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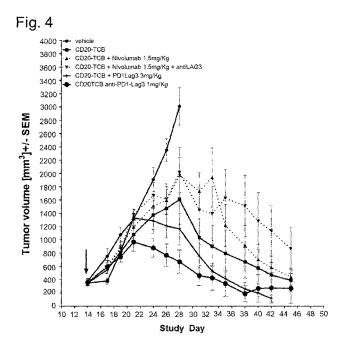
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(54) Title: COMBINATION THERAPY EMPLOYING A PD1-LAG3 BISPECIFIC ANTIBODY AND A CD20 T CELL BISPECIFIC ANTIBODY



(57) **Abstract:** The invention relates to combination therapies employing anti-PD1/anti-LAG3 bispecific antibody and a CD20 T cell-activating bispecific antibody, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies.

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Combination therapy employing a PD1-LAG3 bispecific antibody and a CD20 T cell bispecific antibody

FIELD OF THE INVENTION

The present invention relates to combination therapies employing a PD1-LAG3 bispecific antibody and a CD20 T cell-activating bispecific antibody, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies.

5 BACKGROUND

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B-cell proliferative disorders describe a heterogeneous group of malignancies that includes both leukemias and lymphomas. Lymphomas develop from lymphatic cells and include two main categories: Hodgkin lymphomas (HL) and the non-Hodgkin lymphomas (NHL). In the United States, lymphomas of B cell origin constitute approximately 80-85% of all non-Hodgkin lymphoma cases, and there is considerable heterogeneity within the B-cell subset, based upon genotypic and phenotypic expression patterns in the B-cell of origin. For example, B cell lymphoma subsets include the slow-growing indolent and incurable diseases, such as Follicular lymphoma (FL) or chronic lymphocytic leukemia (CLL), as well as the more aggressive subtypes, mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL). Despite the availability of various agents for the treatment of B-cell proliferative disorders, there is an ongoing need for development of safe and effective therapies to prolong remission and improve cure rates in patients.

An anti-CD20/anti-CD3 bispecific antibody is a molecule that targets CD20 expressed on B cells and CD3 epsilon chain (CD3ɛ) present on T cells. Simultaneous binding leads to T-cell activation and T-cell mediated killing of B cells. In the presence of CD20⁺ B cells, whether circulating or in tissue, pharmacologically active doses of a CD20-CD3 bispecific antibody will trigger T-cell activation and associated cytokine release. Parallel to B cell depletion in the peripheral blood, CD20 T cell-activating bispecific antibody leads to a transient decrease of T cells in the peripheral blood within 24 hours after the first administration and to a peak in cytokine release, followed by rapid T-cell recovery and return of cytokine levels to baseline within 72 hours. Two major reported escape mechanisms during treatment with a T cell-activating bispecific antibody include increased frequencies of regulatory T cells (T_{regs}) and increased levels of PD-L1 expression on B-precursor cells. T_{regs} suppress effector T cell

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activation through CTLA4 and other mechanisms. However, even when T cells are fully activated, upregulation of PD1 will lead to inhibitory signaling after binding to PD-L1 expressed by the tumor cells. These mechanisms induce effector T cell suppression and exhaustion or dysfunction, which can be treated with checkpoint blockade.

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Exhausted T cells are characterized by sustained expression of the inhibitory molecule PD-1 (programmed cell death protein 1) and it has been found that blockade of PD-1 and PD-L1 (PD-1 ligand) interactions can reverse T cell exhaustion and restore antigen-specific T cell responses. However, targeting the PD-1–PD-L1 pathway alone does not always result in reversal of T cell exhaustion, possibly due to resistance mechanisms, immunosuppressive activity of MDSC, and/or regulatory T cells.

Lymphocyte activation gene-3 (LAG3 or CD223) was initially discovered in an experiment designed to selectively isolate molecules expressed in an IL-2-dependent NK cell line (Triebel F et al., Cancer Lett. 235 (2006), 147–153). LAG3 is a unique transmembrane protein with structural homology to CD4 with four extracellular immunoglobulin superfamilylike domains (D1-D4). The membrane-distal IgG domain contains a short amino acid sequence, the so-called extra loop that is not found in other IgG superfamily proteins. The intracellular domain contains a unique amino acid sequence (KIEELE, SEQ ID NO:103) that is required for LAG3 to exert a negative effect on T cell function. LAG3 can be cleaved at the connecting peptide (CP) by metalloproteases to generate a soluble form, which is detectable in serum. Like CD4, the LAG3 protein binds to MHC class II molecules, however with a higher affinity and at a distinct site from CD4 (Huard et al. Proc. Natl. Acad. Sci. USA 94 (1997), 5744-5749). LAG3 is expressed by T cells, B cells, NK cells and plasmacytoid dendritic cells (pDCs) and is upregulated following T cell activation. It modulates T cell function as well as T cell homeostasis. Subsets of conventional T cells that are anergic or display impaired functions express LAG3. LAG3⁺ T cells are enriched at tumor sites and during chronic viral infections (Sierro et al Expert Opin. Ther. Targets 15 (2011), 91-101). It has been shown that LAG3 plays a role in CD8 T cell exhaustion (Blackburn et al. Nature Immunol. 10 (2009), 29-37). Thus, there is a need for antibodies that antagonize the activity of LAG3 and can be used to generate and restore immune response to tumors.

By targeting both PD-1 and LAG-3 on dysfunctional tumour-specific T lymphocytes, PD1-LAG3 aims to restore an effective anti-tumor immune-response and to provide survival benefit to more cancer patients than the currently available checkpoint inhibitors. By preferentially targeting PD-1/LAG-3 co-expressing dysfunctional T cells and potentially reduced targeting of LAG-3 expressing Tregs in the tumor microenvironment, PD1-LAG3 BsAb might avoid reinvigorating Treg mediated immunosuppressive effects while restoring the anti-tumor immune response.

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While effective CD20-expressing cancer therapies exist, suboptimal response, relapsed-refractory disease, and/or resistance to one or more therapeutic agents have remained a challenge. Further, patients with higher risk and cytogenetic abnormalities still have less than optimal response to approved therapies and shorter duration of response and progression free survival. Accordingly, there is a need for more effective, safe, and durable targeted combination therapies for the treatment of hematological malignancies.

