

**Antibodies that modulate a biological activity expressed by a cell**

The invention relates to the field of antibodies. In particular it relates to the field of therapeutic antibodies for the treatment of diseases involving aberrant cells. More in particular it relates to antibodies that bind extracellular parts of two or more membrane associated proteins and thereby modulate biological activity expressed by a cell.

Cancer is still a major cause of death in the world, in spite of the many advances that have been made in the treatment of the disease and the increased knowledge of the molecular events that lead to cancer. Colorectal cancer (CRC), for instance, is the third most common cancer worldwide. In 2008, 1.23 million people were diagnosed with the disease. It is the second most common cancer in Europe, with around 447,000 new cases diagnosed in 2012 (13% of the total). Colorectal cancer is the fourth most common cause of cancer death, estimated to be responsible for 608,000 (EU 148,000) deaths per annum. While some new treatments have been advanced in CRC many have failed clinical testing; metastatic CRC is still largely incurable with conventional treatments. Melanoma is another example of a cancer that occurs very frequently. When detection is not early enough the cancer is likely to metastasize at which stage it is very hard to treatment. Immune-intervention treatments have been shown to be effective to at least some of the patients with metastasized melanoma. Non-small cell lung cancer is a cancer type that is rarely discovered at an early enough stage for surgery. Also these types of cancers have been successfully treatment with immune-intervention treatments.

Traditionally, most cancer drug discovery has focused on agents that block essential cell functions and kill dividing cells. However, in cases of advanced cancer, no matter how aggressively applied, even to the point where patients suffer life-threatening side-effects from the treatment, chemotherapy rarely results in a complete cure. In most cases the tumors in the patients stop growing or temporarily shrink (referred to as remission) only to start proliferating again, some times more rapidly (referred to as relapse), and become increasingly more difficult to treat. More recently the focus of cancer drug development has moved away from

broadly cytotoxic chemotherapy to targeted cytostatic therapies with less toxicity. Treatment of advanced cancer has been validated clinically in leukemia and some other cancers. However, in a majority of carcinomas, targeted approaches are still proving not effective enough to completely abolish cancer in the majority of the patients.

Targeting of cancers has been achieved using a variety of different methods including for instance small molecules directed towards signaling proteins on which the cancer depends for survival and/or growth; vaccines with tumor specific proteins; cell therapies with immune cells that actively kill tumor cells and antibodies that target cytotoxic molecules to the tumor; interfere with signaling and/or that (re)direct the immune system of the host to the tumor cells.

The present invention provides novel means and methods for (re)directing immune system components. The invention also relates to means and methods for modulating a biological activity expressed by cells.

## SUMMARY OF THE INVENTION

The invention provides a method for interfering with Programmed Cell Death 1 protein (PD-1) and Lymphocyte activation 3 (LAG3) mediated inhibition in a PD-1 and/or LAG3 positive cell, the method comprising contacting said cell with an antibody or a functional part, derivative and/or analogue thereof that comprises

- a variable domain that can bind to an extracellular part of PD-1 and
- a variable domain that can bind to an extracellular part of LAG3, thereby inhibiting PD-1 and/or LAG3 mediated activity in said cell.

The invention also provides a method for stimulating the formation, stability and/or activity of an immunological synapse comprising providing a system that comprises at least two cells capable of associating with each other via an immunological synapse and providing said system with an antibody or a functional part, derivative and/or analogue thereof that comprises

- a variable domain that can bind to an extracellular part of PD-1 and
- a variable domain that can bind to an extracellular part of LAG3, thereby stimulating the formation, stability and/or activity of an immunological synapse between said at least two cells.

Further provided is an antibody or variant thereof that comprises a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of LAG3.

5 In a preferred embodiment of the invention the binding of said PD-1 binding variable domain to PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1. In a preferred embodiment of the invention the binding of said LAG3 binding variable domain to LAG3 blocks the binding of LAG3 to MHC class II.

10 The LAG-3 binding variable domain is preferably a variable domain that binds to LAG-3 extracellular domain 1, 2, 3 or 4, preferably domain 1 or domain 2.

Further provided is an antibody or variant thereof that comprises a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of LAG3; wherein the variable domain that can bind PD-1 comprises a heavy chain variable region with a CDR3 region that  
15 comprises the amino acid sequence of the CDR3 of a variable heavy chain region of one of the VH depicted for MF6076; ; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076 or MF6974. In a preferred embodiment said variable domain that can bind PD-1  
20 comprises a heavy chain variable region comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a variable heavy chain region of one of the VH depicted for MF6076;; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930;  
25 MF6076, MF6974 or MF6226, preferably MF6930, MF6076 or MF6974.

Further provided is an antibody or variant thereof that comprises a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of LAG3; wherein the variable domain that can bind PD-1 comprises a heavy chain variable region that comprises the amino acid  
30 sequence of the variable heavy chain region as depicted for MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076 or MF6974, having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions,

deletions, substitutions or a combination thereof with respect the amino acid sequence of the VH as depicted for MF.

The variable domain that can bind to an extra-cellular part of LAG3 preferably comprises a heavy chain variable region with a CDR3 region that  
 5 comprises the amino acid sequence of the CDR3 region of the variable heavy chain region as depicted for MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096;  
 10 MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096. Preferably variable domain that can bind to an extra-cellular part of LAG3 and that comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a variable heavy chain region of one of the VH depicted for  
 15 MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096.

20 The variable domain that can bind to an extra-cellular part of LAG3 preferably comprises a heavy chain variable region that comprises the amino acid sequence of the variable heavy chain region as depicted for MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097;  
 25 MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137,, preferably MF7139; MF7524, MF7133, MF7518 or MF7096 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with  
 30 respect the amino acid sequence of the indicated MF.

An antibody of the invention preferably comprises a heavy chain variable region comprising an amino acid sequence of an MF as depicted in figure 3. In a preferred embodiment the antibody further comprises a light chain variable region that comprises an amino acid sequence of a light chain variable region depicted in

figure 1. In a preferred embodiment the light chain comprises an amino acid sequence as depicted in figure 1A. In a preferred embodiment the heavy chain comprises a constant region of an IgG1 antibody, preferably a human IgG1 antibody. In a preferred embodiment the CH2 region of said IgG1 constant region is engineered to reduce ADCC and/or CDC activity of the antibody. In a preferred embodiment the CH2 region comprises a sequence as depicted in figure 2E. In a preferred embodiment the CH3-region of the antibody is engineered to facilitate heterodimerization of the heavy chains. In a preferred embodiment one heavy chain comprises a sequence as depicted in figure 2F and another heavy chain comprises a sequence as depicted in figure 2G.

Further provided is a pharmaceutical composition that comprises one or more antibodies or variants thereof of the invention.

Also provided is a nucleic acid molecule or a collection of nucleic acid molecules that codes for a heavy chain(s) or a heavy chain variable region(s) of an antibody of the invention or a variant thereof.

Also provided is a nucleic acid molecule or collection of nucleic acid molecules that codes for an antibody of the invention.

Also provided is a cell comprising one or more nucleic acid molecules that alone or together code for an antibody or a variant thereof of the invention. Also provided are methods of producing an antibody or a variant thereof of the invention using a cell as described, preferably together with the harvesting of the antibody or variant thereof from a culture of the cells.

Further provided is a cell system that comprises an antibody or variant thereof of the invention.

Also provided is a method for the treatment of an individual that has a disease involving aberrant cells such as cancer or has a chronic infection with a virus or parasite, the method comprising administering an antibody or a variant thereof of the invention, preferably a bispecific antibody or variant thereof of the invention, to the individual in need thereof.

The invention further provides an antibody or variant thereof of the invention; preferably a bispecific antibody or variant thereof of the invention, for use in the treatment of an individual that has disease involving aberrant cells such as cancer, or a chronic infection with a virus or parasite.

Further provided is an antibody of the invention, or a variant thereof that maintains the binding specificity of said antibody, or a nucleic acid molecule or functional equivalent of the invention, for use as a medicament.

5 The invention further provides an antibody of the invention or a variant of said antibody that maintains the binding specificity of the antibody of the invention, for use in the treatment of an individual that has a disease that involves aberrant cells such as cancer, or that has an infection, preferably an infection with a virus or parasite.

10 Further provided is a use of an antibody or variant according to the invention for the preparation of a medicament for the treatment or prevention of cancer and/or an infection, preferably an infection with a virus or parasite.

In a preferred embodiment the parasite is an intracellular parasite.

15 Further provided is a method of stimulating an immune response in an individual against an aberrant cell in said individual, the method comprising providing (administering to) said individual with an antibody or a variant thereof, preferably a bispecific antibody or a variant thereof of the invention. The aberrant cell is preferably a cancer cell, a virus-infected cell, a parasite or a parasite infected cell. In a preferred embodiment the cell is a cancer cell or a neoplastic cell.

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## DETAILED DESCRIPTION OF THE INVENTION

T-cell exhaustion is mediated by several inhibitory receptors including programmed cell death protein 1 (PD1), T cell immunoglobulin and mucin domain 25 3 (TIM-3), and LAG3. LAG3's main ligand is MHC class II, to which it binds with higher affinity than CD4. The protein negatively regulates cellular proliferation, activation, and homeostasis of T cells, in a similar fashion to CTLA-4 and PD-1 and has been reported to play a role in Treg suppressive function. LAG3 reportedly also helps maintain CD8+ T cells in a tolerogenic state. LAG3 is known under a number of different names such as Lymphocyte Activating 3; Lymphocyte-Activation Gene 30 3; CD223 Antigen; Protein FDC; CD223; LAG-3; or FDC. External Ids for LAG3 are: HGNC: 6476; Entrez Gene: 3902; Ensembl: ENSG00000089692; OMIM: 153337; and UniProtKB: P18627. LAG-3 is closely related to CD4. LAG-3 is located on the human chromosome 12 (12p13.32) adjacent to the CD4 gene, and its

sequence is approximately 20% identical to CD4. The LAG-3 protein binds a nonholomorphic region of major histocompatibility complex 2 (MHC class II) with greater affinity than CD4. LAG-3 is one of the various immune-checkpoint receptors that are coordinately upregulated on both regulatory T cells (Tregs) and  
5 anergic T cells. LAG-3 can negatively regulated T cell proliferation, activation and homeostasis.

Programmed Cell Death 1 protein (PD-1) is a cell surface receptor that belongs to the CD28 family of receptors and is expressed on T cells and pro-B cells. PD-1 is presently known to bind two ligands, PD-L1 and PD-L2. PD-1, functioning  
10 as an immune checkpoint, plays an important role in down regulating the immune system by inhibiting the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is thought to be accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing  
15 apoptosis in regulatory T cells (suppressor T cells). PD-1 is also known under a number of different aliases such as PDCD1; Programmed Cell Death 1; Systemic Lupus Erythematosus Susceptibility 2; Protein PD-1; HPD-1; PD1; Programmed Cell Death 1 Protein; CD279 Antigen; CD279; HPD-L; HSLE1; SLEB2; and PD-1. External Ids for PD-1 are HGNC: 8760; Entrez Gene: 5133; Ensembl:  
20 ENSG00000188389; OMIM: 600244; and UniProtKB: Q15116. New classes of drugs that block the activity of PD-1, the PD-1 inhibitors, activate the immune system to attack tumors and are therefore used with success to treat some types of cancer.

PD-L1 is a type 1 transmembrane protein that plays a role in suppressing an  
25 immune response during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. The binding of PD-L1 to PD-1 or B7.1 (CD80) transmits an inhibitory signal which reduces the proliferation of the PD-1 expressing T cells. PD-1 is thought to be able to control the accumulation of foreign antigen specific T cells through apoptosis. PD-L1 is  
30 expressed by a variety of cancer cells and the expression thereof is thought to be at least in part responsible for a dampening of an immune response against the cancer cell. PD-L1 is a member of the B7-family of protein and is known under a variety of other names such as CD274 Molecule; CD274 Antigen; B7 Homolog 1; PDCD1 Ligand 1; PDCD1LG1; PDCD1L1; B7H1; PDL1; Programmed Cell Death 1

Ligand 1; Programmed Death Ligand 1; B7-H1; and B7-H. External Ids for CD274 are HGNC: 17635; Entrez Gene: 29126; Ensembl: ENSG00000120217; OMIM: 605402; UniProtKB: Q9NZQ7.

PD-L2 is a second ligand for PD-1. Engagement of PD-1 by PD-L2 inhibits T  
5 cell receptor (TCR)-mediated proliferation and cytokine production by CD4+ T  
cells. At low antigen concentrations, PD-L2/PD-1 binding inhibits B7-CD28 signals.  
At high antigen concentrations, PD-L2/PD-1 binding reduces cytokine production.  
PD-L expression is up-regulated on antigen-presenting cells by interferon gamma  
treatment. It is expressed in some normal tissues and a variety of tumors. PD-L1  
10 and PD-L2 are thought to have overlapping functions and regulate T cell  
responses. The protein is known under a number of other names such as  
Programmed Cell Death 1 Ligand 2; B7 Dendritic Cell Molecule; Programmed  
Death Ligand 2; Butyrophilin B7-DC; PDCD1 Ligand 2; PD-1 Ligand 2; PDCD1L2;  
B7-DC; CD273; B7DC; PDL2; PD-1-Ligand 2; CD273 Antigen; BA574F11.2; and  
15 Btdc. External Ids for PD-L2 are HGNC: 18731; Entrez Gene: 80380; Ensembl:  
ENSG00000197646; OMIM: 605723; and UniProtKB: Q9BQ51.

Reference to sequence identifiers is done to identify which protein is targeted.  
An antibody of the invention also recognizes at least some variants thereof such as  
allelic variants, splice variants and mutant variants thereof as long as the epitope  
20 recognized by the respective variable domain of the antibody has not been affected.  
Some of the alternative names may or may not have also been used to refer to other  
proteins. The names are given for reference purposes only. An antibody of the  
invention binds to the protein as expressed on cells. It can also bind to variants of  
the protein as long as the epitope to which the antibody binds is available. Thus  
25 splicing variants or mutant proteins (if any) will also be bound as long as the  
epitope is available. The fact that the antibody binds to the indicated protein  
means that it can bind to protein as a property and does not imply that the  
antibody is actually bound to the target, although it can be. It also does not mean  
that the antibody does not bind to other proteins. The invention discloses an  
30 antibody or variant thereof, which is preferably a bispecific antibody or variant  
thereof, that binds an extracellular part of a member of the PD-1 (first membrane  
protein) and an extracellular part of LAG3 (second membrane protein). Such a  
(bispecific) antibody is further also referred to as "an antibody or bispecific

antibody of the invention". Also provided are compositions and kits of parts comprising two or more (bispecific) antibodies as described herein.

Antibodies typically bind their target via the so-called antigen binding site. An unmodified antigen-binding site is typically formed by and present in the  
5 variable domain of the antibody. The variable domain contains said antigen-binding site. A variable domain that binds an antigen is a variable domain comprising an antigen-binding site that binds the antigen.

In one embodiment an antibody variable domain comprises a heavy chain variable region (VH) and a light chain variable region (VL). The antigen-binding  
10 site can be present in the combined VH/VL variable domain, or in only the VH region or only the VL region. When the antigen-binding site is present in one of the two regions of the variable domain, the counterpart variable region can contribute to the folding and/or stability of the binding variable region, but does not significantly contribute to the binding of the antigen itself.

As used herein, antigen-binding refers to the typical binding capacity of an  
15 antibody to its antigen. Binding of an antibody to an antigen can be assessed in various ways. One way is to incubate the antibody with the antigen (preferably cells expressing the antigen), removing unbound antibody (preferably by a wash step) and detecting bound antibody by means of a labeled antibody that binds to  
20 the bound antibody.

Antigen binding by an antibody is typically mediated through the complementarity determining regions (CDR) of the antibody and the specific three-dimensional structure of both the antigen and the variable domain allowing these two structures to bind together with precision (an interaction similar to a lock and  
25 key), as opposed to random, non-specific sticking of proteins. As an antibody typically recognizes part of an antigen called the epitope of an antigen, and as such epitope may be present in other compounds as well, antibodies according to the present invention may recognize other proteins as well, if such other compounds contain the same epitope. Hence, the term "binding" does not exclude binding of the  
30 antibodies to another protein or protein(s) that contain the same epitope. Such other protein(s) is preferably not a human protein.

A protein of the invention such as an antibody typically does not bind to other proteins than the specified target protein on the membrane of cells in a post-natal, preferably adult human.

The term “antibody” as used herein means a proteinaceous molecule, preferably belonging to the immunoglobulin class of proteins, containing one or more variable domains that bind an epitope on an antigen, where such domains are derived from or share sequence homology with the variable domain of an antibody.

5 Antibodies for therapeutic use are preferably as close to natural antibodies of the subject to be treated as possible (for instance human antibodies for human subjects). Antibody binding can be expressed in terms of specificity and affinity. The specificity determines which antigen or epitope thereof is specifically bound by the binding domain. The affinity is a measure for the strength of binding to a  
10 particular antigen or epitope. Preferably, the affinity of the separate arms of the antibodies according to the invention is in the nanomolar range. Antibodies such as the bispecific antibodies of the present invention typically comprise the constant domains (Fc part) of a natural antibody, which may be engineered as described elsewhere herein to, for instance reduce ADCC and/or CDC activity. An antibody of  
15 the invention is typically a bispecific full length antibody, preferably of the human IgG subclass.

A variable domain is composed of the variable region of a heavy chain and a variable region of a light chain. The variable region of a heavy chain is typically formed by a rearranged VDJ region. A variable region of a light chain is typically  
20 formed by a rearranged VJ region. The VDJ/VJ regions can now also be artificially produced using for instance the large body of sequence information that is available of functional antibodies.

An antibody of the invention is preferably a “full length” antibody. The term ‘full length’ according to the invention is defined as comprising an essentially  
25 complete antibody, without one or more artificially added moieties which a size of larger than 20 amino acid residues, such as for instance additional antigen binding sites or additional activation sites or additional ligands or additional ligand-binding moieties. A full length antibody, however, does not necessarily have all functions of an intact antibody. For the avoidance of doubt, a full length antibody  
30 contains two heavy and two light chains. Each chain contains constant (C) and variable (V) regions, which can be broken down into domains designated CH1, CH2, CH3, VH for the heavy chain, and CL, VL for the light chain. The domains of the heavy chains are preferably present in the order of a natural antibody (VH-CH1-CH2-CH3, meaning that the VH domain is adjacent to the CH1 domain,

followed by a CH2 domain and subsequently followed by a CH3 domain). The domains of the light chains are also preferably present in the order of a natural antibody (VL-CL: meaning that the VL domain is adjacent to the CL domain). An antibody binds to antigen via the variable domains contained in the Fab fragment  
5 portion. The antibody can interact with molecules and cells of the immune system through the constant domains, mostly through the Fc portion.

In some embodiments, an antibody of the invention is an IgG, preferably a full length IgG. Full length IgG antibodies are preferred because of their typically favorable half-life and the desire to stay as close to fully autologous (human)  
10 molecules for reasons of immunogenicity. In some embodiments, an antibody of the invention is a full length IgG1, a full length IgG2, a full length IgG3 or a full length IgG4 antibody.

Full length antibodies according to the invention encompass antibodies  
15 wherein mutations may be present that provide desired characteristics or are just alternatives to the ones in the original chain. Such mutations should not be deletions of substantial portions of any of the regions. However, antibodies wherein one or several amino acid residues are acid inserted, deleted, substituted or a combination thereof, without essentially altering the antigen binding  
20 characteristics of the resulting antibody are embraced within the term "full length antibody". For instance, an IgG antibody can have 1-20 amino acid residue insertions, substitutions, deletions or a combination thereof in the constant region.

An antibody or a functional part, derivative and/or analogue thereof of the invention is preferably a bispecific antibody or a functional part, derivative and/or  
25 analogue thereof. In a preferred embodiment it is a bispecific IgG antibody with reduced effector function. In a preferred embodiment an antibody of the invention is a bispecific full length antibody. An antibody of the invention is preferably a bispecific full length IgG antibody, preferably mutated in the CH2/lower hinge region to reduce effector function. IgG1 which is mutated in the CH2/lower hinge  
30 region to reduce effector function is favored based on its long circulatory half-life in man. In order to prevent any immunogenicity in humans it is preferred that the bispecific antibody according to the invention is a human antibody.

