGTTT CCCCTGGCCCCTAGCTCTAAGTCTAC TAGCGGAGGCACCGCCGCTCTGGGC TGCCTGGTCAAGGACTACTTCCCCGA GCCCCTGACCGTCAGCTGGAATAGC GGCGCTCTGACTAGCGGAGTGCACA CCTTCCCCGCCGTGCTGCAGTCTAG CGGCCTGTATAGCCTGTCTAGCGTC GTGACCGTGCCTAGCTCTAGCCTGG GCACTCAGACCTATATCTGTAAACGTG AACCACAAGCCCTCTAACACTAAGGT GGACAAGCGGGTGGAACTAAGTCC TGCGATAAGACTCACACCTGTCCCTCC CTGCCCTGCCCTGAGGCTGCCGGA GGACCTAGCGTGTTCTGTCCACC TAAGCCTAAAGACACCCTGATGATCT CTAGGACCCCCGAAGTGACCTGCGT GGTGGTGGACGTCTCACACGAGGAC CCTGAAGTGAAGTTTAATTGGTACGT GGACGGCGTGGAACTGCACAACGCT AAGACTAAGCCTAGAGAGGAACAGTA TAACTCTACCTATAGGGTCGTCAGCG TGCTGACAGTGCTGCACCAGGACTG GCTGAACGGGAAAGAGTATAAGTGTA</p>
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		AAGTGTCTAACAAGGCCCTGCCAGC CCCTATCGAAAAGACTATCTCTAAGG CTAAGGGGCAGCCTAGAGAACCCCA AGTGTGCACTCTGCCCCCTAGTAGAG AAGAGATGACTAAGAATCAGGTGTCA CTGAGCTGTGCCGTGAAGGGCTTCT ACCCTAGCGATATCGCCGTGGAGTG GGAGAGCAACGGCCAGCCCGAGAAC AACTACAAGACCACCCCCCAGTGCT GGACAGCGACGGCAGCTTCTTCCTG GTGAGCAAGCTGACCGTGGACAAGT CCAGGTGGCAGCAGGGCAACGTGTT CAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTC CCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 57 (Combined)	LCDR1	RASQSIGSSLH
SEQ ID NO: 58 (Combined)	LCDR2	YASQSFS
SEQ ID NO: 59 (Combined)	LCDR3	HQSSSLPFT
SEQ ID NO: 60 (Kabat)	LCDR1	RASQSIGSSLH
SEQ ID NO: 61 (Kabat)	LCDR2	YASQSFS
SEQ ID NO: 62 (Kabat)	LCDR3	HQSSSLPFT
SEQ ID NO: 63 (Chothia)	LCDR1	SQSIGSS
SEQ ID NO: 64 (Chothia)	LCDR2	YAS
SEQ ID NO: 65 (Chothia)	LCDR3	SSSLPF
SEQ ID NO: 66 (IMGT)	LCDR1	QSIGSS
SEQ ID NO: 67 (IMGT)	LCDR2	YASQSFSGVP
SEQ ID NO: 68 (IMGT)	LCDR3	HQSSSLPFT
SEQ ID NO: 69	VL	EIVLTQSPDFQSVTPKEKVTITCRASQS IGSSLHWYQQKPDQSPKLLIKYASQSF SGVPSRFSGSGSGTDFTLTINSLEAED AAAYYCHQSSSLPFTFGPGTKVDIK
SEQ ID NO: 70	DNA VL	GAGATCGTGCTGACCCAGTCACCCG ACTTTCAGTCAGTGACCCCTAAAGAA