SUMMARY OF THE INVENTION

The present invention relates to combination therapies employing an anti-CD20/anti-CD3 bispecific antibody and a bispecific antibody comprising a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3). It has been found, that the anti-PD1/anti-LAG3 bispecific antibodies as described herein are advantageous over anti-PD1 antibodies as they provide better selectivity and efficacy. These anti-PD1/anti-LAG3 bispecific antibodies are further characterized in that they show a reduced sink effect (as shown by reduced internalization by T cells), they preferentially bind to conventional T cells as to Tregs and are able to rescue T cell effector functions from Treg suppression, they show increased tumor-specific T cell effector functions and increased tumor eradication *in vivo*. Based on these properties they are advantageous to be used in combination with T cell bispecific antibodies, in particular anti-CD20/anti-CD3 bispecific antibodies.

Described herein is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating cancer, in particular CD20 expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with an anti-PD1/anti-LAG3 bispecific antibody.

The invention provides an anti-CD20/anti-CD3 bispecific antibody for use in a method as defined herein before, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein a first antigen binding domain specifically binding to PD1 comprises a VH domain comprising

- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- 35 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.

Furthermore, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with an anti-PD1/anti-LAG3 bispecific antibody and wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fc domain that is an IgG Fc domain, particularly an IgG1 Fc domain or an IgG4 Fc domain, and wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor. More particularly, the anti-PD1/anti-LAG3 bispecific antibody comprises an Fc domain of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In one aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as described herein before, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising

- (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
- 25 (b) a VH domain comprising

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- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as disclosed herein, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen-binding domain specifically binding to PD1 comprising the VH domain comprising

the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO:10.

In a further aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use as described herein, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising

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- (a) a VH domain comprising the amino acid sequence of SEQ ID NO:17 and a VL domain comprising the amino acid sequence of SEQ ID NO:18, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.
- In an additional aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as described herein, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 27 and a VL domain comprising the amino acid sequence of SEQ ID NO: 28, or
- 15 (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 29 and a VL domain comprising the amino acid sequence of SEQ ID NO: 30, or
 - (c) a VH domain comprising the amino acid sequence of SEQ ID NO: 31 and a VL domain comprising the amino acid sequence of SEQ ID NO: 32, or
- (d) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and a VL domaincomprising the amino acid sequence of SEQ ID NO: 34.

Furthermore, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as disclosed herein, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises

- a first antigen binding domain specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,
- and a second antigen binding domain specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

In a further aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fab fragment specifically binding to PD1 and a Fab fragment specifically binding to LAG3. In one aspect, the anti-PD1/anti-LAG3 bispecific antibody comprises a Fab

fragment specifically binding to PD1, wherein the variable domains VL and VH are replaced by each other so that VL is part of the heavy chain and VH is part of the light chain.

In another aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as disclosed herein before, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises monovalent binding to PD-1 and monovalent binding to LAG3.

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In a further aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method as disclosed herein before, wherein the anti-PD1/anti-LAG3 bispecific antibody is a humanized or chimeric antibody. In particular, the anti-PD1/anti-LAG3 bispecific antibody is a humanized antibody. Furthermore, provided is an anti-PD1/anti-LAG3 bispecific antibody as described herein before, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises an Fc domain comprising a modification promoting the association of the first and second subunit of the Fc domain. In one aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein the first subunit of the Fc domain comprises knobs and the second subunit of the Fc domain comprises holes according to the knobs into holes method. In particular, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

In a particular aspect, provided is a anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises

- (a) a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38, or
- 25 (b) a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 39, and a second light chain comprising an amino acid sequence of SEQ ID NO:40.

More particularly, the anti-PD1/anti-LAG3 bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38.

Furthermore, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is for use

in combination with an anti-PD1/anti-LAG3 bispecific antibody and wherein the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_HCD20) and a light chain variable region (V_LCD20). In one aspect, the anti-CD20/anti-CD3 bispecific antibody comprises first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46. 10 More particularly, the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:48. In one aspect, the anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer comprises a second antigen binding domain 15 comprises a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. In particular, the second antigen binding domain comprises a heavy chain variable region (V_HCD20) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) 20 comprising the amino acid sequence of SEQ ID NO:56. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer comprises a third antigen binding domain that binds to CD20. In another aspect, the anti-CD20/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions 25 that reduce binding to an Fc receptor and/or effector function.

In one particular aspect, the anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer is glofitamab. In another particular aspect, the anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer is mosunetuzumab.

In a further aspect, provided is an anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20 expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with anti-PD1/anti-LAG3 bispecific antibody and wherein the combination is for administration at intervals from about one week to three weeks.

In yet another aspect, the anti-CD20/anti-CD3 bispecific antibody is for use in a method of treating CD20 expressing cancer, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of

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time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody. Preferably, the Type II anti-CD20 antibody is obinutuzumab.

In one further aspect, provided is a composition comprising an anti-PD1/anti-LAG3
bispecific antibody for use in the treatment of CD20 expressing cancer, wherein said treatment comprises administration of said composition comprising an anti-PD1/anti-LAG3 bispecific antibody in combination with a composition comprising an anti-CD20/anti-CD3 bispecific antibody, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,

- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and
- 15 a VL domain comprising

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- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, the composition comprises an anti-PD1/anti-LAG3 bispecific antibody comprising a first antigen-binding domain specifically binding to PD1 comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO: 10. In one further aspect, the composition comprises an anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising

- 25 (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
- 30 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
 - (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
- 35 (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and

a VL domain comprising

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- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.
- In one aspect, the composition comprises an anti-PD1/anti-LAG3 bispecific antibody comprising an antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.

In one particular aspect, the composition comprises an anti-PD1/anti-LAG3 bispecific antibody comprising

a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,

and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

Furthermore, provided is a composition comprising an anti-PD1/anti-LAG3 bispecific antibody for use in the treatment of CD20 expressing cancer, wherein said treatment comprises administration of said composition comprising an anti-PD1/anti-LAG3 bispecific antibody in combination with a composition comprising an anti-CD20/anti-CD3 bispecific antibody, wherein the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_HCD20) and a light chain variable region (V_LCD20). In one aspect, the anti-CD20/anti-CD3 bispecific antibody comprises first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46. More particularly, the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:48. In one aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a second antigen binding domain comprises a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID

NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. In particular, the second antigen binding domain comprises a heavy chain variable region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:56. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CD20. In another aspect, the anti-CD20/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In one particular aspect, the anti-CD20/anti-CD3 bispecific antibody is glofitamab. In another particular aspect, the anti-CD20/anti-CD3 bispecific antibody is mosunetuzumab.