The term 'bispecific' (bs) means that one part of the antibody (as defined above) binds to one epitope on an antigen whereas a second part binds to a

different epitope on either the same antigen, or a different antigen. The different epitopes are typically present on different antigens. The different epitopes can, however, also be present on the same antigen. According to the present invention, said first and second antigens are in fact two different proteins. A preferred

5 bispecific antibody is an antibody that comprises parts of two different monoclonal antibodies and consequently can bind to two different epitopes, preferably on two different antigens. Dependent on the expression level, (sub-)cellular localization and stoichiometry of the two antigens recognized by a bispecific antibody, both Fab arms of the antibody may or may not simultaneously bind their epitope. One arm of

10 the bispecific antibody typically contains the variable domain of one antibody and the other arm contains the variable domain of another antibody (i.e. one arm of the bispecific antibody is formed by one heavy chain paired with one light chain whereas the other arm is formed by a different heavy chain paired with a light chain). The heavy chain variable regions of the bispecific antibody of the invention

15 are typically different from each other, whereas the light chain variable regions are preferably the same in the bispecific antibodies of the invention. A bispecific antibody wherein the different heavy chain variable regions are associated with the same or a common, light chain variable region is also referred to as a bispecific antibody with a common light chain variable region (cLcv). It is preferred that the

20 light chain constant region is also the same. Such bispecific antibodies are referred to as having a common light chain (cLc). Further provided is therefore a bispecific antibody according to the invention, wherein both arms comprise a common light chain.

Bispecific antibodies as described herein preferably comprise a common

25 light chain variable domain, preferably a common light chain. The term 'common light chain' according to the invention refers to light chains which may be identical or have some amino acid sequence differences while the binding specificity of the full length antibody is not affected. It is for instance possible within the scope of the definition of common light chains as used herein, to prepare or find light chains

30 that are not identical but still functionally equivalent, e.g., by introducing and testing conservative amino acid changes, changes of amino acids in regions that do not or only partly contribute to binding specificity when paired with the heavy chain, and the like. The terms 'common light chain', 'common LC', 'cLC', 'single light chain' with or without the addition of the term 'rearranged' are all used

herein interchangeably. The terms 'common light chain variable region', 'common VL', 'common LCv', 'cLCv', 'single VL' with or without the addition of the term 'rearranged' are all used herein interchangeably. It is a preferred aspect of the present invention that a bispecific antibody has a common light chain (variable region) that can combine with at least two, and preferably a plurality of heavy chains (variable regions) of different binding specificity to form antibodies with functional antigen binding domains (WO2009/157771). The common light chain (variable region) is preferably a human light chain (variable region). A common light chain (variable region) preferably has a germline sequence. A preferred germline sequence is a light chain variable region that is frequently used in the human repertoire and has good thermodynamic stability, yield and solubility. A preferred germline light chain is O12. A common light chain is preferably the rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 (Figure 1A). The common light chain variable region is preferably the variable region of the rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01. A common light chain preferably comprises a light chain variable region as depicted in figure 1B, or 1D with 0-5 amino acid insertions, deletions, substitutions, additions or a combination thereof. The common light preferably further comprises a light chain constant region, preferably a kappa light chain constant region. A nucleic acid that encodes the common light chain can be codon optimized for the cell system used to express the common light chain protein. The encoding nucleic acid can deviate from a germ-line nucleic acid sequence.

In a preferred embodiment the light chain comprises a light chain region comprising the amino acid sequence of an O12 / IgV $\kappa$ 1-39\*01 gene segment as depicted in figure 1A with 0-10, preferably 0-5 amino acid insertions, deletions, substitutions, additions or a combination thereof. The phrase "O12 light chain" will be used throughout the specification as short for "a light chain comprising a light chain variable region comprising the amino acid sequence of an O12 / IgV $\kappa$ 1-39\*01 gene segment as depicted in figure 1A with 0-10, preferably 0-5 amino acid insertions, deletions, substitutions, additions or a combination thereof. IgV $\kappa$ 1-39 is short for Immunoglobulin Variable Kappa 1-39 Gene. The gene is also known as Immunoglobulin Kappa Variable 1-39; IGKV139; IGKV1-39; O12a or O12. External Ids for the gene are HGNC: 5740; Entrez Gene: 28930; Ensembl: ENSG00000242371. A preferred amino acid sequence for IgV $\kappa$ 1-39 is given in

figure 1E. This lists the sequence of the V-region. The V-region can be combined with one of five J-regions. Figure 1B and 1D describe two preferred sequences for IgV $\kappa$ 1-39 in combination with a J-region. The joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or  
 5 IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01 (nomenclature according to the IMGT database worldwide web at imgt.org).

It is preferred that the O12 / IgV $\kappa$ 1-39\*01 comprising light chain variable region is a germline sequence. It is further preferred that the IGJ $\kappa$ 1\*01 or /IGJ $\kappa$ 5\*01 comprising light chain variable region is a germline sequence. In a  
 10 preferred embodiment, the IGKV1-39/jk1 or IGKV1-39/jk5 light chain variable regions are germline sequences.

In a preferred embodiment the light chain variable region comprises a germline O12 / IgV $\kappa$ 1-39\*01. In a preferred embodiment the light chain variable region comprises the kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or IgV $\kappa$ 1-  
 15 39\*01/IGJ $\kappa$ 5\*01. In a preferred embodiment a IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01. The light chain variable region preferably comprises a germline kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or germline kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01, preferably a germline IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01.

Mature B-cells that produce an antibody with an O12 light chain often  
 20 produce a light chain that has undergone one or more mutations with respect to the germline sequence, i.e. the normal sequence in non-lymphoid cells of the organism. The process that is responsible for these mutations is often referred to as somatic (hyper)mutation. The resulting light chain is referred to as an affinity matured light chain. Such light chains, when derived from an O12 germline  
 25 sequence are O12-derived light chains. In this specification, the phrase "O12 light chains" will include O12-derived light chains, The mutations that are introduced by somatic hypermutation can of course also be introduced artificially in the lab. In the lab also other mutations can be introduced without affecting the properties of the light chain in kind, not necessarily in amount. A light chain is at least an O12  
 30 light chain if it comprises a sequence as depicted in figure 1A, figure 1B; figure 1D or figure 1E with 0-10, preferably 0-5 amino acid insertions, deletions, substitutions, additions or a combination thereof. In a preferred embodiment the O12 light chain is a light chain comprising a sequence as depicted in figure 1A; 1B; 1D or 1E with 0-9, 0-8, 0-7, 0-6, 0-5, 0-4 amino acid insertions, deletions,

substitutions, additions or a combination thereof. In a preferred embodiment the O12 light chain is a light chain comprising a sequence as depicted in figure 1A, figure 1B; figure 1D or figure 1E with 0-5, preferably 0-4, more preferably 0-3 amino acid insertions, deletions, substitutions, additions or a combination thereof.

5 In a preferred embodiment the O12 light chain is a light chain comprising a sequence as depicted in figure 1A, figure 1B; figure 1D or figure 1E with 0-2, more preferably 0-1, most preferably 0 amino acid insertions, deletions, substitutions, additions or a combination thereof. In a preferred embodiment the O12 light chain is a light chain comprising a sequence as depicted in figure 1A or figure 1B with  
10 the mentioned amino acid insertions, deletions, substitutions, additions or a combination thereof. In a preferred embodiment the light chain comprises the sequence of figure 1A. In a preferred embodiment the light chain variable region comprises the sequence of figure 1B.

The common light chain (variable region) can be a lambda light chain and  
15 this is therefore also provided in the context of the invention, however a kappa light chain is preferred. The constant part of a common light chain of the invention can be a constant region of a kappa or a lambda light chain. It is preferably a constant region of a kappa light chain, preferably wherein said common light chain is a germline light chain, preferably a rearranged germline human kappa light  
20 chain comprising the IgVK1-39 gene segment, most preferably the rearranged germline human kappa light chain IgVK1-39\*01/IGJK1\*01 (Figure 1). The terms rearranged germline human kappa light chain IgVk1-39\*01/IGJk1\*01, IGKV1-39/IGKJ1, huVk1-39 light chain or in short huVk1-39, or simply 1-39 are used interchangeably throughout the application. Obviously, those of skill in the art will  
25 recognize that “common” also refers to functional equivalents of the light chain of which the amino acid sequence is not identical. Many variants of said light chain exist wherein mutations (deletions, substitutions, additions) are present that do not influence the formation of functional binding regions.

IgVk1-39 is short for Immunoglobulin Variable Kappa 1-39 Gene. The gene  
30 is also known as Immunoglobulin Kappa Variable 1-39; IGKV139; IGKV1-39; O12a or O12. External Ids for the gene are HGNC: 5740; Entrez Gene: 28930; Ensembl: ENSG00000242371. A preferred amino acid sequence for IgVk1-39 is given in figure 1. This lists the sequence of the V-region. The V-region can be combined with one of five J-regions. Figure 1 describes two preferred sequences for IgVk1-39 in

combination with a J-region. The joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01 (nomenclature according to the IMGT database worldwide web at [imgt.org](http://imgt.org)).

5           A common light chain variable region is preferably linked to a kappa light chain constant region. In a preferred embodiment the light chain comprises the kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01. In a preferred embodiment a IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01.

10           A cell that produces a common light chain can produce for instance rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 and a light chain comprising the variable region of the mentioned light chain fused to a lambda constant region.

15           Bispecific antibodies or variants thereof as described herein preferably have one heavy chain variable region/light chain variable region (VH/VL) combination that binds an extracellular part of PD-1 and a second VH/VL combination that binds an extracellular part of LAG3. In a preferred embodiment the VL in said first VH/VL combination is similar to the VL in said second VH/VL combination. In a more preferred embodiment, the VLs in the first and second VH/VL combinations are identical. In a preferred embodiment, the bispecific antibody is a full length  
20           antibody which has one heavy/light (H/L) chain combination that binds an extracellular part of PD-1 and one H/L chain combination that binds an extracellular part of LAG3. In a preferred embodiment the light chain in said first H/L chain combination is similar to the light chain in said second H/L chain combination. In a more preferred embodiment, the light chains in the first and  
25           second H/L chain combinations are identical.

30           Several methods have been published to favor the production of the bispecific antibody or vice versa, the monospecific antibodies. In the present invention it is preferred that the cell favors the production of the bispecific antibody over the production of the respective monospecific antibodies. Such is typically achieved by modifying the constant region of the heavy chains such that they favor heterodimerization (i.e. dimerization with the heavy chain of the other heavy/light chain combination) over homodimerization. In a preferred embodiment the bispecific antibody of the invention comprises two different immunoglobulin heavy chains with compatible heterodimerization domains. Various compatible

heterodimerization domains have been described in the art. The compatible heterodimerization domains are preferably compatible immunoglobulin heavy chain CH3 heterodimerization domains. When wildtype CH3 domains are used, co-expression of two different heavy chains (A and B) and a common light chain will result in three different antibody species, AA, AB and BB. AA and BB are designations for the two mono-specific, bivalent antibodies, and AB is a designation for the bispecific antibody. To increase the percentage of the desired bispecific product (AB) CH3 engineering can be employed, or in other words, one can use heavy chains with compatible hetero-dimerization domains, as defined hereunder.

10 The art describes various ways in which such hetero-dimerization of heavy chains can be achieved. One way is to generate 'knob into hole' bispecific antibodies. See US Patent Application 20030078385 (Arathoon et al.).

The term 'compatible hetero-dimerization domains' as used herein refers to protein domains that are engineered such that engineered domain A' will preferentially form heterodimers with engineered domain B' and vice versa, homodimerization between A'-A' and B'-B' is diminished.

15

In US13/866,747 (now issued as US 9,248,181), US14/081,848 (now issued as US 9,358,286) and PCT/NL2013/050294 (published as WO2013/157954); incorporated herein by reference) methods and means are disclosed for producing bispecific antibodies using compatible heterodimerization domains. These means and methods can also be favorably employed in the present invention. Specifically, a bispecific antibody of the invention preferably comprises mutations to produce essentially only bispecific full length IgG molecules. Preferred mutations are the amino acid substitutions L351K and T366K (EU numbering) in the first CH3 domain (the 'KK-variant' heavy chain) and the amino acid substitutions L351D and L368E in the second domain (the 'DE-variant' heavy chain), or vice versa. It was previously demonstrated in our US 9,248,181 and US 9,358,286 patents as well as the WO2013/157954 PCT application that the DE-variant and KK-variant preferentially pair to form heterodimers (so-called 'DEKK' bispecific molecules).

20

25

30 Homodimerization of DE-variant heavy chains (DEDE homodimers) hardly occurs due to repulsion between the charged residues in the CH3-CH3 interface between identical heavy chains.

Bispecific antibodies can be generated by (transient) transfection of a plasmid encoding a light chain and two different heavy chains that are CH3 engineered to

ensure efficient hetero-dimerization and formation of the bispecific antibodies. The production of these chains in a single cell leads to the favored formation of bispecific antibodies over the formation of monospecific antibodies. Preferred mutations to produce essentially only bispecific full length IgG1 molecules are amino acid substitutions at positions 351 and 366, e.g. L351K and T366K (numbering according to EU numbering) in the first CH3 domain (the 'KK-variant' heavy chain) and amino acid substitutions at positions 351 and 368, e.g. L351D and L368E in the second CH3 domain (the 'DE-variant' heavy chain), or vice versa.

The Fc region mediates effector functions of an antibody, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). Depending on the therapeutic antibody or Fc fusion protein application, it may be desired to either reduce or increase the effector function. Reduced effector functions are preferred in the present invention. Reduced effector function can be desired when an immune response is to be activated, enhanced or stimulated as in some of the embodiments of the invention. Antibodies with reduced effector functions can be used to target cell-surface molecules of immune cells, among others.

Binding of IgG to the FcγRs or C1q was found to require residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain (Figure 2D) are relevant for FcγRs and C1q binding. Substitutions into human IgG1 of IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 were shown to greatly reduce ADCC and CDC (Armour et al., 1999. *Eur J Immunol.* 29(8):2613-24; Shields et al., 2001. *J Biol Chem.* 276(9):6591-604). Furthermore, Idusogie et al. demonstrated that alanine substitution at different positions, including K322, significantly reduced complement activation (Idusogie et al., 2000. *J Immunol.* 164(8):4178-84).

Due to their reduced effector functions, IgG4 antibodies represent an IgG subclass for receptor blocking without cell depletion. IgG4 molecules can exchange half-molecules in a dynamic process termed Fab-arm exchange. This phenomenon can occur between therapeutic antibodies and endogenous IgG4. The S228P mutation is an example of a mutation that ensures reduced capacity to Fab-arm exchange. (Labrijn. et al., 2009. *Nat Biotechnol.* 27(8):767-71).

Antibodies with reduced effector functions are preferably IgG antibodies comprising a modified CH2/lower hinge region, for instance to reduce Fc-receptor

interaction or to reduce C1q binding. In some embodiments the antibody of the invention is an IgG antibody with a mutant CH2 and/or lower hinge domain such that interaction of the bispecific IgG antibody to a Fc-gamma receptor is reduced. An antibody comprising a mutant CH2 region is preferably an IgG1 antibody. Such  
5 a mutant IgG1 CH2 and/or lower hinge domain preferably comprise an amino substitution at position 235 and/or 236 (numbering according to EU numbering), preferably an L235G and/or G236R substitution (Figure 2E).

A variant of an antibody or bispecific antibody as described herein comprises a functional part, derivative and/or analogue of the antibody or bispecific antibody.  
10 The variant maintains the binding specificity of the (bispecific) antibody. The functional part, derivative and/or analogue maintains the binding specificity of the (bispecific) antibody. Binding specificity is defined by capacity to bind an extracellular part of PD-1 and LAG3 as described herein.

A functional part of an antibody, or preferably a functional part of a bispecific  
15 antibody as described herein is a part comprising a variable domain that binds an extracellular part of PD-1 and a variable domain that binds an extracellular part of LAG3. A suitable part is for instance an F(ab')<sub>2</sub> fragment as created by digestion of a bispecific antibody with pepsin. Other parts comprising said variable domains are included in the present invention.

A functional derivative of an antibody, or preferably a functional derivative of a bispecific antibody as described herein is a protein comprising a variable domain that binds an extracellular part of PD-1 and a variable domain that an  
20 extracellular part of LAG3 that are linked by a linker. The variable domains may be variable domains as such, or Fab fragments or variable domain like molecules such as single chain Fv fragments comprising a VH and a VL linked together via a  
25 linker. Other examples of variable domain like molecules are so-called single domain antibody fragment. A single-domain antibody fragment (sdAb) is an antibody fragment with a single monomeric variable antibody region. Like a whole antibody, it is able to bind selectively to a specific antigen. With a molecular weight  
30 of only 12–15 kDa, single-domain antibody fragments are much smaller than common antibodies (150–160 kDa) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa, two variable regions, one from a light and one from a heavy chain). Single-domain

antibodies by themselves are not much smaller than normal antibodies (being typically 90-100kDa). Single-domain antibody fragments are mostly engineered from heavy-chain antibodies found in camelids; these are called VHH fragments (Nanobodies®). Some fishes also have heavy-chain only antibodies (IgNAR, 'immunoglobulin new antigen receptor'), from which single-domain antibody fragments called VNAR fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulin G (IgG) from humans or mice into monomers. Although most research into single-domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been shown to bind specifically to target epitopes. Other non-limiting examples of variable domain-like molecules are VHH, Human Domain Antibodies (dAbs) and Unibodies. Preferred functional parts are parts that comprise variable domains comprising a heavy chain variable region and a light chain variable region. Non-limiting examples of such variable domains are F(ab)-fragments and Single chain Fv fragments. Bispecific formats for variable domain(-like) linkage are for instance Human Serum Albumine (HSA) bound to two different scFv; bispecific mini-antibodies comprising two different scFv bound together via a dimerization motifs or self-associating secondary structures such as helix bundles or coiled coils to bring about dimerization of the scFv fragments (Morrison (2007) Nat. Biotechnol 25:1233-34). Examples of suitable HSA linkers and method for coupling scFv to the linker are described in WO2009/126920.

An antibody or functional part, derivative and/or analogue thereof or preferably a bispecific antibody or functional part, derivative and/or analogue thereof of the present invention is preferably used in humans. To this end an antibody or functional part, derivative and/or analogue thereof of the invention is preferably a human or humanized antibody. Tolerance of a human to a polypeptide is governed by many different aspects. Immunity, be it T-cell mediated, B-cell mediated or other is one of the variables that are encompassed in tolerance of the human for a polypeptide. The constant region of a bispecific antibody of the present invention preferably comprises a human heavy chain constant region, preferably comprising a sequence as depicted in figure 2; and a human light chain constant region, preferably comprising a sequence as depicted in figure 1C. The constant region may contain one or more, preferably not more than 10, preferably not more than 5 amino-acid differences with the constant region of a naturally occurring

human antibody. It is preferred that the constant part is entirely derived from a naturally occurring human antibody. Various antibodies produced herein are derived from common light chain mice immunized with the respective target as described in WO2009/157771. Various antibodies produced herein are derived from  
5 a human antibody variable domain library. As such these variable domains are human. The unique CDR regions may be derived from humans, be synthetic or derived from another organism. The variable region is at least a human variable region when it has with the exception of the CDR regions, an amino acid sequence that is identical to an amino acid sequence of the variable region of a naturally  
10 occurring human antibody. In such embodiments the VH of a variable domain of an antibody that binds an extracellular part of PD-1 or LAG3, or a light chain in an antibody of the invention may contain one or more, preferably not more than 10, preferably not more than 5 amino-acid differences with the variable region of a naturally occurring human antibody, not counting possible differences in the amino  
15 acid sequence of the CDR regions. Such mutations also occur in nature in the context of somatic hypermutation.

Antibodies may be derived from various animal species, at least with regard to the heavy chain variable region. It is common practice to humanize such e.g. murine heavy chain variable regions. There are various ways in which this can be  
20 achieved among which there are CDR-grafting into a human heavy chain variable region with a 3D-structure that matches the 3-D structure of the murine heavy chain variable region; de-immunization of the murine heavy chain variable region, preferably done by removing known or suspected T- or B- cell epitopes from the murine heavy chain variable region. The removal is typically by substituting one or  
25 more of the amino acids in the epitope for another (typically conservative) amino acid, such that the sequence of the epitope is modified such that it is no longer a T- or B-cell epitope.

De-immunized murine heavy chain variable regions are less immunogenic in humans than the original murine heavy chain variable region. Preferably a  
30 variable region or domain of the invention is further humanized, such as for instance veneered. By using veneering techniques, exterior residues which are readily encountered by the immune system are selectively replaced with human residues to provide a hybrid molecule that comprises either a weakly immunogenic or substantially non-immunogenic veneered surface. An animal as used in the

invention is preferably a mammal, more preferably a primate, most preferably a human.

An antibody or bispecific antibody or functional part, derivative and/or analogue thereof according to the invention preferably comprises a constant region of a human antibody. According to differences in their heavy chain constant domains, antibodies are grouped into five classes, or isotypes: IgG, IgA, IgM, IgD, and IgE. These classes or isotypes comprise at least one of said heavy chains that is named with a corresponding Greek letter. In a preferred embodiment the invention provides an antibody according to the invention wherein said constant region is selected from the group of IgG constant regions, i.e. selected from the group consisting of IgG1, IgG2, IgG3 and IgG4. Preferably said constant region is preferably an IgG4 or IgG1 constant region (Figure 2), more preferably a mutated IgG1 constant region. Some variation in the constant region of IgG1 occurs in nature and/or is allowed without changing the immunological properties of the resulting antibody. Typically between about 1-10 amino acid insertions, deletions, substitutions or a combination thereof are allowed in the constant region. The constant region may be mutated as indicated herein for enabling efficient heterodimerization, for reducing effector function or for other reasons including half-life, stability and the like.