		AAAGTGACTATCACCTGTAGGGCCTC CCAGTCTATCGGCTCTAGCCTGCACT GGTATCAGCAGAAGCCCGATCAGTC ACCTAAGCTGCTGATTAAGTACGCCT CTCAGTCCTTTAGCGGCGTGCCCTCT AGGTTTAGCGGCTCAGGCTCAGGCA CCGACTTCACCCTGACTATCAATAGC CTGGAAGCCGAGGACGCCGCTGCCT ACTACTGTCATCAGTCAAGTAGCCTG CCCTTCACCTTCGGCCCTGGCACTAA AGTGGATATTAAG
SEQ ID NO: 71	Light Chain	EIVLTQSPDFQSVTPKEKVTITCRASQS IGSSLHWYQQKPDQSPKLLIKYASQSF SGVPSRFSGSGSGTDFTLTINSLEAED AAAYYCHQSSSLPFTFGPGTKVDIKRT VAAPSVFIFPPSDEQLKSGTASVCLLN NFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 72	DNA Light Chain	GAGATCGTGCTGACCCAGTCACCCG ACTTTCAGTCAGTGACCCCTAAAGAA AAAGTGACTATCACCTGTAGGGCCTC CCAGTCTATCGGCTCTAGCCTGCACT GGTATCAGCAGAAGCCCGATCAGTC ACCTAAGCTGCTGATTAAGTACGCCT CTCAGTCCTTTAGCGGCGTGCCCTCT AGGTTTAGCGGCTCAGGCTCAGGCA CCGACTTCACCCTGACTATCAATAGC CTGGAAGCCGAGGACGCCGCTGCCT ACTACTGTCATCAGTCAAGTAGCCTG CCCTTCACCTTCGGCCCTGGCACTAA AGTGGATATTAAGCGTACGGTGGCC GCTCCCAGCGTGTTTCATCTTCCCCC CAGCGACGAGCAGCTGAAGAGCGGC ACCGCCAGCGTGGTGTGCCTGCTGA ACAACTTCTACCCCCGGGAGGCCAA

		GGTGCAGTGGAAGGTGGACAACGCC CTGCAGAGCGGCAACAGCCAGGAGA GCGTCACCGAGCAGGACAGCAAGGA CTCCACCTACAGCCTGAGCAGCACC CTGACCCTGAGCAAGGCCGACTACG AGAAGCATAAGGTGTACGCCTGCCA GGTGACCCACCAGGGCCTGTCCAGC CCCGTGACCAAGAGCTTCAACAGGG GCGAGTGC
First part from mAb1		
SEQ ID NO: 73 (Combined)	HCDR1	GGTFKSYAIS
SEQ ID NO: 74 (Combined)	HCDR2	NIIPMTGQTYYAQKFQG
SEQ ID NO: 75 (Combined)	HCDR3	AAYHPLVFDN
SEQ ID NO: 76 (Kabat)	HCDR1	SYAIS
SEQ ID NO: 77 (Kabat)	HCDR2	NIIPMTGQTYYAQKFQG
SEQ ID NO: 78 (Kabat)	HCDR3	AAYHPLVFDN
SEQ ID NO: 79 (Chothia)	HCDR1	GGTFKSY
SEQ ID NO: 80 (Chothia)	HCDR2	IPMTGQ
SEQ ID NO: 81 (Chothia)	HCDR3	AAYHPLVFDN
SEQ ID NO: 82 (IMGT)	HCDR1	GGTFKSYA
SEQ ID NO: 83 (IMGT)	HCDR2	IIPMTGQT
SEQ ID NO: 84 (IMGT)	HCDR3	ARAAYHPLVFDN
SEQ ID NO: 85	VH	EVQLVQSGAEVKKPGSSVKVSKASG GTFKSYAISWVRQAPGQGLEWMGNIIP MTGQTYYAQKFQGRVTITADESTSTAY MELSSLRSEDTAVYYCARAAYHPLVFD NWGQGTLVTVSS
SEQ ID NO: 86	DNA VH	GAGGTGCAGCTGGTGCAGTCAGGCG CCGAAGTGAAGAAACCCGGCTCTAG CGTGAAAGTCAGCTGTAAAGCTAGTG GCGGCACCTTCAAGTCCTACGCTATT AGCTGGGTCAGACAGGCCCCAGGTC AGGGCCTGGAGTGGATGGGCAATAT

		TATCCCTATGACCGGTCAGACCTACT ACGCTCAGAAATTTTCAGGGTAGAGTG ACTATCACCGCCGACGAGTCTACTAG CACCGCCTATATGGAAGTGTCTAGCC TGAGATCAGAGGACACCGCCGTCTA CTACTGCGCTAGAGCCGCCTATCACC CCCTGGTGTTCGATAACTGGGGTCA GGGCACCCTGGTCACCGTGTCTAGC
SEQ ID NO: 87	Heavy Chain	EVQLVQSGAEVKKPGSSVKVSKASG GTFKSYAISWVRQAPGQGLEWMGNIIP MTGQTYIAQKFQGRVTITADESTSTAY MELSSLRSEDTAVYYCARAAHPLVFD NWGQGTLVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKHTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPCREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 88	DNA Heavy Chain	GAGGTGCAGCTGGTGCAGTCAGGCG CCGAAGTGAAGAAACCCGGCTCTAG CGTGAAAGTCAGCTGTAAAGCTAGTG GCGGCACCTTCAAGTCCTACGCTATT AGCTGGGTCAGACAGGCCCCAGGTC AGGGCCTGGAGTGGATGGGCAATAT TATCCCTATGACCGGTCAGACCTACT ACGCTCAGAAATTTTCAGGGTAGAGTG ACTATCACCGCCGACGAGTCTACTAG CACCGCCTATATGGAAGTGTCTAGCC TGAGATCAGAGGACACCGCCGTCTA