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In yet another aspect, provided is a composition comprising an anti-PD1/anti-LAG3 bispecific antibody for use in the treatment of CD20 expressing cancer, wherein said treatment comprises administration of said composition comprising an anti-PD1/anti-LAG3 bispecific antibody in combination with a composition comprising an anti-CD20/anti-CD3 bispecific antibody, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody. Preferably, the Type II anti-CD20 antibody is obinutuzumab.

In a further aspect, provided is a pharmaceutical product comprising (A) a first composition comprising as active ingredient an anti-CD20/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient an anti-PD1/anti-LAG3 bispecific antibody and a pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous, treatment of a disease, in particular CD20 expressing cancer.

In another aspect, provided is a pharmaceutical composition comprising a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody for use in the combined, sequential or simultaneous, treatment of a disease, in particular CD20 expressing cancer. In particular, the pharmaceutical composition is for use in the treatment of B-cell proliferative disorders, in particular a disease selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).

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In another aspect, provided is the use of a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody in the manufacture of a medicament for treating or delaying progression of a proliferative disease, in particular for treating a CD20 expressing cancer, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising

- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- 10 (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- 15 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one further aspect, the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising

(a) a VH domain comprising

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- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
- 25 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
 - (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and
- 30 a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another aspect, provided is the use of a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody in the manufacture of a 35 medicament for treating or delaying progression of a proliferative disease, in particular for

treating a CD20 expressing cancer, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10, and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

In yet another aspect, provided is the use of a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody in the manufacture of a medicament for treating or delaying progression of a proliferative disease, in particular for treating a CD20 expressing cancer, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody. Preferably, the Type II anti-CD20 antibody is obinutuzumab.

- In a further aspect, provided is a method for treating CD20 expressing cancer in a subject comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 bispecific antibody, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and
- 25 a VL domain comprising

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- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, provided is the method, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising

(a) a VH domain comprising

- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and
- a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,

- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
- (b) a VH domain comprising

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- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another aspect, provided is the method, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10, and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

In one aspect, provided is the method, wherein the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_HCD20) and a light chain variable region (V_LCD20). In one aspect, the anti-CD20/anti-CD3 bispecific antibody comprises first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46. More particularly, the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:48. In one aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a second antigen binding domain comprises a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. In particular, the second antigen binding domain comprises a heavy chain variable region (V_HCD20) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable

region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:56. In a further aspect, the

anti-CD20/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CD20. In another aspect, the anti-CD20/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In one particular aspect, the anti-CD20/anti-CD3 bispecific antibody is glofitamab. In another particular aspect, the anti-CD20/anti-CD3 bispecific antibody is mosunetuzumab.

In yet another aspect, provided is a method for treating CD20 expressing cancer in a subject comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 bispecific antibody, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody. Preferably, the Type II anti-CD20 antibody is obinutuzumab.

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In one aspect, the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered intravenously or subcutaneously. In another aspect, the anti-CD20/anti-CD3 bispecific antibody is administered concurrently with, prior to, or subsequently to the anti-PD1/anti-LAG3 bispecific antibody.

In any of the above aspects the subject is preferably a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and **1B** are schematic illustrations of particular anti-PD1/anti-LAG3 bispecific antibodies (Fig. 1A) and a particular anti-CD20/anti-CD3 bispecific antibody (Fig. 1B) as used in the Examples. These molecules are described in more detail in Examples 2 and 1, respectively. **Fig. 1A** shows the anti-PD1/anti-LAG3 bispecific antibodies in 1+1 format, wherein the PD1 binding domain comprises a crossFab (with VH/VL domain exchange) and the LAG3 binding domain comprises CH1 and CK domains with amino acid mutations to support correct pairing ("charged variants"). The Fc part comprises the knob into hole mutations (illustrated by the black arrow) and the amino acid mutations L234A, L235A and P329G almost completely abolishing Fcγ receptor binding of the human IgG1 Fc domain. In **Fig. 1B** an exemplary bispecific anti-CD20/anti-CD3 antibody in 2+1 format is shown (named CD20 TCB).

Figure 2 shows the effect of an anti-PD1/anti-LAG3 bispecific antibody (PD1-LAG3 BsAb) in combination with CD20 TCB on cytotoxic Granzyme B release by human CD4 T cells

cocultured with a B cell-lymphoblatoid cell line (ARH77). PD1-LAG3 BsAb is compared with PD-1 antibodies (nivolumab, pembrolizumab and the parental PD-1 antibody.

Figure 3 shows the protocol of the *in vivo* efficacy study of PD1-LAG3 BsAb vs. PD1 antibodies in combination with CD20 TCB in WSU-DLCL2-bearing fully humanized NSG mice. In the table below the subgroups of mice receiving different combinations are defined. The experiment is described in Example 4.

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Figure 4 shows the results of the study. Humanized NSG mice were injected s.c. with 1.5 x 10⁶ WSU-DLCL2 cells expressing CD20. After the tumors reached an average volume of around 350-400 mm³ (on day 14), mice were randomized to 6 groups receiving: A) phosphate-buffer saline (PBS; vehicle) as control; B) CD20-TCB (0.15 mg/kg once/week i.v.), C) CD20-TCB (0.15 mg/kg once/week i.v.) + Nivolumab (1.5mg/kg once/week i.v.), D) CD20-TCB (0.15 mg/kg once/week i.v.) + Nivolumab (1.5mg/kg once/week i.p.) + anti-LAG3 (1.5mg/kg once/week i.v.), E) CD20-TCB (0.15 mg/kg once/week i.v.) + PD1-LAG3 BsAb (1.5 mg/kg once/week i.v.), F) CD20-TCB (0.15 mg/kg once/week i.v.) + PD1-LAG3 BsAb (3 mg/kg once/week i.v.). Tumor volume was measured by digital caliper 3 times a week. Data is shown as average tumor volume and standard error of mean (+/-SEM).