Rational methods have evolved toward minimizing the content of non-human residues in the human context. Various methods are available to successfully graft the antigen-binding property of an antibody onto another antibody. The binding properties of antibodies may rest predominantly in the exact sequence of the CDR3 region, often supported by the sequence of the CDR1 and CDR2 regions in the variable domain combined with the appropriate structure of the variable domain as a whole. Various methods are presently available to graft CDR regions onto a suitable variable domain of another antibody. Some of these methods are reviewed in J.C. Almagro<sup>1</sup> and J. Fransson (2008) *Frontiers in Bioscience* 13, 1619-1633, which is included by reference herein.

The light chain variable region of a variable domain comprising a variable heavy chain sequence as depicted in Figure 3, is preferably a germline light chain of or based on O12, preferably the rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or a fragment or a functional derivative thereof (nomenclature according to the IMGT database worldwide web at [imgt.org](http://imgt.org)). The

terms rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01, IGKV1-39/IGKJ1, huV $\kappa$ 1-39 light chain or in short huV $\kappa$ 1-39 are used. The light chain can have 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or combination thereof. The mentioned 1, 2, 3, 4 or 5 amino acid substitutions are preferably conservative amino acid substitutions, the insertions, deletions, substitutions or combination thereof are preferably not in the CDR3 region of the VL chain, preferably not in the CDR1, CDR2 or CDR3 region or FR4 region of the VL chain. A preferred sequence for the common light chain is depicted in figure 1.

Various methods are available to produce bispecific antibodies. One method involves the expression of two different heavy chains and two different light chains in a cell and collecting antibody that is produced by the cell. Antibody produced in this way will typically contain a collection of antibodies with different combinations of heavy and light chains, some of which are the desired bispecific antibody. The bispecific antibody can subsequently be purified from the collection. The ratio of bispecific to other antibodies that are produced by the cell can be increased in various ways. In a preferred embodiment of the invention, the ratio is increased by expressing not two different light chains but two essentially identical light chains in the cell. The two essentially identical light chains can be light chains with essentially the same light chain variable regions and different light chain constant regions or, preferably, two essentially identical light chain constant regions. This concept is in the art also referred to as the “common light chain” method. When the essentially identical light chains work together with the two different heavy chains allowing the formation of variable domains with different antigen-binding sites and concomitant different binding properties, the ratio of bispecific antibody to other antibody that is produced by the cell is significantly improved over the expression of two essentially different light chains. The ratio of bispecific antibody that is produced by the cell can be further improved by stimulating the pairing of two different heavy chains with each other over the pairing of two identical heavy chains. The art describes various ways in which such hetero-dimerization of heavy chains can be achieved. A preferred method is described in US provisional application 61/635,935, which has been followed up by US regular application No. 13/866,747 and PCT application No. PCT/NL2013/050294 (WO 2013/157954 A1), which are incorporated herein by reference. Methods and means are disclosed for producing bispecific antibodies (from a single cell), whereby means are provided

that favor the formation of bispecific antibodies over the formation of monospecific antibodies. These methods can also be favorably employed in the present invention. Thus the invention provides a method for producing a bispecific antibody according to the invention (from a single cell), wherein said bispecific antibody comprises two

5 CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) a first nucleic acid molecule encoding a 1st CH3 domain comprising heavy chain, b) a second nucleic acid molecule encoding a 2nd CH3 domain comprising heavy chain, wherein said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain comprising

10 heavy chains, said method further comprising the step of culturing said host cell and allowing for expression of said two nucleic acid molecules and harvesting said bispecific antibody from the culture. Said first and second nucleic acid molecules may be part of the same nucleic acid molecule, vector or gene delivery vehicle and may be integrated at the same site of the host cell's genome. Alternatively, said

15 first and second nucleic acid molecules are separately provided to said cell. The host cell comprises at least one light chain, and preferably a common light chain.

A preferred embodiment provides a method for producing a bispecific antibody according to the invention from a single cell, wherein said bispecific antibody comprises two CH3 domains that are capable of forming an interface, said

20 method comprising providing:

- a cell having a) a first nucleic acid molecule encoding a heavy chain comprising an antigen binding site that can bind to an extracellular part of PD-1 and that contains a 1st CH3 domain, and b) a second nucleic acid molecule encoding a heavy chain comprising an antigen-binding site that can bind to an

25 extracellular part of LAG3 and that contains a 2nd CH3 domain, wherein said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domains,

said method further comprising the step of culturing said cell and allowing for expression of the proteins encoded by said two nucleic acid molecules and

30 harvesting said bispecific IgG antibody from the culture. In a particularly preferred embodiment, said cell also has a third nucleic acid molecule encoding a common light chain. Said first, second and third nucleic acid molecule may be part of the same nucleic acid molecule, vector or gene delivery vehicle and may be integrated at the same site of the host cell's genome. Alternatively, said first, second and third

nucleic acid molecules are separately provided to said cell. A preferred common light chain is based on O12, preferably it is the rearranged germline human kappa light chain IgV $\kappa$ 1 39\*01/IGJ $\kappa$ 1\*01, as described above. Means for preferential pairing of said 1st and said 2nd CH3 domain are preferably the corresponding mutations in the CH3 domain of the heavy chain coding regions. The preferred mutations to produce essentially only bispecific antibodies are the amino acid substitutions L351K and T366K (numbering according to EU numbering) in the first CH3 domain and the amino acid substitutions L351D and L368E in the second CH3 domain, or vice versa (Figure 2). Further provided is therefore a method according to the invention for producing a bispecific antibody, wherein said first CH3 domain comprises the amino acid substitutions L351K and T366K (numbering according to EU numbering) and wherein said second CH3 domain comprises the amino acid substitutions L351D and L368E, said method further comprising the step of culturing said cell and allowing for expression of proteins encoded by said nucleic acid molecules and harvesting said bispecific antibody from the culture. Also provided is a method according to the invention for producing a bispecific antibody, wherein said first CH3 domain comprises the amino acid substitutions L351D and L368E (numbering according to EU numbering) and wherein said second CH3 domain comprises the amino acid substitutions L351K and T366K, said method further comprising the step of culturing said cell and allowing for expression of said nucleic acid molecules and harvesting said bispecific antibody from the culture. Antibodies that can be produced by these methods are also part of the present invention. The CH3 hetero-dimerization domains are preferably IgG1 hetero-dimerization domains. The heavy chain constant regions comprising the CH3 hetero-dimerization domains are preferably IgG1 constant regions.

A variable domain that “blocks” the binding of PD-1 to PD-L1 and/or PD-L2 interferes with binding of PD-1 to PD-L1 and/or PD-L2. Such a variable domain can bind PD-1. Such a blocking variable domain can bind an epitope on PD-1 and competes with PD-L1 and/or PD-L2 for binding to the epitope. Such a blocking variable domain and PD-L1 and/or PD-L2 can also bind to different epitopes on PD-1. In such cases the blocking activity can for instance be due to diminished binding of the PD-L1 and/or PD-L2, displacement of PD-L1 and/or PD-L2 when it is already bound to PD-1 or can prevent binding to PD-1 through steric hindrance. All these

and other mechanisms can, at least partially, prevent that said binding partner binds to said first membrane protein.

A variable domain that “blocks” the binding of LAG3 to MHC class II interferes with binding of LAG3 to MHC class II. Such a variable domain binds LAG3. Such a blocking variable domain binds an epitope on LAG3 and competes with MHC class II for binding to the epitope. Such a blocking variable domain and MHC class II can also bind to different epitopes on LAG3. In such cases the blocking activity can be due to diminished binding of the PD-L1 and/or PD-L2, displacement of MHC class II when it is already bound to LAG3 or prevent binding to LAG3 through steric hindrance. All these and other mechanisms can, at least partially, prevent that said binding partner bind to said first membrane protein.

The LAG3 ectodomain is composed of four Ig-like domains (D1–D4). The first two N-terminal domains of LAG-3 (D1 and D2) are capable of binding MHC class II. In the present invention it was found that an antibody comprising a variable domain that binds an extracellular part of PD-1 as specified herein and a variable domain that binds an extracellular part of LAG-3 is effective in stimulating an immune response and/or stimulating the formation, stability and/or activity of an immunological synapse. This is so when the variable domain that binds LAG-3 binds extracellular domain 1, extracellular domain 2, extracellular domain 3 or extracellular domain 4 of LAG-3. It is preferred that the variable domain that binds LAG-3 binds extracellular domain 1 or extracellular domain 2. Such variable domains are more effective in the context of an antibody as described herein. In one embodiment the variable domain that binds LAG-3 binds extracellular domain 2 of LAG-3.

A variable domain that blocks the binding of a specific binding pair (i.e. PD-1/PD-L1; PD-1/PD-L2 or LAG3/MHC class II) as described herein typically reduces binding of the pair when compared to the binding in the absence of the variable domain. This is preferably measured in an in vitro assay. Typically this is done by incubating the variable domain with the membrane protein that it can bind to and subsequently incubating the mixture with the other member of the pair. The binding of the pair is then compared with the binding of the pair in the absence of the variable domain. A variable domain can completely prevent the binding of the first membrane protein to a binding partner thereof. It can also partially prevent the binding of the binding pair. A variable domain that blocks the binding of a

specific binding pair of membrane proteins preferably reduces binding of the pair by at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, and more preferably at least 90% when compared to the binding in the absence of the variable domain. Blocking of binding by a variable domain is defined  
5 herein as the blocking obtained using a bivalent monoclonal antibody comprising said two of the same of said variable domains. The variable domain of course also blocks the binding when present in an antibody comprising said variable domain and a variable domain that binds a second membrane protein.

Specific variable domains that can bind an extracellular part of PD-1 and  
10 that at least partially block the binding of PD-1 to PD-L1 and/or PD-L2 are variable domains that comprise the amino acid sequence of the VH of: MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076 or MF6974. Specific variable domains that  
15 can bind an extracellular domain of LAG3 and that block the binding of LAG3 to MHC class II are variable domains that comprise the amino acid sequence of the VH of MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of  
20 Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096.

The invention also provides a method of engaging and/or activating T-cells comprising providing a system comprising a T-cell and a cell (second cell) to which  
25 said T-cell is to be engaged or activated, and providing said system with at least one antibody, preferably at least one bispecific antibody, that comprises a variable domain that can bind PD-1 and a variable domain that can bind LAG3 and incubating said system under conditions that are permissive for the T-cell to become engaged and/or activated. In some embodiments, said method is an *in vitro*  
30 method. The cell to which said T-cell is to be engaged or activated is preferably an immune cell, for example an antigen presenting cell, a macrophage, a neoplastic cell, a virus infected cell, or an intracellular parasite infected cell. Engaging and/or activating T-cells directs T-cells to a specific target. Activating a T-cell is activating the T-cell receptor of said T-cell. Engaging a T-cell typically is activating a T-cell.

Engagement can also direct an already activated T-cell to a target specified by the antibody. Conditions that are permissive for said T-cell to become engaged and/or activated are typically culture conditions but can also be incubation in a non-human animal. The conditions are such that the T-cell is not engaged in the  
5 absence of the antibody. If collections of T-cells are measured some of these can be already engaged or activated provided that the collection contains sufficient T-cells that are not engaged or activated.

An antibody of the invention can bring two cells together in close proximity that allows the interactions between the cells mediated by proteins other than the  
10 PD-1 and LAG3 bound by the antibody of the invention. One such interaction is an interaction of a T-cell receptor of one cell and MHC on the other cell.

In one aspect the invention provides a method for interfering with PD-1 and/or LAG3 mediated inhibition in a PD-1 and/or LAG3 positive cell, the method comprising contacting said cell with an antibody or a functional part, derivative  
15 and/or analogue thereof that comprises

- a variable domain that can bind to an extracellular part of PD-1 and
  - a variable domain thereof that can bind to an extracellular part of LAG3,
- thereby inhibiting PD-1 and/or LAG3 mediated activity in said cell.

A LAG3 or PD-1 positive cell expresses the membrane protein on the cell  
20 membrane in amounts that can be detected, typically by means of immune fluorescence with a monoclonal antibody specific for the membrane protein. The PD-1 positive cell is a T-cell. The LAG3 cell is preferably a T-cell, more preferably a so-called exhausted T-cell. T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections and cancer. It is defined by poor effector  
25 function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. An antibody of the invention interferes with PD-1 and LAG3 mediated inhibition by binding to the respective membrane proteins and preventing stimulation of the proteins by the respective binding  
30 partners of the protein. Known binding partners for PD-1 are PD-L1 and PD-L2. A known binding partner of LAG3 is MHC class II. The antibody blocks the interaction of PD-1 with PD-L1 and/or PD-L2; and/or LAG3 and MHC class II and thereby at least in part prevents the inhibitory activity of the PD-1 in the PD-1 positive cell; and/or the inhibitory activity of LAG3 in a LAG3 positive cell. In a

preferred embodiment of the invention the binding of said PD-1 binding variable domain to PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1. In a preferred embodiment of the invention the binding of said LAG3 binding variable domain to LAG3 blocks the binding of LAG3 to MHC class II. Inhibition of the PD-1 and/or LAG3 mediated activity in said cell can be measured in various ways. Typically, though not necessarily, the activity is measured by measuring activation of CD4<sup>+</sup> or CD8<sup>+</sup> T cells. This can be done by using healthy CD4<sup>+</sup> or CD8<sup>+</sup> T cells, but an effect on exhaustion is typically best measured on exhausted T-cells. Such T-cells are, for instance, positive for LAG3. Activity is preferably measured in HIV-specific T-cells, preferably collected from subjects with progressive disease. Proliferation is a suitable parameter. Proliferation rates can be determined in the presence and the absence of the antibody. Differences in proliferation rates are a measure for the level of inhibition of the activity of LAG3 and or PD-1 in these cells. Other examples of suitable T-cells are TIL collected from subjects with non-small-cell lung carcinoma (NSCLC). Interferon-gamma production is a suitable parameter. Interferon-gamma production can be determined in the presence and the absence of the antibody. Differences in Interferon-gamma production are a measure for the level of inhibition of the activity of LAG3 and or PD-1 in these cells. An increase in the proliferation and /or interferon-gamma production is indicative for inhibition of the activity of LAG3 and or PD-1 in these cells. In a preferred embodiment the increase is an increase of at least 10%, preferably at least 20% more preferably at least 40% more preferably at least 80% over the level or rate detected in the absence of the antibody.

The invention further provides a method for stimulating the formation, stability and/or activity of an immunological synapse comprising providing a system that comprises at least two cells capable of associating with each other via an immunological synapse and providing said system with an antibody or a functional part, derivative and/or analogue thereof that comprises

- a variable domain that can bind to an extracellular part of PD-1 and
- a variable domain that can bind to an extracellular part of LAG3,

thereby stimulating the formation, stability and/or activity of an immunological synapse between said at least two cells. The antibody facilitates the formation, stability and/or activity of an immunological synapse by binding to PD-1 and/or LAG3 on a cell that contains the PD-1 or LAG3 on the cell membrane. The

binding inhibits the activity of PD-1 and/or LAG3. This has the effect that the formation, stability and/or activity of an immunological synapse is stimulated. The variable domain that can bind PD-1 preferably blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1. The variable domain that can bind LAG3  
5 preferably blocks the binding of LAG3 to MHC class II. Said two cells are cells that are capable of forming an immunological synapse. At least one of the cells is a T-cell receptor positive cell. The other cell is typically, though not necessarily an antigen presenting cell. An immunological synapse forms as a result of the tight apposition of a T cell with an antigen-presenting cell (APC) and it is the site where  
10 the T-cell receptor (TCR) is triggered by its antigen ligand, the peptide-MHC complex present in the APC membrane. The immunological synapse in the T-cell membrane typically has three concentric rings of membrane receptors and their underlying cytoskeletal and signaling proteins. The inner circle, or central supramolecular activation cluster (cSMAC), concentrates most of the TCR and  
15 CD28, and it is surrounded by the peripheral SMAC that is formed by integrins. Finally, the most external ring or distal SMAC (dSMAC) is where proteins with large ectodomains are located, such as CD43 and CD45, far from the cSMAC.

The invention further provides an antibody or variant thereof that comprises a variable domain that can bind to an extracellular part of PD-1 and a variable  
20 domain that can bind to an extracellular part of LAG3.

In a preferred embodiment of a method, antibody (or variant thereof) or use of the invention the variable domain that binds PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2. The variable domain that binds LAG3 preferably blocks the binding of LAG3 to MHC class II. Preferably both variable domains block the  
25 binding of the respective binding partners.

The variable domain that binds an extracellular part of PD-1 is preferably defined as a variable domain that when in a bivalent monospecific antibody format that comprises two of said variable domains that bind PD-1, inhibits PD-1/PD-L1 mediated inhibition of T cell receptor mediated activation of a Jurkat cell in a  
30 range of 20-150% when compared to the inhibition obtained with the antibody Nivolumab on a Jurkat cell.

The inhibition of PD-1 inhibition of TCR mediated activation of the Jurkat cell is preferably in the range of 50-150%, preferably 80-150%, more preferably 100-150% when compared to the inhibition obtained with the antibody Nivolumab on

said Jurkat cell. In a preferred embodiment the inhibition is at least 100% when compared to the inhibition obtained with the antibody Nivolumab on said Jurkat cell. PD-1 inhibition of TCR mediated activation of Jurkat cells is preferably measured by measuring an immune dampening effect of PD-1/PD-L1 binding in  
5 Jurkat cells that are incubated under conditions that would, but for the presence of the antibody or functional part, derivative and/or analogue thereof, be activated via the T-cell receptor.

The invention further provides a composition or kit of parts comprising two or more antibodies or functional parts, derivatives and/or analogues thereof, that  
10 comprise a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of a LAG3;

wherein a first and a second of said antibodies or functional parts, derivatives and/or analogues thereof bind

- different epitopes on PD-1;  
15 - different epitopes on LAG3; or  
- different epitopes on PD-1 and LAG3. Embodiments comprising a method, a use, a composition or kit of parts involving two or more antibodies or functional parts, derivatives and/or analogues thereof that have variable domains that bind PD-1 and LAG3 as specified in this paragraph are also referred to as "Oligoclonics"  
20 embodiments. Examples of such Oligoclonics embodiments are embodiments with said first and second antibody. 'Oligoclonics' is a registered trademark. General methods for making such Oligoclonics® products are disclosed in WO 2013/157953 and WO2004/009618 and are incorporated here by reference.

In Oligoclonics embodiments the first and second antibody comprise variable  
25 domains that bind PD-1 and LAG3. Antibodies that have variable domains that bind the same PD-1 or LAG3 can bind the same individual protein, but this is not necessarily so. An antibody of the invention that binds to PD-1 or LAG3 binds an epitope on said protein. An epitope is the part of an antigen, in this case the membrane protein that is recognized by the antibody. First and second antibodies  
30 that bind different epitopes on a membrane protein can bind the same individual protein on the membrane. To this end the different epitopes are preferably non-overlapping epitopes. In other words the different epitopes are sufficiently separated on the membrane protein that two antibodies can bind simultaneously to the same individual protein. It was surprisingly found that Oligoclonics (a

combination of a first and second or more antibodies) can be more effective than the same amount of each of the antibodies alone.

Preferably at least one of the two or more antibodies or functional parts, derivatives and/or analogues comprises a PD-1 binding variable domain that blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1. In a preferred embodiment at least two of the two or more antibodies or functional parts, derivatives and/or analogues comprise a PD-1 binding variable domain that blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1.

Preferably at least one of the two or more antibodies or functional parts, derivatives and/or analogues comprises a LAG3 binding variable domain that blocks the binding of LAG3 to MHC class II. In a preferred embodiment at least two of the two or more antibodies or functional parts, derivatives and/or analogues comprise a LAG3 binding variable domain that blocks the binding of LAG3 to MHC class II.

Preferably at least one of the two or more antibodies or functional parts, derivatives and/or analogues comprises a PD-1 binding variable domain that blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1 and a LAG3 binding variable domain that blocks the binding of LAG3 to MHC class II. Preferably at least two of the two or more antibodies or functional parts, derivatives and/or analogues comprise a PD-1 binding variable domain that blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1 and a LAG3 binding variable domain that blocks the binding of LAG3 to MHC class II.

In one aspect the invention provides methods and uses as described herein wherein two or more antibodies or functional parts, derivatives and/or analogues thereof are used and wherein the two or more antibodies or functional parts, derivatives and/or analogues thereof comprise a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of a LAG3;

wherein a first and a second of said antibodies or functional parts, derivatives and/or analogues thereof bind

- different epitopes on PD-1;
- different epitopes on LAG3; or
- different epitopes on PD-1 and LAG3. The preference for blocking binding of PD-L1 and/or PD-L1 by the variable domains that bind PD-1; and the blocking of

binding of LAG3 to MHC class II by the variable domains that bind LAG3 are the same as a described in the Oligoclonics embodiments.