		<p>CTACTGCGCTAGAGCCGCCTATCACC CCCTGGTGTTCGATAACTGGGGTCA GGGCACCCTGGTCACCGTGTCTAGC GCTAGCACTAAGGGCCCCTCAGTGTT CCCCCTGGCCCCTAGCTCTAAGTCTA CTAGCGGGCGGCACCGCCGCTCTGGG CTGCCTGGTGAAAGACTACTTCCCCG AGCCCGTGACCGTGTGCATGGAATAG CGGCGCTCTGACTAGCGGAGTGCAC ACCTTCCCCGCCGTGCTGCAGTCTA GCGGCCTGTATAGCCTGTCTAGCGT GGTGACCGTGCCTAGCTCTAGCCTG GGCACTCAGACCTACATCTGTAACGT GAACCACAAGCCCTCTAACACTAAGG TGGACAAGCGGGTGGAACCTAAGTC CTGCGATAAGACTCACACCTGTCCCC CCTGCCCTGCCCCTGAGGCTGCCGG AGGACCTAGCGTGTTCTGTTCACAC CTAAGCCTAAGGACACCCTGATGATC TCTAGGACCCCCGAAGTGACCTGCG TGGTGGTGGATGTGTCTCACGAGGA CCCTGAAGTGAAGTTCAATTGGTACG TGGACGGCGTGGAAGTGCAACACGC TAAGACTAAGCCTAGAGAGGAACAGT ATAACTCCACCTATAGAGTGGTGTCA GTGCTGACCGTGCTGCATCAGGACT GGCTGAACGGCAAAGAGTATAAGTGT AAAGTCTCTAACAAGGCCCTGCCAGC CCCTATCGAAAAGACTATCTCTAAGG CTAAGGGCCAGCCTAGAGAACCTCA GGTGTACACCCTGCCCCCCTGTAGA GAAGAGATGACTAAGAATCAGGTGTC CCTGTGGTGTCTGGTGAAAGGCTTCT ACCCTAGCGATATCGCCGTGGAATG GGAGTCTAACGGCCAGCCCGAGAAC AACTATAAGACTACCCCCCCTGTGCT</p>
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		GGATAGCGACGGCTCATTCTTCCTGT ACTCTAAGCTGACCGTGGACAAGTCT AGGTGGCAGCAGGGCAATGTGTTTA GCTGTAGCGTGATGCACGAGGCCCT GCATAATCACTACACTCAGAAGTCAC TGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 89 (Combined)	LCDR1	SGSSSNIGNHYVN
SEQ ID NO: 90 (Combined)	LCDR2	RNNHRPS
SEQ ID NO: 91 (Combined)	LCDR3	QSWDYSGFSTV
SEQ ID NO: 92 (Kabat)	LCDR1	SGSSSNIGNHYVN
SEQ ID NO: 93 (Kabat)	LCDR2	RNNHRPS
SEQ ID NO: 94 (Kabat)	LCDR3	QSWDYSGFSTV
SEQ ID NO: 95 (Chothia)	LCDR1	SSSNIGNHY
SEQ ID NO: 96 (Chothia)	LCDR2	RNN
SEQ ID NO: 97 (Chothia)	LCDR3	WDYSGFST
SEQ ID NO: 98 (IMGT)	LCDR1	SSNIGNHY
SEQ ID NO: 99 (IMGT)	LCDR2	RNN
SEQ ID NO: 100 (IMGT)	LCDR3	QSWDYSGFSTV
SEQ ID NO: 101	VL	DIVLTQPPSVSGAPGQRVTISCSGSSS NIGNHYVNWYQQLPGTAPKLLIYRNNH RPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSWDYSGFSTVFGGGTKL TVL
SEQ ID NO: 102	DNA VL	GATATCGTCCTGACTCAGCCCCCTAG CGTCAGCGGCGCTCCCGGTCAGAGA GTGACTATTAGCTGTAGCGGCTCTAG CTCTAATATCGGTAATCACTACGTGA ACTGGTATCAGCAGCTGCCCGGCAC CGCCCCTAAGCTGCTGATCTATAGAA ACAATCACCGGCCTAGCGGCGTGCC CGATAGGTTTAGCGGATCTAAGTCAG GCACTAGCGCTAGTCTGGCTATCACC GGA CTGCAGTCAGAGGACGAGGCCG