In **Figures 5A** to **5F** the measurements of tumor volumes (mm³ +/- SEM), over a period from day 14 to day 45, are shown for each individual animal showing homogeneity of anti-tumor response in the groups treated with PD1-LAG3 BsAb. The tumor growth curves are shown for the vehicle group in **Fig. 5A**, for CD20 CD3 TCB alone (0.15 mg/kg) in **Fig. 5B**, for the combination of CD20 CD3 TCB with Nivolumab (1.5 mg/kg) in **Fig. 5C**, for the combination of CD20 CD3 TCB with nivolumab (1.5 mg/kg) and anti-LAG3 (1.5 mg/kg) in **Fig. 5D**, for the combination of CD20 CD3 TCB with PD1/LAG3 BsAb in **Fig 5E** (1.5 mg/kg) and in **Fig. 5F** (3 mg/kg PD1/LAG3 BsAb).

Figure 6 shows that the combination of CD20 CD3 TCB and PD1/LAG3 BsAb at 3 mg/kg resulted in a statistical significant tumor protection as compared to the treatment in combination with nivolumab or nivolumab + anti-LAG3. For this analysis tumor volume data were transformed introducing a new end point: we evaluated if the last observed tumor volume of each animal was below 800 mm³ or not providing a binary readout and a percentage of low size tumor. This endpoint was then subjected to pairwise group comparisons based on a Chi² test.

Figure 7 shows the protocol of the *in vivo* efficacy study of CD20 TCB in combination with PD1-LAG3 BsAb or with pembrolizumab + anti-LAG3 in OCI-Ly18 bearing fully humanized NSG mice. In the table below the subgroups of mice receiving different combinations are defined. The experiment is described in Example 5.

Figure 8 shows the results of the study. Humanized NSG mice were injected s.c. with OCI-Ly18 lymphoma cells expressing CD20. After the tumors reached an average volume of around 200 mm³ (on day 10), mice were randomized and the therapies injected. The measurements of tumor volumes (mm³ +/- SEM), are shown as mean volume within the group of mice. The tumor size was measured until there were at least 6 (for vehicle) or 7 (for treatment groups) mice/group/timepoint. Vehicle was followed until day 26 while the treatment groups until day 35. Data is shown as average tumor volume and standard error of mean (+/-SEM).

In **Figures 9A** to **9D** the measurements of tumor volumes (mm³ +/- SEM), over a period from day 10 to day 35, are shown for each individual animal. The tumor growth curves are shown for the vehicle group in **Fig. 9A**, for CD20 CD3 TCB alone in **Fig. 9B**, for the combination of CD20 CD3 TCB with PD1-LAG3 BsAb in **Fig. 9C**, and for the combination of CD20 CD3 TCB with pembrolizumab and anti-LAG3 in **Fig. 9D**.

Figure 10 shows the protocol of the *in vivo* efficacy study of CD20 TCB alone compared to the combination with PD1-LAG3 BsAb in OCI-Ly18 bearing fully humanized NSG mice, when a pre-treatment with obitunuzumab is used. In the table below the subgroups of mice receiving different combinations are defined. The experiment is described in Example 6.

Figure 11 shows the results of the study. Humanized NSG mice were injected s.c. with OCI-Ly18 lymphoma cells expressing CD20. After the tumors reached an average volume of around 400 mm³ (on day 17), mice were randomized and the therapies injected according to the experiment layout. The measurements of tumor volumes (mm³ +/- SEM), are shown as mean volume within the group of mice. The tumor size was measured until day 35 for treatment groups, while until day 26 for the vehicle group.

In **Figures 12A** to **12C** the measurements of tumor volumes (mm³ +/- SEM), over a period from day 17 to day 35, are shown for each individual animal. The tumor growth curves are shown for the vehicle group in **Fig. 12A**, for obinutuzumab and CD20 CD3 TCB in **Fig. 12B**, and for the combination of obinutuzumab and CD20 CD3 TCB with PD1-LAG3 BsAb in **Fig. 12C**.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

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Unless defined otherwise, technical and scientific terms used herein have the same meaning as generally used in the art to which this invention belongs. For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa.

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

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The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g. containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

The term "monospecific" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen. The term "bispecific" means that the antibody is able to specifically bind to at least two distinct antigenic determinants, for example two binding sites each formed by a pair of an antibody heavy chain variable domain (VH) and an antibody light chain variable domain (VL) binding to different antigens or to different epitopes on the same antigen. Such a bispecific antibody is a 1+1 format. Other bispecific antibody formats are 2+1 formats (comprising two binding sites for a first antigen or epitope) or 2+2 formats (comprising two binding sites for a second antigen or epitope). Typically, a bispecific antibody comprises two antigen binding sites, each of which is specific for a different antigenic determinant.

The term "valent" as used within the current application denotes the presence of a specified number of binding domains in an antigen binding molecule. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding domain, four binding domains, and six binding domains, respectively, in an antigen binding molecule. The bispecific antibodies according to the invention are at least "bivalent" and may be "trivalent" or "multivalent" (e.g. "tetravalent" or "hexavalent"). In a particular aspect, the antibodies of the present invention have two or more binding sites and are bispecific. That is, the antibodies may be bispecific even in cases where there are more than two binding sites (i.e. that the antibody is trivalent or multivalent).

The terms "full length antibody", "intact antibody", and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure. "**Native antibodies**" refer to naturally occurring immunoglobulin molecules

with varying structures. For example, native IgG-class antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a light chain constant domain (CL), also called a light chain constant region. The heavy chain of an antibody may be assigned to one of five types, called α (IgA), δ (IgD), ϵ (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ 1 (IgG1), γ 2 (IgG2), γ 3 (IgG3), γ 4 (IgG4), α 1 (IgA1) and α 2 (IgA2). The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

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An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. 15 Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies, triabodies, tetrabodies, cross-Fab fragments; linear antibodies; single-chain antibody molecules (e.g. scFv); multispecific antibodies formed from antibody fragments and single domain antibodies. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 20 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')2 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen binding domains that may be bivalent or bispecific, see, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., 25 Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 30 6,248,516 B1). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies. Antibody fragments can be made by various techniques, including but not limited to proteolytic 35

digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or

phage), as described herein.