An antibody or a part, derivative, or analogue thereof, preferably comprises two variable domains as described. Such an antibody is preferably a bispecific  
5 antibody or a functional part derivative or analogue thereof. Two or more antibodies or functional parts, derivatives and/or analogues thereof can be linked together. Various methods are known in the art. A suitable method is conjugation. In addition, the technology of making multi-specific antibodies has progressed to also include bispecific antibodies that have the same overall structure as a normal  
10 mono-specific antibody but wherein the two arms of the antibody each bind a different target. The bispecific antibody or functional part, derivative and/or analogue thereof preferably has two heavy chains with compatible heterodimerization domains. The light chain is preferably a common light chain. The antibody is preferably a full length bispecific antibody that consists of two  
15 heavy chains with compatible heterodimerization domains. The light chain is preferably a common light chain.

As used herein, the term “conjugate” refers to two or more molecules that have been covalently joined, optionally by a linking region. For example, in some embodiments, a conjugate is a first protein or non-protein moiety joined to a second  
20 protein or non-protein moiety by a linking region. For example, in some embodiments of a binding molecule of the invention it comprises or consists of two or more antibodies that have been covalently joined. A conjugate is not limited to a first and second moiety but in some embodiments may also have a third, fourth or more moieties joined by further linking regions. As described elsewhere in this  
25 application, examples of protein moieties include, but are not limited to: a polypeptide, a peptidomimetic or an antibody (or antibody part, derivative, or analogue, as described elsewhere in the application). Examples of non-protein moieties include, but are not limited to aptamers. Numerous types of linker can be used, and the linker will be selected to be appropriate according to the molecule  
30 types in the conjugate and on the desired properties of the linker (length, flexibility, resistance to protease activity and other similar characteristics). Such linkers may comprise nucleotides, polypeptides, or a suitable synthetic material. For example, a linker may be a flexible peptide linker. In certain embodiments, the linker may be a cleavable linker, allowing the parts of the conjugate to be

separated from each other. In other embodiments, a peptide linker might be a helical linker. Various examples and kits for linking proteins and other molecules are well known in the art. As used herein, the term “fusion protein” refers to a protein that comprises two or more polypeptides or proteins that have been joined at the DNA level by recombination and are expressed together as a single polypeptide. A fusion protein may also comprise a peptide linking region also encoded by the DNA and expressed together with the fusion protein. A peptide linker that is part of a fusion protein may be designed to have particular characteristics such as flexibility, hydrophilicity, protease-resistance, cleavability etc. All these properties can be designed within the DNA sequence and methods for designing linkers are well known in the art. For example, antibodies can be linked together by methods well-known in the art, and as described herein, to form bispecific or multi-targeting antibodies. Furthermore, bispecific antibodies can be constructed by various methods known in the art, for example, by using technology such as Biclomics® (see for instance WO2013/157954). A bispecific monoclonal antibody (BsMAb, BsAb) typically comprises binding domains of two different monoclonal antibodies and consequently binds to two different epitopes. Biclomics® molecules, but also other full length IgG bispecific antibodies have two different antigen binding specificities encoded by two different variable regions of a full length IgG molecule of a Fab of a scFv. Biclomics® can be produced by co-transfection of individual cells with genetic constructs encoding two different common light chain (cLC) antibodies as detailed elsewhere herein. CH3 engineering ensures efficient hetero-dimerization and formation of essentially pure bispecific antibodies.

25       An antibody of the present invention is preferably a bispecific antibody. Antibodies typically bind their target via the so-called antigen binding site. An unmodified antigen-binding site is typically formed by and present in a variable domain of the antibody. A variable domain contains the antigen-binding site. A variable domain that can bind an antigen is a variable domain comprising an antigen-binding site that can bind to an antigen.

30       An antibody variable domain typically comprises a heavy chain variable region (VH) and a light chain variable region (VL). The antigen-binding site can be present in the combined VH/VL variable domain, or in only the VH region or only the VL region. When the antigen-binding site is present in one of the two regions of

the variable domain, the counterpart variable region can contribute to the folding and/or stability of the binding variable region, but does not significantly contribute to the binding of the antigen itself.

As used herein, antigen-binding refers to the typical binding capacity of an antibody to its antigen. Binding of an antibody to an antigen can be assessed in various ways. One way is to incubate the antibody with the antigen (preferably cells expressing the antigen), removing unbound antibody (preferably by a wash step) and detecting bound antibody by means of a labeled antibody that binds to the bound antibody.

Antigen binding by an antibody is typically mediated through the complementarity determining regions (CDR) of the antibody and the specific three-dimensional structure of both the antigen and the variable domain allowing these two structures to bind together with precision (an interaction similar to a lock and key), as opposed to random, non-specific sticking of proteins. As an antibody typically recognizes part of an antigen called the epitope of an antigen, and as such epitope may be present in other compounds as well, antibodies according to the present invention may recognize other proteins as well, if such other compounds contain the same epitope. Hence, the term "binding" does not exclude binding of the antibodies to another protein or protein(s) that contain the same epitope. Such other protein(s) is preferably not a human protein.

An antibody typically does not bind to other proteins than the specified target protein on the membrane of cells in a post-natal, preferably adult human.

A variable domain in an antibody or a functional part, derivative and/or analogue thereof of the invention that can bind an extracellular part of PD-1 binds to PD-1 and, under otherwise identical conditions, at least 100-fold lower to the extracellular part of another member of the CD28 family of the same species. A variable domain of an antibody or a functional part, derivative and/or analogue thereof that binds PD-1 binds to PD-1 and, under otherwise identical conditions, at least a 100-fold lower to the CD28, CTLA4, ICOS, BTLA, NKp30 and TMIGD2 of the same species. Considering that PD-1 is a cell surface protein, the binding is typically assessed on cells that express a member on a cell surface.

A variable domain in an antibody or a functional part, derivative and/or analogue thereof of the invention that can bind an extracellular part of LAG3 binds to LAG3 and, under otherwise identical conditions, at least 100-fold lower to the

extracellular part of CD4 of the same species. Considering that LAG3 is a cell surface protein, the binding is typically assessed on cells that express a member on a cell surface.

The invention also provides a method for the treatment of an individual that  
5 has a cancer, the method comprising administering an antibody or a functional part, derivative and/or analogue of the invention or a bispecific antibody of the invention to the individual in need thereof. The individual is preferably an individual that has a cancer. In some embodiments, the cancer is a cancer that comprises cancer cells that express a membrane protein. In a preferred embodiment  
10 the cancer is a cancer that comprises cancer cells that express PD-L1 and/or PD-L2. The cancer is preferably an adenocarcinoma. Preferred cancers are colorectal cancer; pancreatic cancer; lung cancer; breast cancer; liver cancer; prostate cancer; ovarian cancer; cervical cancer; endometrial cancer; head and neck cancer; melanoma; testis cancer; urothelial cancer; renal cancer; stomach cancer; or  
15 carcinoïd cancer. In a preferred embodiment the cancer is colorectal cancer; pancreatic cancer; lung cancer; breast cancer; liver cancer; prostate cancer; ovarian cancer; cervical cancer; endometrial cancer; head and neck cancer; or melanoma. In a particularly preferred embodiment the cancer is colorectal cancer; pancreatic cancer; lung cancer; breast cancer; or liver cancer. In a particularly preferred  
20 embodiment the cancer is a gastrointestinal cancer. In a preferred embodiment the cancer is colorectal cancer. In this embodiment the antibody or functional part, derivative and/or analogue thereof is preferably an antibody with a variable domain that can bind PD-1 and a variable domain that can bind LAG3. A PD-1 binding variable domain preferably blocks the binding of PD-1 to PD-L1 and/or PD-  
25 L2, preferably PD-L1. A LAG3 binding variable domain preferably blocks the binding of LAG3 to MHC class II. Preferably the method comprises two or more of said antibodies or functional parts thereof, as described for an Oligoclones embodiment.

Further provided is a method for stimulating an immune response in an  
30 individual against an aberrant cell in said individual, the method comprising providing said individual with an antibody or a functional part, derivative and/or analogue thereof of the invention. The aberrant cell is preferably a cancer cell, a virus-infected cell, a parasite or a parasite infected cell. In a preferred embodiment the cell is a cancer cell or a neoplastic cell. In this embodiment the antibody or

functional part, derivative and/or analogue thereof is preferably an antibody with a variable domain that can bind PD-1 and a variable domain that can bind LAG3. In this embodiment a PD-1 binding variable domain preferably blocks the binding of PD-1 to PD-L1 and/or PD-L2, preferably PD-L1. A LAG3 binding variable domain  
5 preferably blocks the binding of LAG3 to MHC class II. Preferably the method comprises two or more of said antibodies or functional parts thereof, as described for an Oligoclonics embodiment.

A neoplasm is an abnormal growth of tissue and when it also forms a mass is commonly referred to as a tumor. A neoplasm in the present invention typically  
10 forms a mass. A neoplastic cell is a cell from a neoplasm that has formed a mass. The World Health Organization (WHO) classifies neoplasms into four main groups: benign neoplasms, in situ neoplasms, malignant neoplasms, and neoplasms of uncertain or unknown behavior. Malignant neoplasms are also simply known as cancers.

15 Stimulating an immune response encompasses inducing an immune response and enhancing an already existing immune response. The immune response in an individual can be measured by measuring where applicable; the tumor load of the individual; the virus load of the individual; the parasite load of the individual.

20 Said virus-infected cell is preferably a cell infected with an immune-deficiency virus, a herpes virus, preferably a herpes simplex virus, a varicella-zostervirus, a cytomegalovirus or an Epstein-Barr virus, a papilloma virus, a hepatitis virus, preferably a hepatitis A, B or C virus, a measles virus or an adenoviruses. The virus is preferably a virus known to be able to persist in an  
25 individual. Persistent infections are characterized as those in which the virus is not cleared but remains in specific cells of infected individuals. Persistent infections may involve stages of both silent and productive infection without rapidly killing or even producing excessive damage of the host cells. Persistent virus-host interaction may be a latent, a chronic and/or a slow infection.

30 A parasite-infected cell is a cell that is infected with an intracellular parasite. Such parasites are parasitic microorganisms that are capable of growing and reproducing inside the cells of a host. Some intracellular parasites can also live outside a cell. Such parasites are so-called facultative intracellular parasites. Non-limiting examples are *Listeria monocytogenes*, *Legionella*, certain species of

mycobacterium and *Cryptococcus neoformans*. Preferred intracellular parasites are parasites that cannot grow outside host cells, preferred examples are *Chlamydia*, and closely related species, certain species of mycobacterium such as *Mycobacterium leprae*, certain protozoa, including: Apicomplexans (*Plasmodium* spp., *Toxoplasma gondii* and *Cryptosporidium parvum* and trypanosomatids.

The invention also provides a nucleic acid molecule that encodes an antibody heavy chain variable region according to the invention. The nucleic acid molecule (typically an in vitro, isolated or recombinant nucleic acid molecule) preferably encodes any one of the heavy chain variable regions as depicted in Figure 3 or a heavy chain variable region as depicted in Figure 3 having 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or combination thereof. In a preferred embodiment the nucleic acid molecule comprises a sequence as depicted in Figure 3. The nucleic acid molecule preferably uses codons that are optimized for expression in the antibody producing cell that is to be used. Preferably the nucleic acid encoding a heavy chain variable region as depicted in Figure 3 or a heavy chain variable region as depicted in Figure 3 having 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or combination thereof is codon optimized for expression in a human cell preferably Per.C6TM; or a Chinese hamster, preferably CHO. The invention further provides a nucleic acid molecule that codes for the mentioned heavy chain variable region together with a heavy chain constant region of Figure 2.

A nucleic acid molecule as used in the invention is typically but not exclusively a ribonucleic acid (RNA) or a deoxyribonucleic acid (DNA). Alternative nucleic acids are available for a person skilled in the art. A nucleic acid molecule according to the invention is for instance comprised in a cell. When said nucleic acid molecule is expressed in said cell, said cell can produce an antibody according to the invention. Therefore, the invention in one embodiment provides a cell comprising an antibody according to the invention and/or a nucleic acid molecule according to the invention. An antibody is produced when said cell produces a heavy chain and a light chain. Provided is a cell that can produce an antibody of the invention. The cell preferably comprises a nucleic acid molecule that encodes an antibody heavy chain that comprises an antibody heavy chain variable region that, when combined with a common light chain, can bind said first membrane protein. Said cell preferably further comprises a nucleic acid molecule that encodes

an antibody heavy chain that comprises an antibody heavy chain variable region that, when combined with a common light chain, can bind said second membrane protein. Said cell preferably further comprises a nucleic acid molecule that codes for a common light chain. Said cell is preferably an animal cell, more preferably a mammal cell, more preferably a primate cell, most preferably a human cell. For the purposes of the invention a suitable cell is any cell capable of comprising and preferably of producing an antibody according to the invention and/or a nucleic acid according to the invention.

The invention further provides a cell comprising an antibody according to the invention. Also provided is a cell that comprises one or more nucleic acid molecules that alone or together encode an antibody of the invention. The one or more nucleic acid molecules are expressible nucleic acid molecules meaning that they contain the in cis required signals for RNA transcription and translation of protein coding domains. Preferably said cell (typically an in vitro, isolated or recombinant cell) produces said antibody. In a preferred embodiment said cell is a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NS0 cell or a PER-C6TM cell. In a particularly preferred embodiment said cell is a CHO cell. Further provided is a cell culture that comprises a cell according to the invention. Various institutions and companies have developed cell lines for the large scale production of antibodies, for instance for clinical use. Non-limiting examples of such cell lines are CHO cells, NS0 cells or PER.C6TM cells. These cells are also used for other purposes such as the production of proteins. Cell lines developed for industrial scale production of proteins and antibodies are herein further referred to as industrial cell lines. Thus in a preferred embodiment the invention provides the use of a cell line developed for the large scale production of antibody for the production of an antibody of the invention. The invention further provides a cell for producing an antibody comprising a nucleic acid molecule that codes for a VH, a VL, and/or a heavy chain as depicted in Figures 3, 1 and 2. Preferably said nucleic acid molecule comprises a sequence as depicted in Figures 1 and 2.

The invention further provides a method for producing an antibody comprising culturing a cell of the invention and harvesting said antibody from said culture. Preferably said cell is cultured in a serum free medium. Preferably said cell is adapted for suspension growth. Further provided is an antibody obtainable by a method for producing an antibody according to the invention. The antibody is

preferably purified from the medium of the culture. Preferably said antibody is affinity purified.

A cell of the invention is for instance a hybridoma cell line, a CHO cell, a 293F cell, an NS0 cell or any other cell type known in the art for its suitability for antibody production for clinical purposes, in particular for the production of antibodies used for administration in humans. In a particularly preferred embodiment said cell is a human cell, preferably a cell that is transformed by an adenovirus E1 region or a functional equivalent thereof. A preferred example of such a cell line is the PER.C6TM cell line or equivalent thereof. In a particularly preferred embodiment said cell is a CHO cell or a variant thereof, preferably a variant that makes use of a Glutamine synthetase (GS) vector system for expression of an antibody.

The invention further provides a pharmaceutical composition comprising one or more antibodies or variants thereof according to the invention. The pharmaceutical composition preferably comprises a preferably pharmaceutically acceptable excipient or carrier. An antibody or variant thereof of the invention may further comprise a label, preferably a label for in vivo imaging. Such a label is typically not necessary for therapeutic applications. In for instance a diagnostic setting, a label can be helpful. For instance in visualizing target cells in the body. Various labels are suited and many are well known in the art. In a preferred embodiment the label is a radioactive label for detection. In another preferred embodiment, the label is an infrared label. Preferably the infrared label is suited for in vivo imaging. Various infrared labels are available to the person skilled in the art. Preferred infrared labels are for instance, IRDye 800; IRDye 680RD; IRDye 680LT; IRDye 750; IRDye 700DX; IRDye 800RS IRDye 650; IRDye 700 phosphoramidite; IRDye 800 phosphoramidite (LI-COR USA; 4647 Superior Street; Lincoln, Nebraska).

The amount of antibody according to the invention to be administered to a patient is typically in the therapeutic window, meaning that a sufficient quantity is used for obtaining a therapeutic effect, while the amount does not exceed a threshold value leading to an unacceptable extent of side-effects. The lower the amount of antibody needed for obtaining a desired therapeutic effect, the larger the therapeutic window will typically be. An antibody according to the invention exerting sufficient therapeutic effects at low dosage is, therefore, preferred. The

dosage can be in range of the dosing regimen of Nivolumab. The dosage can also be lower.

An antibody or variant thereof and in particular a bispecific antibody or a variant thereof according to the invention may have fewer side effects than a combination of bivalent monospecific antibodies with the variable domains. Combinations of antibodies that block inhibitory and/or costimulatory molecules benefit patients that do not respond to existing immunotherapies. However, dual blockade of immuno-modulatory receptors (iMODs) has been shown to increase immune-related toxicity. An antibody or variant thereof and in particular a bispecific antibody or a variant thereof according to the invention is suited to address dual blockade of iMODs, as they can exert functional activities that cannot be reproduced by monoclonal antibody combinations, and can more selectively target specific cell populations, which reduces safety liabilities in patients.

In view of the above, a bispecific antibody according to the present invention, or a functional part, derivative and/or analogue thereof, is preferred for therapeutic applications.

The antibodies were produced as bispecific antibodies by cloning them into complementary expression vectors that contain mutations in the CH3 region that drives hetero-dimerization of heavy chains. Many bispecific antibodies were produced at small scale and tested in binding and functional assays on cancer cell lines. An antibody of the invention, particularly a bispecific antibody of the invention can combine low toxicity profiles with high efficacy. An antibody of the invention can be useful in various types and lines of immune targeted therapies. An antibody of the invention can have an increased therapeutic window when compared to an antibody that binds the same antigen(s) with both arms.

Further provided is a use of a bispecific antibody according to the invention or a functional part, derivative and/or analogue thereof, for the preparation of a medicament for the treatment or prevention of aberrant cells, a tumor and/or the formation of metastases. The tumor from which said metastases originate is preferably a tumor that is positive for PD-L1 and/or PD-L2.

Antibodies of the invention can be produced at levels > 50 mg/L after transient transfection in suspension 293F cells. The bispecific antibodies can be purified to greater than 98% purity with yields > 70%. Analytical characterization

studies show bispecific IgG1 antibody profiles that are comparable to bivalent monospecific IgG1.

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

Also provided is a method for the treatment of an individual that has a cancer, the method comprising administering a protein of the invention or a bispecific antibody of the invention to the individual in need thereof.

The invention further provides a protein of the invention or a bispecific antibody of the invention, for use in the treatment of an individual that has cancer.

The antibody or variant thereof of the invention preferably comprises a variable domain that can bind to an extra cellular part of PD-1 and comprises a heavy chain variable region with a CDR3 region that comprises the amino acid sequence of the CDR3 of a variable heavy chain region of one of the VH depicted for MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076, or MF6974. Said variable domain that binds PD-1 preferably comprises a heavy chain variable region comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a variable heavy chain region of one of the VH depicted for MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076, or MF6974.

The antibody or variant thereof preferably comprises a variable domain that can bind to an extra cellular part of PD-1 and comprises a heavy chain variable region that comprises the amino acid sequence of the variable heavy chain region as depicted for MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 in figure 3, preferably MF6256; MF6930; MF6076, MF6974 or MF6226, preferably MF6930, MF6076 or MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect the amino acid sequence of the VH as depicted for MF.

The antibody or variant thereof preferably comprises a variable domain that can bind to an extra-cellular part of LAG3 and that comprises a heavy chain variable region with a CDR3 region that comprises the amino acid sequence of the CDR3 region of the variable heavy chain region as depicted for MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096. Said variable domain that binds LAG3 preferably comprises a heavy chain variable region comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a variable heavy chain region of one of the VH depicted for MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096.

The antibody or variant thereof preferably comprises a variable domain that can bind to an extra cellular part of LAG3 and comprises a heavy chain variable region that comprises the amino acid sequence of the variable heavy chain region as depicted for MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524 of Figure 3, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect the amino acid sequence of the indicated MF.

The invention is further explained in the following examples. These examples do not limit the scope of the invention, but merely serve to clarify the invention.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1.** Common light chain used in mono- and bispecific IgG. Figure 1A:

Common light chain amino acid sequence. Figure 1B: Common light chain variable  
 5 domain DNA sequence and translation (IGKV1-39/jk1). Figure 1C: Common light  
 chain constant region DNA sequence and translation. Figure 1D: IGKV1-39/jk5  
 common light chain variable domain translation. Figure 1E: V-region IGKV1-39A

**Figure 2.** IgG heavy chains for the generation of bispecific molecules. Figure 2A:

10 VH gene. Figure 2B: CH1 region. Figure 2C: hinge region. Figure 2D: CH2 region.  
 Figure 2E: CH2 region containing L235G and G236R silencing substitutions.