		ACTACTACTGTCAGTCCTGGGACTAT AGCGGCTTTAGCACCGTGTTTCGGCG GAGGCACTAAGCTGACCGTGCTG
SEQ ID NO: 103	Light Chain	DIVLTQPPSVSGAPGQRVTISCSGSSS NIGNHYVNWYQQLPGTAPKLLIYRNNH RPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSWDYSGFSTVFGGGTKL TVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKA GVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTE CS
SEQ ID NO: 104	DNA Light Chain	GATATCGTCCTGACTCAGCCCCCTAG CGTCAGCGGCGCTCCCGGTCAGAGA GTGACTATTAGCTGTAGCGGCTCTAG CTCTAATATCGGTAATCACTACGTGA ACTGGTATCAGCAGCTGCCCGGCAC CGCCCCTAAGCTGCTGATCTATAGAA ACAATCACCGGCCTAGCGGCGTGCC CGATAGGTTTAGCGGATCTAAGTCAG GCACTAGCGCTAGTCTGGCTATCACC GGACTGCAGTCAGAGGACGAGGCCG ACTACTACTGTCAGTCCTGGGACTAT AGCGGCTTTAGCACCGTGTTTCGGCG GAGGCACTAAGCTGACCGTGCTGGG TCAGCCTAAGGCTGCCCCCAGCGTG ACCCTGTTCCCCCCCAGCAGCGAGG AGCTGCAGGCCAACAAGGCCACCCT GGTGTGCCTGATCAGCGACTTCTACC CAGGCGCCGTGACCGTGGCCTGGAA GGCCGACAGCAGCCCCGTGAAGGCC GGCGTGGAGACCACCACCCCCAGCA AGCAGAGCAACAACAAGTACGCCGC CAGCAGCTACCTGAGCCTGACCCCC GAGCAGTGGAAGAGCCACAGGTCCT ACAGCTGCCAGGTGACCCACGAGGG

		CAGCACCGTGGAAAAGACCGTGGCC CCAACCGAGTGCAGC
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Throughout the text of this application, should there be a discrepancy between the text of the specification (e.g. **Table 15**) and the sequence listing, the text of the specification shall prevail.

CLAIMS

1. A bispecific antibody suitable for co-expression in a common host cell, wherein the antibody comprises
 - a. a first part which is an immunoglobulin with a first variable light chain of lambda wild type (VL1) and a first variable heavy chain of wild type (VH1), that binds specifically to a first target, and a first constant heavy chain (CH1) with a hetero-dimerization modification, and
 - b. a second part which is an immunoglobulin with a second variable light chain of kappa wild type (VL2) and a second variable heavy chain of wild type (VH2), that binds specifically to a second target, different from the first target, and a second constant heavy chain (CH2) with a hetero-dimerization modification which is complementary to the hetero-dimerization modification of the first constant heavy chain,wherein the first part and the second part, when co-expressed in a common host cell, form a bispecific antibody.
2. The bispecific antibody according to claim 1, wherein the first and second constant heavy chain are human IgA, IgD, IgE, IgG, or IgM, preferably IgD, IgE or IgG, such as human IgG1, IgG2, IgG3, or IgG4, preferably IgG1.
3. The bispecific antibody according to claim 2, wherein the wherein the first variable light chain is of lambda1 type, and the second variable light chain is of kappa 6 type.
4. The bispecific antibody according to claim 3, wherein the first and second constant heavy chain are IgG1, and wherein
 - a. the first constant heavy chain has point mutations generating a knob structure and the second constant heavy has point mutations generating a hole structure, or
 - b. the first constant heavy chain has point mutations generating a hole structure and the second constant heavy has point mutations generating a knob structure, and optionally
 - c. the first and second constant heavy chains have mutations resulting in a disulfide bridge.
5. The bispecific antibody according to any of the preceding claims, comprising a first immunoglobulin VH1 domain, a first immunoglobulin VL1 domain, a second

immunoglobulin VH2 domain and a second immunoglobulin VL2 domain,
wherein:

- a. the first immunoglobulin VH1 domain comprises (e.g. in sequence):
 - i. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
5 the amino acid sequence SEQ ID NO:76, said CDR2 having the
amino acid sequence SEQ ID NO:77, and said CDR3 having the
amino acid sequence SEQ ID NO:78; or
 - ii. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
10 the amino acid sequence SEQ ID NO:79, said CDR2 having the
amino acid sequence SEQ ID NO:80, and said CDR3 having the
amino acid sequence SEQ ID NO:81; and
- b. the first immunoglobulin VL1 domain comprises (e.g. in sequence):
 - i. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
15 the amino acid sequence SEQ ID NO:92, said CDR2 having the
amino acid sequence SEQ ID NO:93, and said CDR3 having the
amino acid sequence SEQ ID NO:94 or
 - ii. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
20 the amino acid sequence SEQ ID NO:95, said CDR2 having the
amino acid sequence SEQ ID NO:96, and said CDR3 having the
amino acid sequence SEQ ID NO:97; and
- c. the second immunoglobulin VH2 domain comprises (e.g. in sequence):
 - i. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
25 the amino acid sequence SEQ ID NO:44, said CDR2 having the
amino acid sequence SEQ ID NO:45, and said CDR3 having the
amino acid sequence SEQ ID NO:46; or
 - ii. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
30 the amino acid sequence SEQ ID NO:47, said CDR2 having the
amino acid sequence SEQ ID NO:48, and said CDR3 having the
amino acid sequence SEQ ID NO:49; and
- d. the second immunoglobulin VL2 domain comprises (e.g. in sequence):
 - i. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
35 the amino acid sequence SEQ ID NO:60, said CDR2 having the
amino acid sequence SEQ ID NO:61, and said CDR3 having the
amino acid sequence SEQ ID NO:62 or
 - ii. hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having
the amino acid sequence SEQ ID NO:63, said CDR2 having the

amino acid sequence SEQ ID NO:64, and said CDR3 having the amino acid sequence SEQ ID NO:65.

- 5 6. The bispecific antibody according to any of the preceding claims, comprising a first immunoglobulin VH1 domain, a first immunoglobulin VL1 domain, a second immunoglobulin VH2 domain and a second immunoglobulin VL2 domain, wherein:
- 10 a. the first immunoglobulin VH1 domain comprises the amino acid sequence SEQ ID NO: 85,
 - b. the first immunoglobulin VL1 domain comprises the amino acid sequence SEQ ID NO: 101,
 - c. the second immunoglobulin VH2 domain comprises the amino acid sequence SEQ ID NO: 53, and
 - 15 d. the second immunoglobulin VL2 domain comprises the amino acid sequence SEQ ID NO: 69.
- 20 7. The bispecific antibody according to any of the preceding claims, comprising a first immunoglobulin heavy chain, a first immunoglobulin light chain, a second immunoglobulin heavy chain and a second immunoglobulin light chain, wherein:
- a. the first immunoglobulin heavy chain comprises the amino acid sequence SEQ ID NO: 87,
 - b. the first immunoglobulin light chain comprises the amino acid sequence SEQ ID NO: 103,
 - 25 c. the second immunoglobulin heavy chain comprises the amino acid sequence SEQ ID NO: 55, and
 - d. the second immunoglobulin light chain comprises the amino acid sequence SEQ ID NO: 71.
- 30 8. A method for selecting a bispecific antibody according to any of claims 1-7, said method comprising;
- a. a first step of selecting the first part, and the second part;
 - b. a second step of co-expressing the first part and the second part in a common host cell, resulting in a bispecific antibody comprising the first part and the second part;
 - 35 c. a third step of purifying the bispecific antibody by removing mismatched fragments from the correctly matched bispecific antibody.