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Papain digestion of intact antibodies produces two identical antigen-binding fragments, called "Fab" fragments containing each the heavy- and light-chain variable domains and also the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. As used herein, Thus, the term "Fab fragment" refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL), and a VH domain and a first constant domain (CH1) of a heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteins from the antibody hinge region. Fab'-SH are Fab' fragments wherein the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites (two Fab fragments) and a part of the Fc region.

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The term "**cross-Fab fragment**" or "xFab fragment" or "crossover Fab fragment" refers to a Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. Two different chain compositions of a crossover Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the variable regions of the Fab heavy and light chain are exchanged, i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab (VLVH). On the other hand, when the constant regions of the Fab heavy and light chain are exchanged, the crossover Fab molecule comprises a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). This crossover Fab molecule is also referred to as CrossFab (CLCH1).

A "single chain Fab fragment" or "scFab" is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL; and wherein said linker is a polypeptide of at least 30 amino acids, preferably between 32 and 50 amino acids. Said single chain Fab fragments are stabilized via the natural disulfide bond between the CL domain and the CH1 domain. In addition, these single chain Fab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

A "crossover single chain Fab fragment" or "**x-scFab**" is a is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CL-linker-VL-CH1 and b) VL-CH1-linker-VH-CL; wherein VH and VL form together an antigen binding domain which binds specifically to an antigen and wherein said linker is a polypeptide of at least 30 amino acids. In addition, these x-scFab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

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A "single-chain variable fragment (scFv)" is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L , or *vice versa*. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, e.g. described in Houston, J.S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

"Scaffold antigen binding proteins" are known in the art, for example, fibronectin and designed ankyrin repeat proteins (DARPins) have been used as alternative scaffolds for antigenbinding domains, see, e.g., Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol 13:245-255 (2009) and Stumpp et al., Darpins: A new generation of protein therapeutics. Drug Discovery Today 13: 695-701 (2008). In one aspect of the invention, a scaffold antigen binding protein is selected from the group consisting of CTLA-4 (Evibody), Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (trans-body); a designed ankyrin repeat protein (DARPin), a variable domain of antibody light chain or heavy chain (single-domain antibody, sdAb), a variable domain of antibody heavy chain (nanobody, aVH), V_{NAR} fragments, a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (V_{NAR} fragments), a human gammacrystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease inhibitors, microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin). CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly CD4+ T-cells. Its extracellular domain has a variable domain-like Ig fold.

Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies (e.g. US7166697B1). Evibodies are around the same size as the isolated variable region of an antibody (e.g. a domain antibody). For further details see Journal of Immunological Methods 248 (1-2), 31-45 (2001). Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see Biochim Biophys Acta 1482: 337-350 (2000), US7250297B1 and US20070224633. An affibody is a scaffold derived from Protein A of Staphylococcus aureus which can be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomization of surface residues. For further details see Protein Eng. Des. Sel. 2004, 17, 455-462 and EP 1641818A1. Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulfide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see Nature Biotechnology 23(12), 1556 - 1561 (2005) and Expert Opinion on Investigational Drugs 16(6), 909-917 (June 2007). A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a permissive surface loop. Examples of engineered transferrin scaffolds include the Transbody. For further details see J. Biol. Chem 274, 24066-24073 (1999). Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1.

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A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. The first single domains were derived from the variable domain of the antibody heavy chain from camelids (nanobodies or V_HH fragments). Furthermore, the term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or V_{NAR} fragments derived from sharks. Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the .beta.-sandwich can be engineered to enable an Adnectin to specifically recognize a

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therapeutic target of interest. For further details see Protein Eng. Des. Sel. 18, 435-444 (2005), US20080139791, WO2005056764 and US6818418B1. Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. 5, 783-797 (2005). Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges - examples of microproteins include KalataBI and conotoxin and knottins. The microproteins have a loop which can beengineered to include upto 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

An "antigen binding molecule that binds to the same epitope" as a reference molecule refers to an antigen binding molecule that blocks binding of the reference molecule to its antigen in a competition assay by 50% or more, and conversely, the reference molecule blocks binding of the antigen binding molecule to its antigen in a competition assay by 50% or more.

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As used herein, the term "antigen binding domain" or "antigen-binding site" refers to the part of the antigen binding molecule that specifically binds to an antigenic determinant. More particularly, the term "antigen-binding domain" refers the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more variable domains (also called variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH). In one aspect, the antigen binding domain is able to bind to its antigen and block or partly block its function. Antigen binding domains that specifically bind to PD1 or to LAG3 include antibodies and fragments thereof as further defined herein. In addition, antigen binding domains may include scaffold antigen binding proteins, e.g. binding domains which are based on designed repeat proteins or designed repeat domains (see e.g. WO 2002/020565).

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope," and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM). The proteins useful as antigens herein can be any native form the proteins from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g. mice and rats), unless otherwise indicated. In a particular embodiment the antigen is a human protein. Where reference

is made to a specific protein herein, the term encompasses the "full-length", unprocessed protein as well as any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g. splice variants or allelic variants.

By "specific binding" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen binding molecule to bind to a specific antigen can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. Surface Plasmon Resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding molecule to an unrelated protein is less than about 10% of the binding of the antigen binding molecule to the antigen as measured, e.g. by SPR. In certain embodiments, an molecule that binds to the antigen has a dissociation constant (Kd) of $\leq 1 \mu M$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-7} M or less, e.g. from 10^{-7} M to 10^{-13} M , e.g. from 10^{-9} M to 10^{-13} M).

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"Affinity" or "binding affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g. an antibody) and its binding partner (e.g. an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g. antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd), which is the ratio of dissociation and association rate constants (koff and kon, respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by common methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

As used herein, the term "high affinity" of an antibody refers to an antibody having a Kd of 10^{-9} M or less and even more particularly 10^{-10} M or less for a target antigen. The term "low affinity" of an antibody refers to an antibody having a Kd of 10^{-8} or higher.