Figure 2F: CH3 domain containing substitutions L351K and T366K (KK). Figure  
 2G; CH3 domain containing substitutions L351D and L368E (DE)

Figure 2A VH: dependent on the MF (target): Figure 3.

15 Figure 2B CH1:

Figure 2C Hinge:

Figure 2D CH2:

Figure 2E CH2 containing L235G and G236R silencing substitutions:

Figure 2F CH3: KK of DEKK

20 Figure 2G CH3: DE of DEKK

**Figure 3.** Amino acid sequences of heavy chain variable regions:

Figure 3A heavy chain variable regions of PD-1 specific clones

Figure 3B heavy chain variable regions of LAG-3 specific clones

25

The notation MF refers to a fab containing a heavy chain variable region as  
 depicted and a common light chain. The amino acid sequence of the light chain is  
 indicated in figure 1A. The underlined sequences indicate per amino acid sequence  
 respectively the CDR1, the CDR2 and the CDR3 region.

30

**Figure 4.** Vector map and features of pIRES-Neo3 (MV1363).

**Figure 5.** Vector map and features of pVAX1.

**Figure 6.** Vector map and features of the phagemid vector MV1473 used to generate 'immune' phage display libraries.

**Figure 7.** Vector map and features of the IgG expression vector MV1452, that was used for expression of the PD-1 and PD-L1 specific Fab arms in the KK-variant heavy chain for bispecific IgG generation.

**Figure 8.** Amino acid sequence of the VH gene that is tetanus toxin specific when combined with the common light chain as MF1337, and that is present in the DE-variant heavy chain that was used to generate PD-L1xTT and PD-1xTT bispecific IgG molecules. The underlined sequences indicate per amino acid sequence respectively the CDR1, the CDR2 and the CDR3 region.

**Figure 9.** Vector map and features of the IgG expression vector MV1377, that was used for expression of the TT specific Fab arm MF1337 in the DE-variant heavy chain for bispecific IgG generation.

**Figure 10. PD-1/PD-L1 blocking assay**

Assessment of the capacity of the anti-PD-1 antibody panel to block the interaction of PD-L1 to coated PD-1 at a concentration of 10 µg/ml bispecific IgG. Data are normalized to data obtained with the bivalent benchmark PD-L1 antibody MPDL3280A at a concentration of 10 µg/ml (100% blocking). A representative example is shown of the PD-1 panel. Maximum binding (normalized to 0% blocking) was established by incubation with a non-PD-1/PD-L1 specific human isotype antibody. All PD-1 variable domains comprising MF sequences depicted in Figure 3 and not represented here block the PD-1/PD-L1 interaction >70%.

**Figure 11**

LAG-3 functional activity of a panel of antibodies in a dose titration in the LAG-3 blockade reporter assay. The relation between domain mapping and LAG-3 blocking activity is shown in the LAG-3 is schematically drawn in the right panel.

**Figure 12**

Stimulation index of IL-2 production of LAG-3xPD-1 antibodies in comparison to their parental bivalent LAG-3 antibody. Each IL-2 value is compared to the negative control antibody (not shown) to determine the SI.

5

**Figure 13**

SEB-stimulation of IL-2 production in healthy donor blood cells is enhanced by anti-LAG-3xPD-1 bispecific antibodies in comparison to the parental PD-1 bivalent antibody and 25F7 LAG-3 reference antibody.

10

**Figure 14**

Comparator anti-LAG-3 antibodies. PG1337P300 is a control antibody that is not expected to bind to the cells and binds tetanus toxoid.

Left hand panel shows binding of the indicated antibodies to 293FF-LAG-3 cells that express LAG-3 on the cell membrane. Right hand panel shows activated human T-cells. Binding of antibody to the cells was detected with PE-labelled anti-IgG F(ab')<sub>2</sub>. 25F7\*, determined affinity ~0.2 nM.

15

**Figure 15**

FACS-based LAG-3 panel characterization. Nineteen LAG-3 specific antibodies were expressed in monovalent (PB LAG-3 × TT) and bivalent (PG) format. Antibody binding was tested on activated T cells and 293FF-LAG-3 stable cell lines. The two panels show an example of MF7116 and MF7431 (25F7\*) mono- and bivalent binding on 293FF-LAG-3 cells. Monovalent binding was determined with a bispecific antibody (PB) where one arm has a VH (indicated by the letters MF) of the indicated LAG-3 antibody. The other arm of the antibody has a VH specific for tetanus toxoid (MF1337). Differences in binding were observed between some bivalent/monovalent formats of LAG-3 panel, but not of 25F7\*.

25

30

**Figure 16**

LAG-3 x PD-1 reporter assay validation: bispecific antibodies show activity in LAG-3 x PD-1 reporter assay.

**Figure 17**

PD-1 + LAG-3 reporter assay screening. Panel A, LAG-3 x PD-1 with functional LAG-3 and PD-1 arms. Panel B, LAG-3 x PD-1 with non-functional PD-1 arm with MF5374. Panel C, LAG-3 x PD-1 with non-functional LAG-3 arms with MF7118 and MF7167. \* indicates surrogate arms.

**Figure 18**

Summary of the results reporter assay screening set out in Figure 18. The four digit number immediately following the letters MG indicates the MF number of the heavy chain variable region of the variable domain of the bispecific antibody. For example, the bispecific antibody with the result 52,22 in the upper left hand corner of the table has one arm with the MF6930 and one arm with MF7518. Values are area under the curve (AUC) as % of control.

**Figure 19**

SEB assay screening: example IL-2 production. Panel A, LAG-3 x PD-1 with functional PD-1 and LAG-3 arms. Panel B, LAG-3 x PD-1 with non-functional PD-1 arm with VH MF5374. Panel C, LAG-3 x PD-1 with non-functional LAG-3 arms with VH MF7118 and MF7167. \* indicates a surrogate antibody.

**Figure 20**

Screening results reporter and SEB assays. Arms ranked on (high to low) functionality as bivalent antibodies in reporter assay. Top in each of the columns a LAG-3 arm, Left in each of the rows a PD-1 arm. As indicated for Figure 19, the four digit number immediately following the letters MG indicates the MF number of the heavy chain variable region of the variable domain of the bispecific antibody. Values are AUC as % of control: White = Top 15 %; Grey = Middle 43 %; Black = Lowest 43 %.

**Figure 21**

Ranking LAG-3 x PD-1; PD-1 arms. Matrix files were used to define the Top 15% of bispecific antibodies based on AUC as compared to positive control in: 1) Reporter assay; 2) SEB screening donor 1 (IL-2 data); 3) SEB screening donor 2 (IL-2 data). Next, it was scored how many bispecific antibodies carrying a specific PD-1 Fab

arm were present in this top 15%. Clones with PD-1 arms having a variable domain with a VH of MF6974 or a VH of MF6076 performed best in combination with most of the LAG-3 arms in the reporter and SEB assays. Color/Grey coding, darker indicates higher rank.

5

**Figure 22**

Ranking LAG-3 x PD-1; LAG-3 arms. Matrix file was used to define the Top 15% (left) and top 25% (right) of Bispecifics based on AUC as compared to positive control in: 1) Reporter assay; 2) SEB screening donor 1 based on IL-2 data; 3) SEB screening donor 2 based on IL-2 data. Next, it was scored how many bispecific antibodies carrying a specific LAG-3 Fab arm were present in this top 15%. Fab arms with same score in Top 15% were further ranked by using Top 25% scores. Color/Grey coding, darker indicates higher rank.

15 **Figure 23**

Ranking of PD1 arms. PD-1 arms (left hand column) based on ranking, sequence diversity and binding affinity.

**Figure 24**

20 Ranking of LAG-3 arms. LAG-3 arms (left hand column) based on ranking, sequence diversity and binding affinity.

**Figure 25.**

Effect of test antibodies on IFN- $\gamma$  production in allogeneic mMLR.Mo-DCs were prepared from CD14+ monocytes cultured for 7 days. Immature DCs were used on day 7 and mature DCs were generated by culturing for a further 3 days in maturation medium before being cultured together with T cells isolated by negative selection and test antibody for 4 days (mMLR). IFN- $\gamma$  was measured in culture supernatants by ELISA. Data are normalized to vehicle control. Four separate MLRs were performed.

30

## EXAMPLES

As used herein “MFXXXX” wherein X is independently a numeral 0-9, refers to a Fab comprising a variable domain wherein the VH has the amino acid  
5 sequence identified by the 4 digits. Unless otherwise indicated the light chain variable region of the variable domain typically has a sequence of Figure 1A, typically 1B. “MFXXXX VH” refers to the amino acid sequence of the VH identified by the 4 digits. The MF further comprises a constant region of a light chain and a constant region of a heavy chain that normally interacts with a constant region of a  
10 light chain. PG refers to a monospecific antibody comprising identical heavy and light chains. PB refers to a bispecific antibody with two different heavy chains. The variable region of the heavy chains differs and typically also the CH3 region, wherein one of the heavy chains has a KK mutation of its CH3 domain and the other has the complementing DE mutation of its CH3 domain (see for reference  
15 PCT/NL2013/050294 (published as WO2013/157954).

### Example: 1

#### Generation of materials for selection and screening

##### 20 **Culturing of cell lines**

Freestyle 293F cells (cat. no. p/n51-0029) were obtained from Invitrogen and routinely maintained in 293 FreeStyle medium. HEK293T (cat. no. ATCC-CRL-11268) cells were purchased from ATCC and routinely maintained in DMEM/F12 (Gibco) supplemented with L-Glutamine (Gibco) and FBS (Lonza), and CHO-S (cat.  
25 no. 11619-012) cell lines were purchased from Gibco and routinely maintained in Freestyle CHO expression medium (Invitrogen) supplemented with L-glutamine.

#### **Generation of PD-1 and LAG-3 expression vectors for immunization, and for generation of stable cell lines and transient transfections**

30 Full length cDNA of each target including unique restriction sites for cloning and kozak consensus sequence for efficient translation was either synthesized, or obtained via PCR amplification on a commercially available expression construct, containing the target cDNA, with specific primers that introduced unique restriction sites for cloning and kozak consensus sequence for

efficient translation. The cDNA of each target was cloned into a eukaryotic expression construct such as pIRES-Neo3 (Clontech; Figure 4) or pVAX1 (Thermo Fisher Scientific; Figure 5) via

NheI/EcoRI, resulting in pIRES-Neo3\_[TARGET\_NAME] and  
 5 pVAX1\_[TARGET\_NAME], respectively. The insert sequences were verified by comparison with NCBI Reference amino acid sequences. The pIRES-Neo3 constructs were used for generation of stable cell lines and transient transfections. The pVAX1 constructs were used for immunization purposes. See TABLE 1 for an overview of the names of the resulting constructs.

10 Amino acid sequence full length huPD-1 insert (both in pIRES-Neo3 and pVAX1) for expression on the cell surface (Identical to GenBank: NP\_005009.2):  
 MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPTTFSPALLVVTEGDNATF  
 TCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRD  
 FHMSVVRARRNDSGTLYCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS  
 15 PRPAGQFQTLVVGVGGLLGSLLVWVLAVICSRAARGTIGARRTGQPLKEDP  
 SAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGS  
 ADGPRSAQPLRPEDGHCSWPL

Of which:

20 MQIPQAPWPVVWAVLQLGWR: signal peptide.  
 PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPS  
 NQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTLYCGAI  
 SLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV: ECD of huPD-  
 1.  
 25 VGVVGGLLGSLLVWVLAVI: Predicted TM region.  
 CSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQ  
 TEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL: Intracellular  
 tail.

30 Amino acid sequence full length macaque (*macaca fascicularis*) PD-1 insert (both in pIRES-Neo3 and pVAX1) for expression on the cell surface (Identical to GenBank: ABR15751.1):  
 MQIPQAPWPVVWAVLQLGWRPGWFLESPDRPWNAPTTFSPALLLVTEGDNATF  
 TCSFSNASESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTRLPNGRD

FHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS  
 PRPAGQFQALVVGVGGLLGSLLVWVLAVICSRAAQGTIEARRTGQPLKEDP  
 SAVPVFSVDYGELDFQWREKTPEPPAPCVPEQTEYATIVFPSGLGTSSPARRGSA  
 DGPRSPRPLRPEDGHCSWPL

5

Of which:

MQIPQAPWPVWVAVLQLGWR: signal peptide.

PGWFLESPDRPWNAPTFSPALLLVTEGDNATFTCSFSNASESFVLNWYRMSPS  
 NQTDKLAAPEDRSQPGQDCRFVTRLNNGRDFHMSVVRARRNDSTYLCGAI

10 SLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQALV: ECD of maPD-  
 1.

VGVVGGLLGSLLVWVLAVI: Predicted TM region.

CSRAAQGTIEARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPAPCVPEQ  
 TEYATIVFPSGLGTSSPARRGSADGPRSPRPLRPEDGHCSWPL: Intracellular

15 tail.

Amino acid sequence full length human LAG-3 insert (both in pIRES-Neo3 and  
 pVAX1) for expression on the cell surface (Identical to GenBank: NP\_002277.4):

MWEAQFLGLLFLQPLWVAPVKPLQPGAIEVPVWVAQEGAPALPCSTIPLQDL

20 SLLRRAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVGP

GGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRDRALS

CRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVP

VRESPHHHLAESFLFPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLPEPT

PLTVYAGAGSRVGLPCRLPAGVGTRSFLLTAKWTPPGGGPDLLVTGDNGDFTLR

25 LEDVSQAQAGTYTCHHLQEQLNATVTLAHTVTPKSFSGPSLGKLLCEVTPV

SGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERLLGAAYVF

TELSSPGAQRSGRAPGALPAGHLLFLILGVLSLLLLVTGAFGFHLWRRQWRPR

RFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEPEQL

30 Of which:

MWEAQFLGLLFLQPLWVAPVKP: signal peptide.

LQPGAIEVPVWVAQEGAPALPCSTIPLQDLSLLRRAGVTWQHQPDSGPPAAA

PGHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGLRSGRLPLQPRVQLDERGRQ

RGDFSLWLRPARRADAGEYRAAVHLRDRALSCRLRLRLGQASMTASPPGSLRA

SDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPIHHHLAESFLFLPQVSPM  
 DSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRLPAGV  
 GTRSFLTAKWTPPGGGPDLLVTGDNGDFTLRLEDVVSQAQAGTYTCHHLQEQQ  
 LNATVTLAIITVTPKSFSGPSLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGP  
 5 WLEAQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQRSGRAPGALPAGH  
 L: ECD.

LLFLILGVLSLLLLVTGAFGF: Predicted TM region.

HLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEQL  
 : Intracellular tail.

10

Amino acid sequence full length rat (*Rattus norvegicus*) LAG-3 insert (both in  
 pIRES-Neo3 and pVAX1) for expression on the cell surface (Identical to GenBank:  
 NP\_997678.2):

MRQDLFLDLLLLQLLWEAPVVSSGPGKELSVVWAQEGAPVHLPCSLEFPHLDP  
 15 NFLRRGWVTWQHRPDSQDQASIPALDLLQGMPSTRRHPPHRYTVLSVAPGGLR  
 SGRQPLLSHVQLEKRGPPRGDFSLWLRPATRKDAGEYHAFVRLPDRDFSCSLR  
 LRVGQASMIASPPGTLKPSDWVILNCSFSRPDRPVSVHWFQGGQSRVPVHNSPR  
 HYLAESFLLLPQVSPLDSGTWGCVLTYRDGFNVSTYNLKVQGLEPVAPLTVYA  
 AEGSRVELPCHLPPVVGTPSLLIAKWTPPGGGPELPTGKSGNFTLQLENVGRA  
 20 QAGTYTCSIHLQGRQLSAAVTLAVITVTPKSFGLPGSPQKLLCEVVPASGEGRFV  
 WRPLSDLSRSSLGPVLELQEAKLLAEQWQCQLYEGQKLLGATVYTAESSSGAW  
 SAKRISGDLKGGHLFLSLILGALALFLLVTGAFGFHLWRRQLLRRRFSALEHGIR  
 PPPVQSKIEELEREPETEMEPETEPDPEPQPEPELEPEESRQL

25 Of which:

MRQDLFLDLLLLQLLWEAPVVSS: signal peptide.

GPGKELSVVWAQEGAPVHLPCSLEFPHLDPNFLRRGWVTWQHRPDSQDQASIP  
 ALDLLQGMPSTRRHPPHRYTVLSVAPGGLRSGRQPLLSHVQLEKRGPPRGDFS  
 LWLRPATRKDAGEYHAFVRLPDRDFSCSLRLRVGQASMIASPPGTLKPSDWVIL  
 30 NCSFSRPDRPVSVHWFQGGQSRVPVHNSPRHYLAESFLLLPQVSPLDSGTWGCV  
 LTYRDGFNVSTYNLKVQGLEPVAPLTVYAAEGSRVELPCHLPPVVGTPSLLIAK  
 WTPPGGGPELPTGKSGNFTLQLENVGRAQAGTYTCSIHLQGRQLSAAVTLAVI  
 TVTPKSFGLPGSPQKLLCEVVPASGEGRFVWRPLSDLSRSSLGPVLELQEAKLL  
 AEQWQCQLYEGQKLLGATVYTAESSSGAWSAKRISGDLKGGHL: ECD.

FLSLILGALALFLLVTGAFGF: Predicted TM region.

HLWRRQLLRRRFSALEHGIRPPPQVQSKIEELEREPETEMEPETEPDPEPQPEPE  
LEPESRQL: Intracellular tail.

- 5 Amino acid sequence full length macaque (*Macaca mulatta*) LAG-3 insert (both in pIRES-Neo3 and pVAX1) for expression on the cell surface (Identical to GenBank: *Macaca mulatta*):

MWEAQFLGLLFLQPLWVAPVKPPQPGAEISVVWAQEGAPAQLPCSPTIPLQDLS  
LLRRAGVTWQHQPDSGPPAPAPGHPPAPGHRPAAPYSWGPRPRRYTVLSVGP  
10 GLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRATVHLRDRALSC  
RLRLRVGQASMTASPPGSLRTSDWVILNCSFSRPDRPASVHWFRSRGQGRVPV  
QGSPHHHLAESFLFLPHVGPMDSGLWGCILTYRDGFNVSIMYNLTVLGLPAT  
PLTVYAGAGSRVELPCRLPPAVGTQSFLTAKWAPPGGGPDLLVAGDNGDFTLR  
LEDVSQAQAGTYICHIRLQGQQLNATVTLAHTVTPKSFGSPGSLGKLLCEVTPA  
15 SGQEHFVWSPNLTPSQRSFSGPWLEAQEAQLLSQPWQCQLHQGETLLGAAVYF  
TELSSPGAQRSGRAPGALRAGHLPLFLILGVLFLLLLVTGAFGFHLWRRQWRPR  
RFSALEQGIHPPQAQSKIEELEQEPELEPEPELERELGPEPEPGPEPEPEQL

Of which:

- 20 MWEAQFLGLLFLQPLWVAPVKP: signal peptide.  
PQPGAEISVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQPDSGPPAPAP  
GHPPAPGHRPAAPYSWGPRPRRYTVLSVGPGLRSGRLPLQPRVQLDERGRQR  
GDFSLWLRPARRADAGEYRATVHLRDRALSCRLRLRVGQASMTASPPGSLRTS  
DWVILNCSFSRPDRPASVHWFRSRGQGRVPVQGSPPHHHLAESFLFLPHVGPMD  
25 SGLWGCILTYRDGFNVSIMYNLTVLGLPATPLTVYAGAGSRVELPCRLPPAVG  
TQSFLTAKWAPPGGGPDLLVAGDNGDFTLRLEDVSQAQAGTYICHIRLQGQQL  
NATVTLAHTVTPKSFGSPGSLGKLLCEVTPASGQEHFVWSPNLTPSQRSFSGPW  
LEAQEAQLLSQPWQCQLHQGETLLGAAVYFTELSSPGAQRSGRAPGALRAGHL:  
ECD.  
30 PLFLILGVLFLLLLVTGAFGF: Predicted TM region.  
HLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPELEPEPELERELGPEPEPG  
PEPEPEQL: Intracellular tail.

### Generation of stable cell lines expressing PD-1 or LAG-3

pIRES-Neo3\_[TARGET\_NAME] expression constructs (TABLE 1) were used to generate CHO-S or Freestyle 293F clones stably expressing the respective proteins. Constructs were transiently transfected in CHO-S and Freestyle 293F  
5 cells using lipofectamine transfection, and screened by FACS using antibodies reacting with the respective proteins. After confirmation of expression, transiently transfected cells were seeded in limiting dilution and cultured under selection pressure relevant for the used expression construct to obtain stable cell clones. After 2-3 weeks of selection, clones were screened by FACS. The selected clones  
10 were expanded by serial passage, retested in FACS and frozen to -150°C. The names of clones that stably express the heterologous proteins are CHO-S\_[TARGET\_NAME] cells or Freestyle 293F\_[TARGET\_NAME] cells. See TABLE 1 for an overview of the constructs used to generate the stable cell lines and their resulting name.