9. The method according to claim 8, wherein the third step of purification results in a bispecific antibody which is at least 60% (mass), 70% (mass), 80% (mass), 85% (mass) pure, such as at least 90% (mass) pure, 95% (mass), 96% (mass), 97% (mass), 98% (mass), or 99% (mass) pure.
- 5
10. A method for manufacturing a bispecific antibody according to any of claims 1-7 by co-expression in a common host cell, said method comprising;
- 10
- a. a first step of generating at least one vector encoding the first part and the second part;
 - b. a second step of introducing the at least one vector into the common host cell;
 - c. a third step of selecting cells specifically expressing the bispecific antibody;
 - d. a forth step of culturing the selected cells under conditions wherein the cells express the bispecific antibody; and
 - 15 e. a fifth step of purifying the bispecific antibody which is at least 60% (mass), 70% (mass), 80% (mass), or 85% (mass) pure, such as at least 90% (mass) pure, 95% (mass), 96% (mass), 97% (mass), 98% (mass), or 99% (mass) pure.
- 20
11. The method according to claim 10, wherein the first step comprises generating a first vector encoding the first part and a second vector encoding the second part.
- 25
12. An expression system comprising at least one vector comprising a polynucleotide encoding the first part or the second part of the bispecific antibody according to any of claims 1-7, and a selectable marker.
- 30
13. The expression system according to claim 12 comprising:
- a. a polynucleotide encoding a first selectable marker (sm I);
 - b. a polynucleotide encoding a second selectable marker (sm II), which differs from the first selectable marker (sm I).
- 35
14. The expression system according to claim 12 or 13, wherein the first selectable marker (sm I) is a folate transporter or a polynucleotide encoding a mutated folate receptor, wherein the mutated folate receptor has a decreased folate binding affinity compared to the wildtype folate receptor and the second selectable marker (sm II) is DHFR.

15. The expression system according to any of claims 12-14, wherein the first selectable marker (sm I) is Hygromycine and the second selectable marker (sm II) is Neo/G418.
- 5 16. The expression system according to any of claims 12-15, comprising two expression vectors wherein:
- a. a first vector comprising polynucleotide encoding at least a first selectable marker (sm I) and at least polynucleotides encoding the first part; and
 - b. a second vector comprising polynucleotide encoding at least a second
10 selectable marker (sm II) and at least polynucleotides encoding the second part.
17. The expression system according to any of claims 12-16, comprising a stop codon downstream of the polynucleotides encoding the heavy chain and a
15 polynucleotide encoding an immunoglobulin membrane anchor located downstream of the stop codon.
18. A method for selecting a common host cell for use in the methods according to any of claims 8-11, comprising
- a. a first step of providing a plurality of host cells, comprising the expression
20 system according to any of claims 12-17; and
 - b. culturing said plurality of host cells under conditions selective for the selectable marker, thereby obtaining a host cell expressing the product of interest.
- 25 19. The method according to claim 18, wherein a selective culture medium is used:
- a. comprising a limiting concentration of folate; and/or
 - b. comprising a folic acid in a concentration of 500nM or less; and/or
 - c. comprising a folic acid in a concentration selected from:
30
 - i. 1000 nM – 100 pM;
 - ii. 100 nM – 1 nM;
 - iii. 15nM – 1nM;
 - iv. 10nM – 1nM; and
 - v. 10 nM – 2.5 nM; and/or
 - d. comprising a DHFR inhibitor; and/or
 - e. comprising an antifolate; and/or
 - f. comprising an antifolate in a concentration of 500 nM or less; and/or
- 35

- g. comprising MTX in a concentration selected from:
- i. 500 nM – 3 nM;
 - ii. 100 nM – 10 nM;
 - iii. 50nM – 10nM; and
 - iv. 50 nM; and/or
- h. comprising a concentration of antifolate up to 20-fold of the folate concentration; and/or
- i. comprising a concentration of antifolate 10 – 20 fold of the folate concentration; and/or
- j. comprising a folic acid in a concentration up to 15 nM and an equimolar concentration up to 20 - fold of MTX.
20. The method according to claim 18 or 19, wherein the host cell comprises the expression system according to claim 17, wherein at least a portion of the first or second part is expressed as a fusion polypeptide comprising the immunoglobulin transmembrane anchor or fragment thereof, and wherein said fusion polypeptide is being displayed on the surface of said host cell, further comprising a step of:
- a. contacting the plurality of host cells with a detection compound binding the fusion polypeptide;
 - b. selecting at least one host cell based upon the presence or amount of the detection compound bound to the cell surface.
21. The method according to claim 20, wherein the detection compound comprises the first or second target, or derivatives thereof, and at least one detection label.
22. The method according to claims 10 or 11, wherein the fifth step of purifying the bispecific antibody comprises affinity chromatography and/or ion exchange chromatography.
23. The method according to claim 22, wherein the chromatography comprises
- a. a first step of capturing;
 - b. a second step of polishing; and optionally
 - c. a third step of further polishing.
24. The method according to claim 23, wherein the first step of capturing is performed with a principle selected from the group consisting of Fc-binding affinity chromatography, such as Protein A or Protein G, lambda light chain