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

"CD20" refers to B-lymphocyte antigen CD20, also known as B-lymphocyte surface antigen B1 or Leukocyte surface antigen Leu-16, and includes any native CD20 from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The amino

acid sequence of human CD20 is shown in Uniprot accession no. P11836 (version 149, SEQ ID NO:61). CD20 is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD expressed on pre-B and mature B lymphocytes. The corresponding human gene is membrane-spanning 4-domains, subfamily A, member 1, also known as MS4A1. This gene encodes a member of the membrane-spanning 4A gene family. Members of this nascent protein family are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns among hematopoietic cells and nonlymphoid tissues. This gene encodes the B-lymphocyte surface molecule which plays a role in the development and differentiation of B-cells into plasma cells. This family member is localized to 11q12, among a cluster of family members. Alternative splicing of this gene results in two transcript variants which encode the same protein. The term "CD20" encompasses "full-length," unprocessed CD20 as well as any form of CD20 that results from processing in the cell. The term also encompasses naturally occurring variants of CD20, e.g., splice variants or allelic variants.

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The terms "anti-CD20 antibody" and "an antibody that binds to CD20" refer to an antibody that is capable of binding CD20 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD20. In one embodiment, the extent of binding of an anti-CD20 antibody to an unrelated, non-CD20 protein is less than about 10% of the binding of the antibody to CD20 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD20 has a dissociation constant (Kd) of $\leq 1 \mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-CD20 antibody binds to an epitope of CD20 that is conserved among CD20 from different species.

By "**Type II anti-CD20 antibody**" is meant an anti-CD20 antibody having binding properties and biological activities of Type II anti-CD20 antibodies as described in Cragg et al., Blood 103 (2004) 2738-2743; Cragg et al., Blood 101 (2003) 1045-1052, Klein et al., mAbs 5 (2013), 22-33, and summarized in Table 1 below.

type I anti-CD20 antibodies	type II anti-CD20 antibodies
Bind class I CD20 epitope	Bind class II CD20 epitope
Localize CD20 to lipid rafts	Do not localize CD20 to lipid rafts
High CDC *	Low CDC *
ADCC activity *	ADCC activity *
Full binding capacity to B cells	Approx. half binding capacity to B cells
Weak homotypic aggregation	Homotypic aggregation
Low cell death induction	Strong cell death induction

TABLE A. Properties of type I and type II anti-CD20 antibodies

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Examples of type II anti-CD20 antibodies include e.g. obinutuzumab (GA101), tositumumab (B1), humanized B-Ly1 antibody IgG1 (a chimeric humanized IgG1 antibody as disclosed in WO 2005/044859), 11B8 IgG1 (as disclosed in WO 2004/035607) and AT80 IgG1.

In one aspect, the Type II anti-CD20 antibody comprises the heavy chain variable region sequence ($V_{\rm H}CD20$) of SEQ ID NO: 55 and the light chain variable region sequence ($V_{\rm L}CD20$) of SEQ ID NO: 56. In another aspect, the Type II anti-CD20 antibody is engineered to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a non-engineered antibody. In one aspect, at least about 40% of the N-linked oligosaccharides in the Fc region of the Type II anti-CD20 antibody are non-fucosylated.

In a particular aspect, the Type II anti-CD20 antibody is obinutuzumab (recommended INN, WHO Drug Information, Vol. 26, No. 4, 2012, p. 453). As used herein, obinutuzumab is synonymous for GA101. The tradename is GAZYVA® or GAZYVARO®. This replaces all previous versions (e.g. Vol. 25, No. 1, 2011, p.75-76), and is formerly known as afutuzumab (recommended INN, WHO Drug Information, Vol. 23, No. 2, 2009, p. 176; Vol. 22, No. 2, 2008, p. 124). In one aspect, the Type II anti-CD20 antibody comprises the heavy chain comprising the amino acid sequence of SEQ ID NO:62 and the light chain comprising the amino acid sequence of SEQ ID NO: 63. In one aspect, the Type II anti-CD20 antibody is tositumomab.

Examples of type I anti-CD20 antibodies include e.g. rituximab, ofatumumab, veltuzumab, ocaratuzumab, ocrelizumab, PRO131921, ublituximab, HI47 IgG3 (ECACC, hybridoma), 2C6 IgG1 (as disclosed in WO 2005/103081), 2F2 IgG1 (as disclosed in WO 2004/035607 and WO 2005/103081) and 2H7 IgG1 (as disclosed in WO 2004/056312).

^{*} if IgG₁ isotype

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The term "humanized B-Ly1 antibody" refers to humanized B-Ly1 antibody as disclosed in WO 2005/044859 and WO 2007/031875, which were obtained from the murine monoclonal anti-CD20 antibody B-Ly1 (variable region of the murine heavy chain (VH): SEQ ID NO:64; variable region of the murine light chain (VL): SEQ ID NO:65 (see Poppema, S. and Visser, L., Biotest Bulletin 3 (1987) 131-139) by chimerization with a human constant domain from IgG1 and following humanization (see WO 2005/044859 and WO 2007/031875). These "humanized B-Ly1 antibodies" are disclosed in detail in WO 2005/044859 and WO 2007/031875.

The term "**reduction**" (and grammatical variations thereof such as "reduce" or "reducing"), for example reduction of the number of B cells or cytokine release, refers to a decrease in the respective quantity, as measured by appropriate methods known in the art. For clarity the term includes also reduction to zero (or below the detection limit of the analytical method), i.e. complete abolishment or elimination. Conversely, "**increased**" refers to an increase in the respective quantity.

A "T-cell antigen" as used herein refers to an antigenic determinant presented on the surface of a T lymphocyte, particularly a cytotoxic T lymphocyte.

A "T cell activating therapeutic agent" as used herein refers to a therapeutic agent capable of inducing T cell activation in a subject, particularly a therapeutic agent designed for inducing T-cell activation in a subject. Examples of T cell activating therapeutic agents include bispecific antibodies that specifically bind an activating T cell antigen, such as CD3, and a target cell antigen, such as CD20 or CD19. Further examples include chimeric antigen receptors (CARs) which comprise a T cell activating domain and an antigen binding moiety that specifically binds to a target cell antigen, such as CD20 or CD19.