15

### Example 2

#### Immunization, selection and screening

##### Mice used for immunizations

For generation of human antibodies binding to huPD-1 and huLAG-3, mice  
20 transgenic for the human VK1-39 light chain (common light chain mice, see W02009/157771) and for a human heavy chain (HC) minilocus (comprising a selection of human V gene segments, all human Ds and all human Js) were immunized. These mice are referred to as 'MeMo®' mice. Mice were immunized with either recombinant protein antigen, or DNA encoding the protein antigen as  
25 briefly described below.

##### Protein immunizations

'MeMo®' mice were immunized by subcutaneous injections with recombinant protein and Gerbu adjuvant MM (Gerbu Biotechnik; cat. no. 3001).  
30 Recombinant huPD-1-Fc (R&D; cat.no. 1086-PD) and huLAG-3-His (Abcam; cat. no. Ab184729) were used for immunizations. Mice were immunized with 40 µg recombinant protein in PBS mixed with 40 µl of adjuvant in a total volume of 100 µl. Subsequently mice were boosted on day 14 and 28 with 20 µg of recombinant protein in PBS together with 20 µl of adjuvant in a total volume of 50 µl. Mouse

serum was collected at day 35 to determine serum titers. Mice with low serum reactivity against the human and/or macaque target received additional cycles of booster immunizations with recombinant human or macaque protein antigen and serum analyses. Each cycle consisted of two weekly immunizations using to 20 µg  
5 of recombinant protein in 50 µl PBS followed one week later by serum collection for titer analysis. Mice showing high serum titers against the human and macaque target received a final boost immunization consisting of daily injections with 20 µg of recombinant protein in 50 µl PBS on three consecutive days. One day after the final injection mouse lymphoid tissue was collected.

10

### **DNA immunizations**

MeMo® mice were immunized by DNA tattooing using a micropigmentation device. DNA tattoo immunizations were performed with 20 µg plasmid DNA encoding the target antigen (pVAX1\_[TARGET\_NAME], TABLE 1). Mice were  
15 immunized with DNA encoding the human target only (PD-1 and LAG-3) or by alternating immunizations with DNA encoding the human and rat (LAG-3) target to obtain species cross-reactive antibodies. Mice were immunized at day 0, 3, 6, 14, 17, 28 and 31. Mouse serum was collected at day 35 to determine serum titers. Mice with low serum reactivity against the human and/or macaque target received  
20 additional cycles of booster immunizations with human DNA antigen, and serum analyses. Each cycle consisted of two weekly DNA immunizations followed one week later by serum collection for titer analysis. Mice showing strong serum reactivity against cells expressing the human and macaque target received a final boost immunization followed after 3 days by collection of lymphoid tissue.

25

### **Determination of serum titers**

Serum titers were determined by FACS analysis using cell lines expressing the human and macaque target antigens.

### **30 Generation of synthetic phage Fab libraries**

Synthetic libraries were constructed based on a repertoire of germline human VH genes that were selected for frequent use in natural repertoires and canonical sequence diversity. Synthetic HCDR3 regions were added to these VH genes using PCR. This was done using forward primers that anneal to framework 1

of the VH genes and include a SfiI restriction site for cloning. Reverse primers included sequences to anneal to framework 3 of the VH genes, followed by randomized sequences to encode HCDR3 diversity and a framework 4 encoding sequence also containing a BstEII and XhoI restriction site for cloning. Synthetic  
5 CDR3 regions were either completely random or encoded a more restricted diversity based on the frequency of use of amino acid residues at certain positions within the HCDR3. PCR products encoding the VH genes were cloned into phage display vectors in fusion with phage M13 gene 3 protein using aforementioned restriction enzymes and also containing a common light chain encoding gene. Large  
10 scale ligation and transformation of E.coli TG1 resulted in large libraries of synthetic Fab fragments displayed on phage which were used for panning on antigens or cells to identify antigen-specific Fab fragments.

#### **Generation of 'immune' phage Fab libraries by RT-PCR from tissues of 15 immunized mice**

Spleen and draining lymph nodes were removed from mice for which a significant humoral response was observed against the respective target proteins. Single cell suspensions were generated from both spleen and inguinal lymph nodes and subsequently these tissues were lysed in Trizol LS Reagent (Thermo Scientific  
20 c#10296028) and stored at -80°C until use.

From successfully immunized mice, the inguinal lymph nodes were used for the construction of 'immune' phage antibody repertoires. RNA was extracted from the single cell suspensions of the lymphoid tissue. 1µg of total RNA was used in a RT reaction using an IgG-CH1 specific primer. The resulting cDNA was then used  
25 to amplify the polyclonal pool of VH-encoding cDNA using in-house adapted VH-specific primers essentially as described in Marks et al. (J Mol Biol. 1991 Dec 5;222(3):581-97). The resulting PCR product was then cloned in a phagemid vector (FIGURE 6) for the display of Fab fragments on phage, as described in de Haard et al. (J Biol Chem. 1999 Jun 25;274(26):18218-30) with the exception that the light  
30 chain (Figure 1A and 1B) was the same for every antibody and was encoded by the vector. After ligation, the phagemids were used to transform E.coli TG1 bacteria and transformed bacteria were plated onto LB-agar plates containing ampicillin and glucose. All phage libraries contained  $>4 \times 10^5$  transformants and had an insert frequency of  $> 90\%$ . Bacteria were harvested after overnight growth and used to

prepare phage according to established protocols (de Haard et al., J Biol Chem. 1999 Jun 25;274(26):18218-30).

**Selection of phage carrying Fab fragments specifically binding to human target protein from synthetic and 'immune' phage Fab libraries using recombinant proteins**

The phage Fab libraries that were generated were used to select target specific Fabs using phage display on directly coated recombinant proteins. For PD-1, huPD-1-Fc (R&D; cat. no. 1086-PD) and huPD-1 biotin (BPS bioscience; cat. no. 71109) were used. For LAG-3, huLAG-3-Fc (R&D; cat. no. 2319-L3), huLAG-3-Fc (Enzo; cat. no. ALX-522-078), huLAG-3-His (Abcam; cat. no. Ab184729) and ratLAG-3 His (SinoBiological; cat. no. 80367-R08H) were used.

For selections with non-biotinylated recombinant protein ('panning selections'), proteins were coated onto the wells of a MAXISORP™ ELISA plate. The MAXISORP™ ELISA plates were blocked with 4% dried skimmed milk (Marvel) in PBS. Phage Fab libraries were also blocked with 4% Marvel and, when Fc tagged recombinant protein was used, also with excess of human IgG to deplete for Fc region binders prior to the addition of the phage library to the coated antigen.

Incubation of the phage library with the coated protein was performed for 1.5 hrs at room temperature under shaking conditions. Plates or tubes were then washed fifteen times with 0.05% Tween-20 in PBS followed by 5 times washing with PBS. Bound phage were eluted for 20 minutes using trypsin, after which trypsin was neutralized with AEBSF trypsin inhibitor (Sigma).

For selections with biotinylated protein ('in-solution selections'), neutravidin was coated onto the well of a MAXISORP™ ELISA plate. The MAXISORP™ ELISA plates were blocked with 1% casein in PBS. In parallel, biotinylated protein and phage Fab libraries were blocked for 30 minutes in 0.5% casein in PBS, containing an excess of human IgG, in separate Eppendorf tubes. Thereafter, the blocked phage and biotinylated protein were mixed and incubated for 2 hours at room temperature. The mixture was thereafter added to the neutravidin coated wells for 20 minutes to capture the phage Fab particles that were bound to biotinylated protein. Plates were then washed fifteen times with 0.05% Tween-20 in PBS followed by 5 times washing with PBS. Bound phage were eluted for 20

minutes using trypsin, after which trypsin was neutralized with AEBSF trypsin inhibitor (Sigma).

The eluates of both selection strategies ('panning and in-solution') were added to *E. coli* TG-1 and incubated at 37°C for phage infection. Subsequently  
5 infected bacteria were plated on agar plates containing Ampicillin and glucose, and incubated at 37°C overnight. Single clones from the selection outputs were screened for target binding in ELISA or FACS depending on the target.

For selections with synthetic phage Fab libraries, a second round selection was performed after rescue of the first round selection output using the same  
10 protocol as outlined above for the first round selection. The same selection antigen that was used in the first round was also used in the second round, with exception of first round raLAG-3-His selections that were followed by a second round selection with huLAG-3-His.

### 15 **Selection of phage carrying Fab fragments specifically binding to human target from 'immune' phage Fab libraries using cells stably expressing the target protein**

Phage Fab libraries that were generated from target immunized mice were selected using phage display on cells expressing the respective target. The stable  
20 cell lines expressing PD-1 or LAG-3 (Table 1) were used for 1<sup>st</sup> round selections. Cells were blocked with 10% FBS in PBS. After blocking, the rescued phage were incubated with blocked cells. Cells plus phage were incubated for 1 hr at 4°C. Washing the cells (5 times) was performed using 1 ml of 10% FBS in PBS. Bound phage were eluted using trypsin for 20 minutes, after which trypsin was  
25 neutralized with AEBSF trypsin inhibitor (Sigma). The eluate was added to *E. coli* TG-1 and incubated at 37°C for phage infection. Subsequently, phage-infected bacteria were plated on agar plates containing ampicillin and glucose, and incubated at 37°C overnight.

### 30 **Screening for Target specific Fab clones in ELISA**

Of single clones, soluble Fab or phage were prepared (J Mol Biol. 1991 Dec 5;222(3):581-97; J Biol Chem. 1999 Jun 25;274(26):18218-30). Obtained soluble Fab or phage samples were diluted (1:5 or 1:10, respectively) in 4% dried skimmed milk (Marvel) in PBS (blockbuffer) and tested for binding in ELISA to wells coated with

the same antigen as was used for selection, or with huLAG-3-His (Abcam; cat. no. Ab184729) for all selection outputs performed with ratLAG-3 His (SinoBiological; cat. no. 80367-R08H).

5 Bound Fabs were detected by staining with an anti-myc antibody (Roche; cat. no. 11667203001) diluted 1:1000 in blockbuffer, followed by a HRP-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch; cat. no. 715-035-150) diluted 1:5000 in blockbuffer. Bound phage were detected by staining with a HRP-conjugated monoclonal anti-M13 antibody (GE healthcare; cat. no. 27-9421-01) diluted 1:5000 in blockbuffer.

10 After each antibody staining, wells were washed with PBS-T (PBS-0.05% v/v Tween 20). Bound secondary antibody was visualized by TMB/H<sub>2</sub>O<sub>2</sub> staining and staining was quantified by means of OD<sub>450nm</sub> measurement. Clones were considered to bind the target when the OD<sub>450nm</sub> was at least three times above the background signal obtained with a negative control Fab.

15 The VH-encoding cDNA's of all target-specific clones were sequenced. A selection of unique clones based on sequence identity and cluster analysis was then analyzed in FACS on binding to PD-L1 expressed on cells as described below for the clones obtained from the cell selection outputs.

## 20 **Screening for Target specific Fab clones in FACS**

Of single clones, selected on cells expressing the respective target, soluble Fab or phage were prepared as described (J Mol Biol. 1991 Dec 5;222(3):581-97; J Biol Chem. 1999 Jun 25;274(26):18218-30). Fab samples were tested for binding in FACS to cells expressing the human and macaque target (Table 1) by incubation  
25 with a mix of 1:5 diluted Fab sample with 1:1000 diluted anti-myc antibody (Gentaur; cat. no. 04-CMYC-9E10) in FACS buffer (0.5% HI-FBS in PBS). Bound Fab/anti-myc complexes were detected by incubation with an APC-conjugated goat anti-mouse IgG antibody (BD Bioscience; cat. no. 550826) diluted 1:500 in FACS buffer.

30 Phage samples were tested for binding in FACS by diluting the phage samples 1:3 in blockbuffer and incubation with target expressing cells for 1 hour. Bound phage were detected by staining with a biotinylated anti-M13 antibody (Fitzgerald, cat. nr. 61R-M101ABTB62-FEZ, 1:125 in FACS buffer, 30 minutes on ice) and PE-labeled streptavidin (Invitrogen, cat. nr. SA1004-4; 1:400 in FACS

buffer for 15 minutes on ice). After each antibody incubation, wells were washed three times with FACS buffer. Stained cells were analysed using a FACS Accuri C6 instrument (Becton and Dickinson). Clones were considered positive when the mean fluorescence intensity was at least three times above the background signal  
5 obtained with a negative control Fab.

### **Example 3**

#### **Characterization huLAG-3 and huPD-1 specific Fab clones in IgG format**

##### **10 Recloning human LAG-3 and PD-1 specific Fab to IgG format**

A selection of unique clones, based on CDR3 sequence and VH germline differences, that bound human and macaque target protein expressed on cells, was then re-cloned to an IgG expression plasmid such as MV1452 (Figure 7), which contained the common light chain (Figure 1), using Sfi1-BstEII digestion and  
15 ligation of the pool of digested cDNA's according to standardized molecular biological techniques.

##### **Expression of bispecific IgG containing a human LAG-3 or human PD-1 specific Fab and a tetanus toxin specific Fab**

20 Bispecific antibodies were generated by transient co-transfection of two plasmids encoding IgG with different VH domains, using a proprietary CH3 engineering technology to ensure efficient hetero-dimerisation and formation of bispecific antibodies. The common light chain present on both plasmids containing the heavy chain is also co-transfected in the same cell. In our co-pending  
25 applications (e.g. WO2013/157954 and WO2013/157953; incorporated herein by reference) we have disclosed methods and means for producing bispecific antibodies from a single cell, whereby means are provided that favor the formation of bispecific antibodies over the formation of monospecific antibodies. These methods can also be favorably employed in the present invention. Specifically, preferred  
30 mutations to produce essentially only bispecific full length IgG molecules are amino acid substitutions at positions 351 and 366, e.g. L351K and T366K (numbering according to EU numbering) in the first CH3 domain (the 'KK-variant' heavy chain) and amino acid substitutions at positions 351 and 368, e.g. L351D and L368E in the second CH3 domain (the 'DE-variant' heavy chain), or vice versa

(Figure 2). It was previously demonstrated in our co-pending applications that the negatively charged DE-variant heavy chain and positively charged KK- variant heavy chain preferentially pair to form heterodimers (so-called 'DEKK' bispecific molecules). Homodimerization of DE-variant heavy chains (DE-DE homodimers) or  
5 KK-variant heavy chains (KK-KK homodimers) hardly occurs due to strong repulsion between the charged residues in the CH3-CH3 interface between identical heavy chains.

VH genes encoding the antibodies binding human LAG-3 and PD-1 described above were cloned into the MV1452 IgG expression vector encoding the  
10 positively charged CH3 domain. A tetanus toxin (TT) targeting antibody (Figure 8) was cloned into the MV1377 IgG expression vector (Figure 9) encoding the negatively charged CH3 domain. For expression of the LAG-3 and PD-1 antibody panel in IgG format, the entire panel was also cloned into the negatively charged CH3 domain vector to be able to produce bivalent LAG-3 or PD-1 IgG. Suspension  
15 growth-adapted 293F Freestyle cells were cultivated in T125 flasks on a shaker plateau until a density of  $3.0 \times 10^6$  cells/ml. Cells were seeded at a density of  $0.3\text{-}0.5 \times 10^6$  viable cells/ml in each well of a 24-deep well plate. The cells were transiently transfected with a mix of two plasmids encoding different antibodies, cloned into the proprietary vector system. Seven days after transfection, the cellular  
20 supernatant was harvested and filtered through a  $0.22 \mu\text{m}$  filter (Sartorius). The sterile supernatant was stored at  $4^\circ\text{C}$  until purification of the antibodies.

### Purification of bispecific IgG

Purification of IgG was performed on a small scale ( $< 500 \mu\text{g}$ ), using protein-  
25 A affinity chromatography. Small scale purifications were performed under sterile conditions in 24 well filter plates using filtration. First, the pH of the medium was adjusted to pH 8.0 and subsequently, IgG-containing supernatants were incubated with protein A Sepharose CL-4B beads (50% v/v) (Pierce) for 2hrs at  $25^\circ\text{C}$  on a shaking platform at 600 rpm. Next, the beads were harvested by filtration. Beads  
30 were washed twice with PBS pH 7.4. Bound IgG was then eluted at pH 3.0 with 0.1 M citrate buffer and the eluate was immediately neutralized using Tris pH 8.0. Buffer exchange was performed by centrifugation using multiscreen Ultracel 10 multiplates (Millipore). The samples were finally harvested in PBS pH7.4. The IgG concentration was measured using Octet. Protein samples were stored at  $4^\circ\text{C}$ .

**IgG quantification using Octet**

To determine the amount of IgG purified, the concentration of antibody was determined by means of Octet analysis using protein-A biosensors (Forte-Bio, according to the supplier's recommendations) using total human IgG (Sigma Aldrich, cat. nr. I4506) as standard.

**Specificity analysis huLAG-3 and huPD-1 IgG**

The antibodies (bivalent LAG-3 antibodies and bispecific PD-1xTT antibodies) were tested for binding in FACS to the stable cell lines expressing the relevant human and macaque orthologs (Table 1) and the wt cells. Therefore, cells were harvested and diluted to  $10^6$  cells/ml in FACS buffer (PBS/0.5%BSA/0.5mM EDTA).  $1-2 \times 10^5$  cells were added to each well in a U-bottom 96 well plate. Cells were centrifuged for 2 minutes at 300 g at 4°C. Supernatant was discarded by inverting the plate(s). 50µl of each IgG sample at a concentration of 10 µg/ml was added and incubated for 1H on ice. Cells were centrifuged once, supernatant was removed and cells were washed twice with 150µl of FACS buffer. 50µl diluted 1:400 goat anti human IgG PE (Invitrogen) was added and incubated for 30 minutes on ice in the dark. After adding FACS buffer, cells were centrifuged once, supernatant was removed and cells were washed twice with FACS buffer. Cells were analyzed on a FACSCanto Flow cytometer (Becton and Dickinson) in a HTS setting. Binding of the antibodies to cells was assessed by measuring the mean fluorescence intensity (MFI) of the stained cell population. Antibodies were considered to bind their target when the MFI was at least five-fold that of the same cell population stained with a (negative control) non-binding antibody (directed to tetanus toxoid).

**Binning huPD-1 specific Fab arms present in the PD-1xTT bispecific IgG on ligand blocking ability**

huPD-1 binding clones were tested for their ability to block the interaction of PD-L1 with PD-1. Therefore PD1-Fc (R&D systems; cat. no. 1086-PD) was coated to a maxisorp plate at 1 µg/ml. Coated wells were blocked with 4% BSA in PBS. Thereafter, 0.55 µg/ml biotinylated PD-L1 (BPS bioscience; cat. no. 71105) was added in the presence or absence of IgG in the range of 0.15 to 20 µg/ml. Bound biotinylated PD-L1 was detected with HRP-conjugated streptavidin (BD bioscience:

cat. no. 554066) diluted 1:2000 in block buffer. After each incubation step, the ELISA plate was washed three times with PBS-T (PBS-0.05% v/v Tween 20). Bound streptavidin was visualized by TMB/H<sub>2</sub>O<sub>2</sub> staining and staining was quantified by means of OD<sub>450nm</sub> measurement. Clones were considered to block the interaction of PD-1 with PD-L1 when the ELISA signal was reduced more than 70% at an IgG (PD-1xTT) concentration of 10 µg/ml, compared to a control in which a TT specific competition antibody was added. See Figure 10 for the results obtained with a representative selection of the PD-1 antibody panel tested as PD-1xTT bispecific molecules.

### **Affinity ranking huLAG-3 and huPD-1 specific Fab arms present in the LAG-3xTT and PD-1xTT bispecific IgG**

Bispecific antibodies that were shown to bind the respective human and macaque orthologs in FACS were ranked on apparent affinity for both orthologs in FACS. Therefore, the stable cell lines expressing the respective orthologs (Table 1) were harvested and diluted to 10<sup>6</sup> cells/ml in FACS buffer (PBS/ 0.5% BSA/ 0.5mM EDTA). Cells were centrifuged for 2 minutes at 300 g at 4°C. Supernatant was discarded by inverting the plate(s). 50 µl of each IgG sample, in a 11-step, 2-fold dilution series ranging from 10 to 0.01 µg/ml, was added and incubated for 1h on ice. Cells were centrifuged once, supernatant was removed and cells were washed twice with 150 µl of FACS buffer. 50 µl diluted 1:400 goat anti human IgG PE (Invitrogen) was added and incubated for 30 minutes on ice in the dark. After adding FACS buffer, cells were centrifuged once, supernatant was removed and cells were washed twice with FACS buffer. Cells were analyzed on a FACSCanto Flow cytometer (Becton and Dickinson) in a HTS setting. Binding of the antibodies to cells was assessed by measuring the mean fluorescence intensity (MFI) of the stained cell population. Antibodies were considered to bind their target when the MFI was at least five-fold that of the same cell population stained with a (negative control) non-binding antibody (directed to tetanus toxoid).