specific affinity chromatography, such as LambdaFabSelect™, kappa light chain specific affinity chromatography, such as KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, such as Capto™ adhere, or Fractogel™ EMD SO₃, and hydrophobic interaction chromatography.

25. The method according to claim 22 or 23, wherein the second step of polishing is performed with a principle selected from the group consisting of Fc-binding affinity chromatography, such as Protein A or Protein G, lambda light chain specific affinity chromatography, such as LambdaFabSelect™, kappa light chain specific affinity chromatography, such as KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, such as Capto™ adhere, or Fractogel™ EMD SO₃, hydrophobic interaction chromatography, and virus inactivation.

26. The method according to claim 25, wherein the third step of further polishing is performed with a principle selected from the group consisting of Fc-binding affinity chromatography, such as Protein A or Protein G, lambda light chain specific affinity chromatography, such as LambdaFabSelect™, kappa light chain specific affinity chromatography, such as KappaSelect™, anti-idiotypic affinity chromatography, such as the first part or the second part, a target based affinity chromatography, such as affinity chromatography using the first target or second target, ion exchange chromatography, such as Capto™ adhere, or Fractogel™ EMD SO₃, hydrophobic interaction chromatography, and virus inactivation, alone or in combination.

27. The method according to any of claims 23, selected from:

- a. a first step of Protein A capturing, such as MabSelect™ SuRe™, a second step of lambda light chain affinity chromatography, such as LambdaFabSelect™, and a third step of kappa light chain affinity chromatography such as KappaSelect™; or
- b. a first step of Protein A, such as MabSelect™ SuRe™, a second step of kappa light chain affinity chromatography such as KappaSelect™, and a third step of lambda light chain affinity chromatography, such as LambdaFabSelect™; or

- c. a first step of kappa light chain affinity chromatography such as KappaSelect™ and a second step of lambda light chain affinity chromatography, such as LambdaFabSelect™; or
- d. a first step of lambda light chain affinity chromatography, such as LambdaFabSelect™ and a second step of kappa light chain affinity chromatography such as KappaSelect™.
28. The method according to any of claims 8-11 or 18-27, wherein the cell line is selected from the group consisting of a CHO cell, a non-producing hybridoma, such as Sp 2/0 or NS0, a human derived cell line, such as HEK or PER.C6, a baby hamster kidney (BHK) derivative, a yeast or filamentous fungi, a prokaryotic bacteria, such as E. coli or Pseudomonas fluorescence, a plant derivative, an algae and a ciliate.
29. A pharmaceutical composition comprising the antibody according to any of claims 1-7 and a pharmaceutically acceptable carrier.
30. A bispecific antibody according to any of claims 1-7, or a pharmaceutical composition according to claim 29, for use as a medicament.
31. A bispecific antibody according to any of claims 1-7, or a pharmaceutical composition accords to claim 29, for use as in the treatment of an inflammasome related disease.
32. The bispecific antibody according to any of claims 1-7, or the pharmaceutical composition according to claim 29, for use as in the treatment of an inflammasome related disease according to claim 31, wherein the inflammasome related disease is selected from the group consisting of sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease, atherosclerosis, vascular dysfunction, skeletal muscle ischemia, pulmonary sarcoidosis, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease and Crohn's disease.
33. A method of treating an inflammasome related disorder comprising administering to a subject afflicted with a inflammasome related disorder an effective amount of a bispecific antibody according to claims 1-7 or a pharmaceutical composition according to claim 29.

34. The method according to claim 33, wherein the inflammasome related disorder is sickle cell disease, vasculopathy, ischemia-reperfusion injury, cardiovascular disease, peripheral artery disease, atherosclerosis, vascular dysfunction, skeletal muscle ischemia, pulmonary sarcoidosis, fibrosis, malaria, hemodialysis-dependent, chronic kidney disease or Crohn's disease.