An "activating T cell antigen" as used herein refers to an antigenic determinant expressed by a T lymphocyte, particularly a cytotoxic T lymphocyte, which is capable of inducing or enhancing T cell activation upon interaction with an antigen binding molecule. Specifically, interaction of an antigen binding molecule with an activating T cell antigen may induce T cell activation by triggering the signaling cascade of the T cell receptor complex. An exemplary activating T cell antigen is CD3.

The term "CD3" refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD3 as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, e.g., splice variants or allelic variants. In one embodiment, CD3 is human CD3, particularly the epsilon subunit of human CD3 (CD3ε).

The amino acid sequence of human CD3ε is shown in UniProt (www.uniprot.org) accession no.

P07766 (version 144), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_000724.1. See also SEQ ID NO: 66. The amino acid sequence of cynomolgus [Macaca fascicularis] CD3ɛ is shown in NCBI GenBank no. BAB71849.1. See also SEQ ID NO: 67.

The term "a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3" "a bispecific antibody that specifically binds PD1 and LAG3", "bispecific antigen binding molecule specific for PD1 and LAG3" or an "anti-PD1/anti-LAG3 antibody" are used interchangeably herein and refer to a bispecific antibody that is capable of binding PD1 and LAG3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PD1 and LAG3.

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The term "PD1", also known as Programmed cell death protein 1, is a type I membrane protein of 288 amino acids that was first described in 1992 (Ishida et al., EMBO J., 11 (1992), 3887–3895). PD-1 is a member of the extended CD28/CTLA-4 family of T cell regulators and has two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273). The protein's structure includes an extracellular IgV domain followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals. This is consistent with binding of SHP-1 and SHP-2 phosphatases to the cytoplasmic tail of PD-1 upon ligand binding. While PD-1 is not expressed on naïve T cells, it is upregulated following T cell receptor (TCR)-mediated activation and is observed on both activated and exhausted T cells (Agata et al., Int. Immunology 8 (1996), 765-772). These exhausted T-cells have a dysfunctional phenotype and are unable to respond appropriately. Although PD-1 has a relatively wide expression pattern its most important role is likely as a coinhibitory receptor on T cells (Chinai et al, Trends in Pharmacological Sciences 36 (2015), 587-595). Current therapeutic approaches thus focus on blocking the interaction of PD-1 with its ligands to enhance T cell response. The terms "Programmed Death 1," "Programmed Cell Death 1," "Protein PD-1," "PD-1," "PDCD1," "PDCD1," "hPD-1" and "hPD-I" can be used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The amino acid sequence of human PD1 is shown in UniProt (www.uniprot.org) accession no. Q15116 (SEQ ID NO:68).

The terms "anti-PD1 antibody" and "an antibody comprising an antigen binding domain that binds to PD1" refer to an antibody that is capable of binding PD1, especially a PD1 polypeptide expressed on a cell surface, with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PD1. In one aspect, the extent of binding of an anti-PD1 antibody to an unrelated, non-PD1 protein is less than about 10% of the binding of the antibody to PD1 as measured, e.g., by radioimmunoassay (RIA) or flow cytometry (FACS) or by

a Surface Plasmon Resonance assay using a biosensor system such as a Biacore® system. In certain aspects, an antigen binding protein that binds to human PD1 has a K_D value of the binding affinity for binding to human PD1 of $\leq 1~\mu\text{M}, \leq 100~\text{nM}, \leq 10~\text{nM}, \leq 1~\text{nM}, \leq 0.1~\text{nM}, \leq 0.1~\text{nM}, \leq 0.01~\text{nM}, \text{or} \leq 0.001~\text{nM}$ (e.g. $10^{-8}~\text{M}$ or less, e.g. from $10^{-8}~\text{M}$ to $10^{-13}~\text{M}$). In one preferred embodiment the respective K_D value of the binding affinities is determined in a Surface Plasmon Resonance assay using the Extracellular domain (ECD) of human PD1 (PD1-ECD) for the PD1 binding affinity. The term "anti-PD1 antibody" also encompasses bispecific antibodies that are capable of binding PD1 and a second antigen.

In a specific aspect, the anti-PD1 antibody is selected from the group consisting of MDX 10 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), PDR001 (spartalizumab), SHR1210 (camrelizumab), MEDI-0680 (AMP-514), REGN2810, and BGB-108. In one particular aspect, the anti-PD1 antibody is pembrolizumab, or an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:76. Pembrolizumab (Merck), also known as MK-3475, Merck 3475, lambrolizumab, SCH-900475, and KEYTRUDA®, is an anti-PD-1 antibody 15 described in WO 2009/114335 (CAS Reg. No. 1374853-91-4). In one particular aspect, the anti-PD1 antibody is nivolumab, or an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:77 and a light chain comprising the amino acid sequence of SEQ ID NO:78. Nivolumab (CAS Registry Number: 946414-94-4, Bristol-Myers Squibb/Ono), also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-20 1 antibody described in WO 2006/121168 (CAS Reg. No. 946414-94-4). In another particular aspect, the anti-PD-1 antibody comprising a heavy chain variable domain VH comprising the amino acid sequence of SEQ ID NO:7 and a light chain variable domain VL comprising the amino acid sequence of SEQ ID NO:8, or a humanized variant thereof. In a particular aspect, the anti-PD-1 antibody comprising a heavy chain variable domain VH comprising the amino acid 25 sequence of SEQ ID NO:9 and a light chain variable domain VL comprising the amino acid sequence of SEQ ID NO:10.

The terms "LAG3" or "Lag-3" or "Lymphocyte activation gene-3" or "CD223" as used herein refer to any native LAG3 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed LAG3 as well as any form of LAG3 resulting from processing in the cell. The term also encompasses naturally occurring variants of LAG3, e.g., splice variants or allelic variants. In one preferred embodiment the term "LAG3" refers to human LAG3. The amino acid sequence of an exemplary processed (without signal sequences) LAG3 is shown in SEQ ID NO:69. The amino acid sequence of an exemplary Extracellular Domain (ECD) LAG3 is shown in SEQ ID NO:70.