Binning huLAG-3 specific Fab arms present in LAG-3xLAG-3 bivalent IgG on domain specificity huLAG-3 binding clones in bivalent IgG format were tested for domain specificity in FACS on HEK293T cells that were transiently transfected with five different pIRES-Neo3 mouse/human LAG-3 hybrid expression constructs, a FL mouse LAG-3 pIRES-Neo3 expression construct (see amino acid insert

sequences below) or the pIRES-Neo3\_huLAG-3 expression construct used for generation of stable huLAG-3 expressing Freestyle 293F cells (Table 1). The same FACS protocol was used as described above during specificity analysis of the antibody panel. For generation of the hybrid constructs the extracellular domain of mouse and human LAG-3 was divided in 5 domains; 4 Ig-like domains based on Uniprot reference sequences P18627 (huLAG-3) and Q61790 (moLAG-3) and 1 hinge domain from end of Ig-like domain 4 to the transmembrane domain. The following amino acid insert sequences were cloned into pIRES-Neo3 (Figure 4) via *NheI/EcoRI*; Text in bold is the signal peptide. Underscored text is the sequence identical to human LAG-3. Text in Italics represent the transmembrane and intracellular domain sequences.

*Amino acid sequence full length mouse LAG-3 insert.*

**MREDLLLGFLLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNL**  
 DPNFLRRGGVIWQHQPDSGQPTPIPALDLHQGMPSRQPAPGRYTVLSVAPGG  
 LRSGRQPLPHVQLEERGLQRGDFSLWLRPALRTDAGEYHATVRLPNRALSCS  
 LRLRVGQASMIASPSGVKLKLSDWVLLNCSFSRPDRPVSVHWFQGGQNRVPVYNS  
 PRHFLAETFLLLPQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVAPLTV  
 YAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEAVG  
 LAQAGTYTCSIHLQGQQLNATVTLAVITVTPKSFGLPGSRGKLLCEVTPASGKER  
 FVWRPLNNLSRSCPGPVLEIQEARLLAERWQCQLYEGQRLLGATVYAAESSGA  
 HSARRISGDLKGGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFSALEHG  
*IQFPFAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL*

*Amino acid sequence mo/huLAG-3 chimeric insert A (Full length mouse LAG-3 sequence in which the mouse signal peptide and Ig-like domain 1 is replaced by the human signal peptide and Ig-like domain 1).*

**MWEAQFLGLLFLQPLWVAPVKPLQPGA****EVVVWAQEGAPAQLPCSPTIPLQ**  
DLSLLRRAGVTWQHQPDSGPPAAAPGHPLAPGHPAAPSSWGPRPRRYTVLSV  
GPGGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRDRA  
LSCRLRLRLGQASMIASPSGVKLKLSDWVLLNCSFSRPDRPVSVHWFQGGQNRVP  
 VYNSPRHFLAETFLLLPQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVA  
 PLTVYAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHL  
 EAVGLAQAGTYTCSIHLQGQQLNATVTLAVITVTPKSFGLPGSRGKLLCEVTPAS

GKERFVWRPLNNLSRSCPGPVLEIQEARLLAERWQCQLYEGQRLLGATVYAAE  
SSGAHSARRISGDLKGGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFS  
ALEHGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

- 5 *Amino acid sequence mo/huLAG-3 chimeric insert B (Full length mouse LAG-3 sequence in which the mouse Ig-like domain 2 is replaced by the human Ig-like domain 2).*

**MREDLLLGFLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNL**  
DPNFLRRGGVIWQHQPDSGQPTPIPALDLHQGMPSRQPAPGRYTVLSVAPGG  
10 LRSGRQPLPHVQLEERGLQRGDFSLWLRPALRTDAGEYHATVRLPNRALSCS  
LRLRVGQASMTASPPGSLRASDWVILNCFSRDPDRPASVHWFRNRGQGRVPVR  
ESPHHHLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSITYNLKVLGLEPVAPL  
TVYAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEA  
VGLAQAGTYTCSIHLQGGQLNATVTLAVITVTPKSFGPLPGSRGKLLCEVTPASG  
15 KERFVWRPLNNLSRSCPGPVLEIQEARLLAERWQCQLYEGQRLLGATVYAAES  
SSGAHSARRISGDLKGGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFSA  
LEHGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

- 20 *Amino acid sequence mo/huLAG-3 chimeric insert C (Full length mouse LAG-3 sequence in which the mouse Ig-like domain 3 is replaced by the human Ig-like domain 3).*

**MREDLLLGFLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNL**  
DPNFLRRGGVIWQHQPDSGQPTPIPALDLHQGMPSRQPAPGRYTVLSVAPGG  
LRSGRQPLPHVQLEERGLQRGDFSLWLRPALRTDAGEYHATVRLPNRALSCS  
25 LRLRVGQASMIASPSGVCLKLSDWVLLNCFSRDPDRPVSVHWFQGGQNRVPVYNS  
PRHFLAETFLLLPQVSPLDSGTWGCVLTYRDGFNVSIMYNLTVLGLEPPTPLTV  
YAGAGSRVGLPCRLPAGVGTRSELTAKWTPPGGGPDLLVTGDNGDFTLRLEDV  
SQAQAGTYTCHIHLEQQQLNATVTLAVITVTPKSFGPLPGSRGKLLCEVTPASGK  
ERFVWRPLNNLSRSCPGPVLEIQEARLLAERWQCQLYEGQRLLGATVYAAESSS  
30 GAHSARRISGDLKGGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFSALE  
HGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

*Amino acid sequence mo/huLAG-3 chimeric insert D (Full length mouse LAG-3 sequence in which the mouse Ig-like domain 4 is replaced by the human Ig-like domain 4).*

**MREDLLLGFLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNL**  
 5 DPNFLRRGGVIWQHQPDSGQPTPIPALDLHQGMPSRQPAPGRYTVLSVAPGG  
 LRSGRQPLHPHVQLEERGLQRGDFSLWLRPALRTDAGEYHATVRLPNRALSCS  
 LRLRVGQASMIASPSGVCLKLSDWVLLNCSFSRPDRPVSVHWFQGGQNRVPVYNS  
 PRHFLAETFLLLQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVAPLTV  
 YAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEAVG  
 10 LAQAGTYTCSIHLQGQQLNATVTLAITVTPKSFGSPGSLGKLLCEVTPVSGQER  
FVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERLLGATVYAAESS  
 GAHSARRISGDLKGGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFSALE  
 HGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

15 *Amino acid sequence mo/huLAG-3 chimeric insert E (Full length mouse LAG-3 sequence in which the mouse Ig-like domain 4 including the hinge up to the transmembrane sequence is replaced by the human Ig-like domain 4 including the hinge).*

**MREDLLLGFLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNL**  
 20 DPNFLRRGGVIWQHQPDSGQPTPIPALDLHQGMPSRQPAPGRYTVLSVAPGG  
 LRSGRQPLHPHVQLEERGLQRGDFSLWLRPALRTDAGEYHATVRLPNRALSCS  
 LRLRVGQASMIASPSGVCLKLSDWVLLNCSFSRPDRPVSVHWFQGGQNRVPVYNS  
 PRHFLAETFLLLQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVAPLTV  
 YAAEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEAVG  
 25 LAQAGTYTCSIHLQGQQLNATVTLAITVTPKSFGSPGSLGKLLCEVTPVSGQER  
FVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERLLGAAVYFTELSS  
PGAQRSGRAPGALPAGHLVLVLILGALSFLLVAGAFGFHWWRKQLLLRRFSAL  
 EHGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

30 Tables 3 and 4 show the bins to which each LAG-3xLAG-3 bivalent IgG was assigned based on domain specificity.

### Reference antibodies

Antibodies that inhibit the function of PD-1 and LAG-3 are known in the art. The information with regard to the anti-PD-1 antibody Nivolumab was generated based on the information disclosed in CA2607147 and was expressed in  
5 CHO-S cells. The anti-LAG-3 antibody 25F7 was regenerated based on information provided in WO2010/019570A2 (Medarex, Inc) recloned in an IgG1 backbone and expressed in 293F Freestyle cells.

### LAG-3 blockade reporter assay

10 The LAG-3 blockade reporter assays were performed using the LAG-3 blockade reporter assay developed by Promega that uses a two cell system based on Raji cells that expressed MHCII and a Jurkat / NFAT-RE Reporter Cell Line overexpressing LAG-3. Activation of Jurkat cells is controlled via superantigen SED, Staphylococcal Enterotoxins D. LAG-3 Jurkat Effector cells were provided by  
15 Promega in a Cell Propagation Model (CPM) format and propagated in RPMI 1640 (+L-glutamine, 10% heat inactivated FBS, 100  $\mu$ M MEM non-essential amino acids, 1 mM sodium pyruvate, 200  $\mu$ g/ml Hygromycin and 500  $\mu$ g/ml G418). Raji cells were propagated in RPMI 1640 (+L-glutamine and 10% heat inactivated FBS). Cells growing in logarithmic phase were harvested and resuspended in RPMI 1640  
20 containing 1% heat inactivated FBS) at a concentration of  $2 \times 10^6$  cells/ml Raji and  $4 \times 10^6$  cells/ml for Jurkat / NFAT-RE cells. Next, 25  $\mu$ l Jurkat / NFAT-RE cell suspension was added to the inner wells of a 96 well plate (Corning, Cat#3917). Next, 25  $\mu$ l test antibody in assay medium (RPMI 1640 containing 1% FBS) in a serial dilution (starting concentration 25  $\mu$ g/ml) was added to each well. Each plate  
25 contained a serial dilution of negative (PG1337) and positive control antibody 25F7 that served as reference controls. Finally 25  $\mu$ l of a 1:1 mixture of Raji cells and (100 ng/ml) SED (Toxin Technologies) were added. Plates were incubated for 6 H at 37°C, 5% CO<sub>2</sub> in 95% relative humidity. 40  $\mu$ l of luciferase (Bio-Glo Luciferase Assay System, cat. no. G794L) was added the next day and the amount of  
30 luciferase activity was measured using aBioTek Synergy 2 Multi-Mode Microplate Reader. LAG-3 antibodies were screened in bivalent format to determine their LAG-3 blocking capacity (Figure 11).

**PD-1 / PD-L1 blockade reporter assay**

The PD-1/PD-L1 blockade reporter assays used were developed by Promega and are based on a two cell system; CHO cells expressing PD-L1, and a T cell activator and a Jurkat / NFAT-RE Reporter Cell Line overexpressing PD-1. The

5 PD-1 / PD-L1 blockade reporter assays were performed using the thaw and use format of Promega. PD-L1 expressing cells (cat. no. C187103) were thawed in 14.5 ml Cell Recovery Medium (DMEM/F12 containing 10% FBS). Next, 50 µl cell suspension was added to the inner wells of a 96 well half area plate (Corning, cat. no. 3688). Plates were incubated overnight at 37°C, 5% CO<sub>2</sub>, in 95% relative

10 humidity. Next day, culture medium was removed and 20 µl test antibody in assay medium (RPMI 1640 containing 4% FBS) in a serial dilution (starting concentration 10 µg/ml) was added to each well. Each plate contained a serial dilution of negative (Ctrl Ab) and positive control antibody (Nivolumab) that served as reference controls. PD-1 effector cells (cat no. C187105) were thawed in 5.9 ml

15 Assay medium and 20 µl cell suspension was added to each well. Plates were incubated for 6 H or overnight at 37°C, 5% CO<sub>2</sub>, in 95% relative humidity. 40 µl of luciferase (Bio-Glo Luciferase Assay System, cat. no. G794L) was added the next day and the amount of luciferase activity was measured using aBioTek Synergy 2 Multi-Mode Microplate Reader. Potency was measured as luciferase activity in

20 comparison to the negative control antibody.

**PBMC isolation**

Human whole blood was obtained from buffy coats (Sanquin) and was diluted 1:1 with PBS. Leucosep tubes (Greiner Bio-One cat. no. 227 290) were filled

25 with 17.5 ml Ficoll-Paque Plus (Amersham Biosciences cat. no. 17-1440-02) warmed at room temperature (RT). Ficoll-Paque Plus was spun down for 30 seconds at 1000 x g at RT. 30 ml of diluted whole blood was poured on top. The tubes were spun at 1000xg for 10 minutes at RT and the mononuclear PBMC interface was harvested, washed twice in PBS and resuspended in 250 µl PBS. The PBMCs were counted

30 and readjusted to 1×10<sup>6</sup>/ml in tissue culture medium (DMEM with 10% FCS) and frozen down by adding an equal volume of ice-cold freeze medium (80% culture medium/20% DMSO). Cells were stored in 1 ml aliquots at -150°C until further use.

**SEB assay**

The functional activity of the bispecific antibodies was determined by using PBMCs stimulated by *Staphylococcus* enterotoxin B (SEB). SEB specifically activates T cells expressing the Vβ3 and Vβ8 T cell receptor chain. PBMCs from 3 donors is thawed, washed, counted and resuspended in culture medium (RPMI1640 plus 10% heat inactivated FBS) to a concentration of  $2 \times 10^6$  cells/ml. Cells were seeded in flat bottom 96-well plates ( $2 \times 10^5$  cells/well) in the presence of SEB (2000 or 125 ng/ml). Antibody serial dilutions starting at 20 μg/ml were added. Each plate contained a serial dilution of negative (Ctrl Ab) and positive control antibody (, nivolumab and LAG-3(25F7) that served as reference controls. Cells were stimulated for 3 days at 37°C, 5% CO<sub>2</sub> in 95% relative humidity prior to being tested for cytokine secretion and/or cell surface expression of antigens.

**Cytokine assays**

ELISA: After stimulation of T-cells or PBMCs at various times, plates were centrifuged and media was removed. Cytokine levels were detected by AlphaLISA in accordance with the manufacturer's instructions (Perkin Elmer). Concentrations were calculated based on the standard curve.

Luminex assay: Another method used to determine cytokine production in vitro was using luminex analysis developed by eBioscience. Levels of IL-2 were measured in culture supernatants following manufacturers' instructions. Results were analyzed by eBioscience analysis software.

**Screening of the PD-1 antibody panel**

VH from the PD- antibody panel were produced in 24 well format and tested as bivalent antibodies in a semi log serial titration (starting concentration 10 μg/ml) in the PD-1/PD-L1 blockade reporter assay to rank the antibodies for blocking potency in comparison to Nivolumab. Based on the activity data antibodies were selected from the PD-1 antibody panel for the subsequent PD1xLAG-3 bispecific screen. The activity of the selected candidates in the reporter assay is shown in Table 2. The PD-1 Fab panel was composed of functional activity variants within two antibody clusters i.e. A and B.

### Screening PD1xLAG-3 antibody panel

VH from the PD-1 and LAG-3 antibody panel were recloned into the charged engineered Fc-silenced vectors such that upon expression of the antibody heavy chains hetero dimerisation of heavy chains is forced resulting in the generation of bispecific antibodies after transfection. The PD-1 Fab arms were cloned in the MV1625 vector whereas the LAG-3 Fab arms were recloned in the MV1624 vector. Fifteen LAG-3 Fab arms representing the different bins (Table 3) were combined with three PD-1 Fab arms (MF6226, MF6930 and MF6256) displaying a range of PD-1 blocking activities (Table 2). Bispecific LAG-3xPD-1 antibodies (Table 4), their bivalent parental LAG-3 antibodies and negative control antibodies were tested for their capacity to activate T cells by a serial dilution of SEB (2000 – 500 – 125 – 31 – 8 – 2 ng/mL). Figure 12 shows the activity of two LAG-3 bivalent antibodies in comparison to LAG-3xPD-1 antibodies with different PD-1 affinity on PBMC cells stimulated with 2 µg/ml SEB. The activity is represented as stimulation index. Each IL-2 value is compared to the negative control antibody to determine the SI (SI, IL-2 of 2 means 200% increase in IL-2 production when compared to the control antibody). LAG-3xPD-1 bispecific antibodies induced more IL-2 in the SEB assay in comparison to the bivalent LAG-3 antibodies. Selections of bispecific antibodies, representing each bin, were subsequently screened in a serial dilution in a SEB assay whereby PBMC were stimulated with 2 µg/ml SEB (Figure 13). The majority of bispecific LAG-3xPD-1 antibodies were more potent than the parental PD-1 bivalent antibody or reference LAG-3 antibody in inducing IL-2 release. LAG-3xPD-1 bispecific antibodies that bound other domains than the MHC Class II interacting domain i.e. domain 3 and 4 were also more potent than the parental PD-1 bivalent antibody or reference LAG-3 antibody in inducing IL-2 release.

### Example 4. Screening of a PD1xLAG-3 antibody panel

The Fab of antibody 25F7, a LAG-3 antibody, was cloned and produced in a human IgG1 format as PG7431 as described above in Example 3. The variable domain of 25F7 was also cloned into a monovalent LAG-3 antibody format as PB22283 wherein the first arm comprised the 25F7 variable domain and the second arm comprised a tetanus toxoid binding variable domain (MF1337). Binding of these

antibodies are shown in Figure 14. PG1337P300 is a control antibody that is not expected to bind to the cells and binds tetanus toxoid.

Binding of these antibodies was compared with the bivalent antibody PG7116 which has two variable domains with MF7116 and a monovalent LAG-3 antibody format wherein the first arm comprised the variable domain of MF7116 and the second arm comprised the tetanus toxoid variable domain comprising MF1337.

Binding of the antibodies in bivalent form is similar as can be seen by comparing the binding of PG7431 with PG7116 in the left hand panel where the antibodies were titrated on Freestyle 293F\_huLAG-3 (293FF LAG-3). The binding of the monovalent variable domain of 25F7 is similar to the binding in bivalent form (compare PB22283 with PG7431 in Figure 15, right hand panel, where the antibodies were titrated on activated T-cells). Binding of monovalent variable domain with MF7116 is reduced when compared to the bivalent antibody PG7116 (compare PB21775 with PG7116, Figure 15, left hand panel).

Accordingly, LAG-3 IgGs were tested in binding assays in bivalent and monovalent formats, and were compared to a 25F7 (PG7431) antibody. LAG-3 antibodies in bivalent format showed similar binding curves as the benchmark antibodies. Monovalent LAG-3×TT antibodies typically showed reduced binding activity compared to the bivalent molecules.

In order to further characterize PD-1×LAG-3 bispecific antibodies, a panel of bispecific PD-1 x LAG-3 antibodies was created as set out in Table 5 and functionally tested in a PD-1×LAG-3 reporter assay.

In the PD1/Lag3 reporter assay, Jurkat Effector Cells as described above (modified to overexpress PD-1 and LAG-3) and target Raji Cells (modified to overexpress PD-L1), SED and LAG-3 antibodies are mixed and incubated. The Jurkat cell line contains a luciferase reporter gene that can become activated through the NFAT (nuclear factor of activated T-cells) pathway. Interaction of the MHCII with LAG-3 will inhibit this signal and blocking the MHCII / LAG-3 interaction by biologics can release the signal as well as the blocking of the PD-1/PD-L1 signal by biologics on the PD-1 receptor.

The PD-1 x LAG-3 bispecific antibodies were titrated in the PD-1/LAG-3 reporter assay and the activity was compared with a bivalent LAG-3 antibody 25F7 and the bivalent PD-1 antibody (both as described above in Example 3). The

bispecific antibodies facilitate the activation of the Jurkat cells at significantly lower concentrations than the monospecific bivalent control antibodies. The activity is comparable to the activity when the two control antibodies are combined, in spite of the fact that the bispecific antibodies are monovalent for each of the targets which, as demonstrated above, can reduce the binding of a monovalent LAG-3 antibody to LAG-3 (see Figure 16).

A representative example of the reporter assay screening is presented in Figure 17: various combinations of LAG-3 and PD-1 variable domains are depicted.

Panel A shows bispecific antibodies with variable domains that bind PD-1 and LAG-3 and that block the binding of natural ligands to the receptor (respectively PD-L1/L2 and MHC class II). The particular bispecific antibodies depicted in panel A allow the activation of the Jurkat reporter cell than a reference having the two monospecific bivalent antibodies to PD-1 and 25F7. Panel B shows bispecific antibodies having the same LAG-3 binding variable domain but now in combination with a PD-1 variable domain that binds PD-1 but that does not block PD-1/PD-L1 signaling. It is clear that the activity of the bispecific antibody in panel A is mediated by at least the arm that binds PD-1 and that blocks PD-1/PD-L1 signaling. Panel C shows the reverse example, the PD-1 arm of panel A in combination with LAG-3 binding variable domains that bind, but that do not block MHC II/LAG-3 signaling. The results shows that the activity of the bispecific antibody depicted in Panel A is mediated at least by a LAG-3 binding arm that blocks the binding of MHC class II to LAG-3.

Figure 18 summarizes the results of the reporter screening and indicates the area under the curve relative to control.

SEB assays were carried out as described in Example 3. Figure 19 sets out a representative example of an SEB titration assay of the PD-1/LAG-bispecific antibodies as described herein. The figure shows the activity of two LAG-3 bivalent antibodies in comparison to PD-1xLAG-3 antibodies with different PD-1 affinity on PBMC cells stimulated with 2 µg/ml SE.

Panel A shows the result of bispecific antibodies with variable domains that bind PD-1 and LAG-3 and that block the binding of natural ligands to the respective receptors. These antibodies facilitate the production of IL-2 in the PBMC cells. Panel B shows the results with bispecific PD-1 x LAG-3 antibodies that have a PD-1 variable domain that binds PD-1 but that does not block the binding of PD-

L1 to the receptor. The LAG-3 variable domain blocks the binding of LAG-3 to MHC II. Panel C shows the results with bispecific antibodies with a variable domain that binds LAG-3 but that does not block the binding of LAG-3 to MHC II. The variable domain that binds PD-1 blocks the binding of PD-1 to PD-L1.

5 A summary of the screening of the bispecific panel set out in Table 5 is depicted in Figure 20. The PD-1 variable domains were ranked top to bottom depending on the activity of the bivalent antibody in the reporter assay (upper panel) and SEB assay based on 2 donors (middle and lower panel) with the PD-1 variable domain with the highest activity in the reporter assay at the top. The  
10 LAG-3 variable domains are ranked high to low in the reporter assay (upper panel) and SEB assay based on 2 donors (middle and lower panel) with the variable domain with the highest activity in the bivalent antibody format in the first column.

The PD-1 arms within bispecific PD-1xLAG3 antibodies were ranked by  
15 determining how many times a given arm within a PD-1xLAG3 bispecific appeared in the top 15% of bispecific antibodies based on percent AUC as compared to positive control in: 1) Reporter assay; 2) SEB screening donor 1 (IL-2 data); 3) SEB screening donor 2 (IL-2 data). See Figures 21 and 23. This ranking is illustrated in the two right most columns of Figure 23.

20 It can be seen that clones with PD-1 arms having a variable domain with a VH of MF6974 or a VH of MF6076 were ranked highest based on the above criteria with most of the LAG-3 arms in the Reporter and SEB assays.

The same approach was taken for bispecific antibodies carrying a specific LAG-3 arms, which were also scored on the basis of how many times they were  
25 present in the top 15% (those arms with same score in the top 15% were further ranked by using the top 25% scores). The final ranking is set out in Figure 22.

Several bivalent, monospecific antibodies (in IgG format) that bind LAG3 domain 1, according to mouse/human LAG-3 hybrid binning experiments described above, scored highest based on AUC percent of a positive control in a reporter  
30 assay. However, the bispecific antibodies binding PD1 and LAG3 that were ranked the highest based on reporter assays and in the SEB assay in IL-2 induction were those that domain 2 (as determined according to the mouse/human LAG-3 hybrid experiments).

This is summarized in Figure 24. Of the top eight bispecifics as ranked based on the PD1/LAG3-reporter assay and SEB, six comprised a LAG3 arm that binds domain 2 (as determined according to the mouse/human LAG-3 hybrid experiments). The activity of the LAG3 arms (on the basis of AUC percent of control) from these 6 bispecifics as determined by LAG3 reporter assay in bivalent monospecific format was typically lower than that of LAG3 arms tested in the same format which were determined to bind domain 1.

### Example 5

#### **Bispecific PD-1 x LAG-3 antibodies enhance IFN $\gamma$ production by CD14+ T cells in a mixed lymphocyte reaction.**

Mixed lymphocyte reaction (MLR) assays are commonly used to understand the effects of antibodies on T-cell activation and proliferation. Such assays aid understanding of whether such compounds will affect the potential of T cells to mount a response in the tumor microenvironment. Here we used an allogeneic MLR protocol with immature DCs to determine the ability of bispecific PD-1 x LAG-3 antibodies to enhance IFN $\gamma$  production by CD14+ T cells, compared with that of benchmark reference antibodies. The responsiveness of the T cells was quantified by measuring the levels of IFN $\gamma$  in culture supernatant.

To this end, human peripheral blood mononuclear cells (PBMCs) from healthy donors were prepared from buffy coats. Immature monocyte-derived dendritic cells (Mo-DCs) were prepared by isolating CD14+ cells (EasySep Stemcell, lot no. 16C69672) using magnetic activated cell sorting (MACS) and culturing these in differentiation medium for seven days. Responder T cells derived from a different donor to that used for the Mo-DCs were prepared from cryopreserved PBMCs on the day required, using a T-cell isolation kit (EasySep Stemcell, lot no. 16D70573) to obtain untouched T cells. Six separate MLRs were performed to provide biological replication.

For the assay,  $1 \times 10^4$  immature Mo-DCs were co-cultured with  $1 \times 10^5$  CD14+ T cells for 4 days, in the presence or absence of test antibody at an end concentration of 10  $\mu\text{g/mL}$ . Cultures were performed in triplicate. Supernatants were collected at the end of the culture period and assessed for IFN $\gamma$  by ELISA

(R&D BioTechne, lot no. 342687) according to the manufacturer's instructions with plates read at 450 nm.

## Results

5

The MLR study comprised experimental groups of a LAG3/PD1 bispecific (PB15307 = MF7137 (LAG3) and MF6930 (PD1)) and LAG3 isotype control group (mono-specific antibody against a LAG-3 (bivalent monospecific antibody PG7431), Tetanus and mIgG1 ). Single cell controls and a vehicle control group were also  
10 included.

Cultures were performed in triplicate to provide technical replicates. At the end of the 4-day culture period supernatant was collected and ELISAs performed to assess effects of the antibodies on the production of IFN- $\gamma$ , according to manufacturer's instructions with plates read at 450 nM.

15

CD14+ cells were sorted on DO and cultured for 7 days, immature DCs were used on D7 and mature DCs were obtained by culturing for an additional 3 days in maturation medium. CD14 positivity was assessed at DO to confirm purity of the initial sort, and in immature and matured DCs at D7 and D10, respectively, to confirm downregulation of CD14 to indicate differentiation to Mo-DCs (data not  
20 shown). Viability and activation markers (CD80, CD83 and CD86) were also assessed on both immature and matured DCs to confirm differentiation and maturation. Mo-DCs (immature or mature) were cultured with responder T cells for 4 days before supernatant was collected and ELISAs performed to assess effects of the test antibodies on production of IFN- $\gamma$ . In the mature MLR (mMLR) the  
25 donor variance was such that the data were normalized to vehicle control for each donor (raw data and normalized).

25

In Figure 25 the results of the test are shown. The bispecific antibody specific for PD-1/LG-3 out-performed IFN- $\gamma$  production by CD14+ T cells in MLR significantly over the control and LAG3 surrogate monospecific antibody.

30

**Table 1:** Expression constructs for each target that were used for DNA immunization (pVAX1 vector based) and for generation of stable Freestyle 293F or CHO-S cell lines (pIRES-neo3 vector based or similar)

| Target | Vectors            | Stable cell line       |
|--------|--------------------|------------------------|
| PD-1   | pVAX1_huPD-1       | NA                     |
|        | pIRES-neo3_huPD-1  | CHO-S_huPD-1           |
|        | pIRES-neo3_maPD-1  | CHO-S_maPD-1           |
| LAG-3  | pVAX1_huLAG-3      | NA                     |
|        | pVAX1_raLAG-3      | NA                     |
|        | pIRES-neo3_huLAG-3 | Freestyle 293F_huLAG-3 |
|        | pIRES-neo3_maLAG-3 | Freestyle 293F_maLAG-3 |

5 hu = human, ma = macaque, ra = rat, NA = not applicable

**Table 2**

Functional activity of PD-1 Fab arms as measured in the PD-1 / PD-L1 blockade reporter assay as a bivalent antibody in comparison to the positive control Nivolumab. Variants of the same cluster (B) that displayed different of PD-1 blocking activity were tested.

| Clone  | CDR3          | Cluster | % Activity of pos control |
|--------|---------------|---------|---------------------------|
| MF6226 | GGYSGYGGDSFDL | A       | 47.77614647               |
| MF6256 | GTVEATLLFDF   | B       | 57.85260834               |
| MF6930 | GTVEATLLFDY   | B       | 51.50445453               |

**Table 3.** Panel of LAG-3 Fab arms describing binning based on FACS profiles, domain binding and LAG-3 blocking activity as bivalent antibody.

| ID   | CDR3H             | VH      | Bin                          | LAG-3 block |
|------|-------------------|---------|------------------------------|-------------|
| 7111 | IPLTGEFDY         | VH4-59  | D1                           | Yes         |
| 7165 | GGTYYYGSGSYTLDY   | VH1-24  | D1                           | Yes         |
| 7116 | DGDNWDVFDI        | VH3-30  | D1                           | Yes         |
| 7100 | ERGWDVFDI         | VH3-30  | D1                           | Yes         |
| 7137 | GGTYYYGSGSYTLDY   | VH1-24  | D1                           | Yes         |
| 7518 | DGSGWDDFDY        | VH1-18  | D1+D4                        | Yes         |
| 7134 | EPNWGVYFDY        | VH7-4-1 | D2                           | Yes         |
| 7146 | DREVGAIYYFDY      | VH1-69  | D2                           | Yes         |
| 7142 | ERDIGSLYYFDS      | VH1-69  | D2                           | Yes         |
| 7185 | DREMFTLYFFDQ      | VH1-69  | D2                           | Yes         |
| 7136 | DSTYYTSGSYSVFDY   | VH3-23  | D2                           | No          |
| 7118 | VPAAATPSGTYYWIFDL | VH3-23  | D3                           | No          |
| 7443 | DTSTWQRGGYKAFDY   | VH3-23  | D3                           | No          |
| 7167 | DRGYDYSYHNWFDP    | VH3-23  | D4                           | No          |
| 7515 | RPGPALGDLDS       | VH1-18  | D4                           | No          |
| 7444 | DTGQSWSNYYHAFDY   | VH3-23  | hu/mo cross-reactive - hu D3 | No          |

5

**Table 4.** Panel of LAG-3 Fab arms describing binning based on FACS profiles and domain binding.

| ID   | CDR3H             | VH     | Bin                         |
|------|-------------------|--------|-----------------------------|
| 7096 | DLLYKWNYYVEGFDI   | VH4-59 | D1                          |
| 7097 | DLLYKWNYYVEGFDI   | VH4-59 | D1                          |
| 7106 | DKAVAGLYYFDS      | VH1-69 | D2                          |
| 7118 | VPAAATPSGTYYWIFDL | VH3-23 | D3                          |
| 7120 | ERELGALYAFDI      | VH1-69 | D2                          |
| 7133 | DRETGTLYYFDY      | VH1-69 | D2                          |
| 7139 | DRAIGTLYYFDY      | VH1-69 | D2                          |
| 7144 | DRDSGGLYYFDS      | VH1-69 | D2                          |
| 7524 | GSILAAQMWGDI      | VH1-18 | hu/mo cross-reactive – mo 1 |

**Table 5.** Overview PB numbers and their MF composition

|              | PD-1    |         |         |
|--------------|---------|---------|---------|
| <b>LAG-3</b> | MF6930  | MF6226  | MF6256  |
| MF7111       | PB15292 | PB16336 | PB15254 |
| MF7116       | PB15296 | PB16367 | PB15258 |
| MF7100       | PB15289 | PB16369 | PB15251 |
| MF7137       | PB15307 | PB16365 | PB15269 |
| MF7518       | PB15383 | PB16364 | PB15347 |
| MF7134       | PB15305 | PB16337 | PB15267 |
| MF7146       | PB15313 | PB16338 | PB15275 |
| MF7142       | PB15311 | PB16339 | PB15273 |
| MF7165       | PB15317 | PB16366 | PB15279 |
| MF7185       | PB15363 | PB16340 | PB15359 |
| MF7136       | PB15306 | PB16341 | PB15268 |
| MF7118       | PB15297 | PB16342 | PB15259 |
| MF7443       | PB15369 | PB16343 | PB15333 |
| MF7444       | PB15403 | PB16346 | PB15393 |
| MF7167       | PB15318 | PB16344 | PB15280 |
| MF7515       | PB15380 | PB16345 | PB15344 |

**Table 5.** Overview of LAG-3 arms and PD-1 arms and which heavy chains the variable domains are associated with in a bispecific antibody

| Target | MF   | Target | MF   |
|--------|------|--------|------|
| LAG-3  | 7096 | PD-1   | 5743 |
|        | 7097 |        | 6076 |
|        | 7100 |        | 6225 |
|        | 7106 |        | 6227 |
|        | 7111 |        | 6930 |
|        | 7116 |        | 6932 |
|        | 7118 |        | 6935 |
|        | 7120 |        | 6974 |
|        | 7133 |        | 6983 |
|        | 7134 |        |      |
|        | 7137 |        |      |
|        | 7139 |        |      |
|        | 7142 |        |      |
|        | 7144 |        |      |
|        | 7167 |        |      |
|        | 7185 |        |      |
|        | 7444 |        |      |
|        | 7518 |        |      |
|        | 7524 |        |      |

## Claims

1. An in vitro method for interfering with Programmed Cell Death 1 protein (PD-1) and Lymphocyte activation 3 (LAG3) mediated inhibition in a PD-1 and/or LAG3 positive cell, the method comprising contacting said cell with an antibody or a functional part, derivative and/or analogue thereof that comprises

- one heavy-light chain combination that can bind to an extracellular part of PD-1 and
- one heavy-light chain combination that can bind to an extracellular part of LAG3, thereby inhibiting PD-1 and/or LAG3 mediated activity in said cell,

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of any one of the VH of MF6076; MF6226; MF6256; MF6930; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, or MF6974, preferably MF6930, and

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

2. The method of claim 1, wherein the binding of said PD-1 binding heavy-light chain combination to PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2.

3. An in vitro method for stimulating the formation, stability and/or activity of an immunological synapse comprising providing a system that comprises at least two cells capable of associating with each other via an immunological synapse and providing said system with an antibody or a functional part, derivative and/or analogue thereof that comprises

- one heavy-light chain combination that can bind to an extracellular part of PD-1 and
- one heavy-light chain combination that can bind to an extracellular part of LAG3,

thereby stimulating the formation, stability and/or activity of an immunological synapse between said at least two cells,

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of any one of the VH of MF6076; MF6226; MF6256; MF6930; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, or MF6974, preferably MF6930, and

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

4. An antibody or variant thereof that comprises one heavy-light chain combination that can bind to an extracellular part of PD-1 and one heavy-light chain combination that can bind to an extracellular part of LAG3,

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of any one of the VH of MF6076; MF6226; MF6256; MF6930; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, or MF6974, preferably MF6930, and

wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

5. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6076.

6. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable

region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6226.

7. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6256.

8. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6930.

9. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6974.

10. The antibody or variant thereof of claim 4, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of the heavy chain variable region of the VH of MF6982.

11. The antibody or variant thereof of any one of claims 4-10, wherein the one heavy-light chain combination that binds PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2.

12. The antibody or variant thereof of any one of claims 4-11, wherein said LAG-3 binding heavy-light chain combination binds to LAG-3 extracellular domain 1, 2, 3 or 4, preferably domain 1 or domain 2, preferably domain 2.

13. The antibody or variant thereof of any one of claims 4-12, wherein the one heavy-light chain combination that binds an extracellular part of PD-1 is defined as a one heavy-light chain combination that when in a bivalent monospecific antibody that comprises two

of said heavy-light chain combinations that bind PD-1, inhibits PD-1/PD-L1 inhibition in a Jurkat cell in a range of 20-150% when compared to the inhibition obtained with the antibody Nivolumab on a Jurkat cell.

14. The antibody or variant thereof of any one of claims 4-13, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076; MF6226; MF6236; MF6256; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, MF6974, or MF6226, preferably MF6930, MF6076 or MF6974, having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

15. The antibody or variant thereof of any one of claims 4-13, wherein the one heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076; MF6226; MF6256; MF6930; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, or MF6974, preferably MF6930.

16. The antibody or variant thereof of any one of claims 4-15, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of any one of the VH of MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096;

wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

17. The antibody or variant thereof of any one of claims 4-15, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096, having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region;

wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

18. The antibody or variant thereof of any one of claims 4-17, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7100; MF7111; MF7116; MF7118; MF7134; MF7136; MF7137; MF7142; MF7146; MF7165; MF7167; MF7185; MF7443; MF7444; MF7515; MF7518; MF7096; MF7097; MF7106; MF7120; MF7133; MF7139; MF7144; or MF7524, preferably MF7518; MF7165; MF7116; MF7096; MF7133; MF7139; MF7524 or MF7137, preferably MF7139; MF7524, MF7133, MF7518 or MF7096.

19. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7139 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions,

substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

20. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7524 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

21. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7133 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

22. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7518 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of

the heavy chain variable region of MF6076 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

23. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7096 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

24. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7139 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region

CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region: and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

25. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7524 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region: and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

26. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of

the heavy chain variable region of MF7133 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region: and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

27. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7518 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region: and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

28. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7096 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region: and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

29. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7139 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

30. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7524 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF

31. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7133; having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the

indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

32. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7518 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

33. The antibody or variant thereof of any one of claims 4 to 18, wherein the antibody comprises:

one heavy-light chain combination that can bind to an extracellular part of LAG-3 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7096 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the indicated MF, wherein the one heavy-light chain combination that can bind to an extracellular part of LAG3 comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT, and wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region; and

one heavy-light chain combination that can bind to an extracellular part of PD-1 which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF.

34. An antibody or variant thereof that comprises a heavy-light chain combination that can bind to an extracellular part of PD-1 and which comprises

a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076 or MF6930 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH of the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region, and

a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

35. The antibody or variant thereof of claim 34, wherein the heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6076.

36. The antibody or variant thereof of claim 34, wherein the heavy-light chain combination that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6930.

37. The antibody or variant thereof of any one of the preceding claims which comprises a light chain variable region having a sequence that is at least 80% identical to the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR  
FSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPPTFGQGTKVEIK,

wherein the CDR1 comprises sequence QSISSY, CDR2 comprises sequence AAS and CDR3 comprises sequence QQSYSTPPT.

38. The antibody or variant thereof of any one of the preceding claims which comprises a light chain variable region having the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR  
FSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPPTFGQGTKVEIK.

39. The method of any one of claims 1 to 3, wherein the antibody or variant thereof is an antibody according to any one of claims 4 to 38.

40. An antibody or variant thereof of any one of claims 4 to 38 for use as a medicament.

41. An antibody or variant thereof of any one of claims 4 to 38 for use in a method for the treatment of cancer or an infection with a pathogen, such as a virus or parasite.

42. A composition comprising at least one antibody or variant thereof according to any one of claims 4 to 38.

43. A pharmaceutical composition comprising at least one antibody or variant thereof of any one of claims 4 to 38 and a pharmaceutically acceptable carrier, diluent or excipient.

44. A nucleic acid molecule with a length of at least 15 nucleotides encoding at least one CDR region of an antibody or variant of any one of claims 4 to 38.

45. A nucleic acid molecule according to claim 44 encoding at least a heavy chain variable region of an antibody or variant of any one of claims 4 to 38.

46. A nucleic acid molecule according to claim 44 or 45, encoding a heavy chain variable region of any one of the VH of MF6076; MF6226; MF6256; MF6930; MF6974; or MF6982, preferably MF6256; MF6930; MF6076, or MF6974, preferably MF6930.

47. A nucleic acid molecule encoding an antibody or variant of any one of claims 4 to 38.

48. A vector comprising a nucleic acid molecule or functional equivalent thereof according to any one of claims 44-47.

49. The vector according to claim 48, wherein the vector comprising a nucleic acid molecule encoding an antibody or variant of any one of claims 4 to 38.

50. An isolated or recombinant cell, or a non-human animal, comprising a nucleic acid molecule according to any one of claims 44-47 or a vector according to claim 48 or 49.

51. An antibody or variant thereof that comprises a heavy-light chain combination that can bind to an extracellular part of PD-1 and which comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF6974 having at most 10, and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions, or a combination thereof, with respect to the amino acid sequence of the VH as depicted for the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region;

wherein the a heavy-light chain combination comprises a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

52. Use of an antibody or variant of any one of claims 4 to 38, a composition according to claim 42 or 43, a nucleic acid molecule according to claim 47, or a vector according to claim 49 in the manufacture of a medicament for treating a cancer or an infection with a pathogen in a subject in need thereof, wherein the cancer or an infection with a pathogen comprises cells that express PD-L1 and/or PD-L2 or wherein the cancer or pathogen is PD-1/PD-L1/2 and LAG-3/MHCII responsive.