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The terms "anti-LAG3 antibody" and "an antibody that binds to LAG3" refer to an antibody that is capable of binding LAG3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting LAG3. In one aspect, the extent of binding of an anti-LAG3 antibody to an unrelated, non-LAG3 protein is less than about 10% of the binding of the antibody to LAG3 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to LAG3 has a dissociation constant (Kd) of $\leq 1 \mu M_s \leq 100$ $nM_{s} \le 10 \text{ nM}_{s} \le 1 \text{ nM}_{s} \le 0.1 \text{ nM}_{s} \le 0.01 \text{ nM}_{s} \text{ or } \le 0.001 \text{ nM}_{s} \text{ (e.g. } 10^{-8} \text{ M or less, e.g. from } 10^{-8} \text{ M}_{s}$ to 10⁻¹³ M, e.g., from 10⁻⁹ M to 10⁻¹³ M). In certain aspects, an anti-LAG3 antibody binds to an epitope of LAG3 that is conserved among LAG3 from different species. In one preferred embodiment, an "anti-LAG3 antibody", "an antibody that specifically binds to human LAG3", and "an antibody that binds to human LAG3" refers to an antibody specifically binding to the human LAG3 antigen or its Extracellular Domain (ECD) with a binding affinity of a K_D-value of 1.0 x 10⁻⁸ mol/l or lower, in one embodiment of a K_D-value of 1.0 x 10⁻⁹ mol/l or lower, in one embodiment of a K_D -value of 1.0 x 10⁻⁹ mol/l to 1.0 x 10⁻¹³ mol/l. In this context the binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden) e.g. using the LAG3 extracellular domain. The term "anti-LAG3 antibody" also encompasses bispecific antibodies that are capable of binding LAG3 and a second antigen. In one aspect, the anti-LAG3 antibody is relatlimab or BMS-986016, or an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:27 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:28.

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A "blocking" antibody or an "antagonist" antibody is one that inhibits or reduces a biological activity of the antigen it binds. In some embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen. For example, the bispecific antibodies of the invention block the signaling through PD-1 and LAG3 so as to restore a functional response by T cells (e.g., proliferation, cytokine production, target cell killing) from a dysfunctional state to antigen stimulation.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antigen binding molecule to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and which determine antigen

binding specificity, for example "complementarity determining regions" ("CDRs"). Generally, antibodies comprise six CDRs: three in the VH (CDR-H1, CDR-H2, CDR-H3), and three in the VL (CDR-L1, CDR-L2, CDR-L3). Exemplary CDRs herein include:

- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96
 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987));
 - (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b
 (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., Sequences of Proteins of Immunological
 Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)); and
 (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. J. Mol. Biol. 262: 732-745 (1996)).

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Unless otherwise indicated, the CDRs are determined according to Kabat et al., supra. One of skill in the art will understand that the CDR designations can also be determined according to Chothia, supra, McCallum, supra, or any other scientifically accepted nomenclature system.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al.. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g., residues 82a, 82b, and 82c, etc., according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3).

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

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human

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

The term "**chimeric**" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ respectively.

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

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

The term "**monoclonal antibody**" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal

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

The term "Fc domain" or "**Fc region**" herein is used to define a C-terminal region of an antibody heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Particularly, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. The amino acid sequences of the heavy chains are always presented with the C-terminal lysine, however variants without the C-terminal lysine are included in the invention.

An IgG Fc region comprises an IgG CH2 and an IgG CH3 domain. The "CH2 domain" of a human IgG Fc region usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. In one embodiment, a carbohydrate chain is attached to the CH2 domain. The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain. The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced "protuberance" ("knob") in one chain thereof and a corresponding introduced "cavity" ("hole") in the other chain thereof; see US Patent No. 5,821,333, expressly incorporated herein by reference). Such variant CH3 domains may be used to promote heterodimerization of two non-identical antibody heavy chains as herein described. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The "**knob-into-hole**" technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001).

Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment a knob modification comprises the amino acid substitution T366W in one of the two subunits of the Fc domain, and the hole modification comprises the amino acid substitutions T366S, L368A and Y407V in the other one of the two subunits of the Fc domain. In a further specific embodiment, the subunit of the Fc domain comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc domain comprising the hole modification additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in the formation of a disulfide bridge between the two subunits of the Fc region, thus further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

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A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990)).

The term "effector functions" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

An "activating Fc receptor" is an Fc receptor that following engagement by an Fc region of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcyRIIIa (CD16a), FcyRI (CD64), FcyRIIa (CD32),

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and FcαRI (CD89). A particular activating Fc receptor is human FcγRIIIa (see UniProt accession no. P08637, version 141).

The term "**peptide linker**" refers to a peptide comprising one or more amino acids, typically about 2 to 20 amino acids. Peptide linkers are known in the art or are described herein. Suitable, non-immunogenic linker peptides are, for example, $(G_4S)_n$, $(SG_4)_n$ or $G_4(SG_4)_n$ peptide linkers, wherein "n" is generally a number between 1 and 10, typically between 2 and 4, in particular 2. Peptide linkers of particular interest are (G4S) (SEQ ID NO:71), $(G_4S)_2$ or GGGGSGGGGS (SEQ ID NO:72), $(G4S)_3$ (SEQ ID NO:73) and $(G_4S)_4$ (SEQ ID NO:74), more particularly $(G_4S)_2$ or GGGGSGGGGS (SEQ ID NO:72).

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By "fused to" or "connected to" is meant that the components (e.g. an antigen binding domain and a FC domain) are linked by peptide bonds, either directly or via one or more peptide linkers.

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

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide (protein) sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN. SAWI or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX

operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

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

In certain aspects, **amino acid sequence variants** of the bispecific antibodies of the invention provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the bispecific antibodies. Amino acid sequence variants of the bispecific antibodies may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the molecules, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding. Sites of interest for substitutional mutagenesis include the HVRs and Framework (FRs). Conservative substitutions are provided in Table C under the heading "Preferred Substitutions" and further described below in reference to amino acid side chain classes (1) to (6). Amino acid substitutions may be introduced into the molecule of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE B

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu

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

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

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;

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- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes 10 for another class.

The term "amino acid sequence variants" includes substantial variants wherein there are amino acid substitutions in one or more hypervariable region residues of a parent antigen binding molecule (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antigen binding molecule and/or will have substantially retained certain biological properties of the

parent antigen binding molecule. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antigen binding molecules displayed on phage and screened for a particular biological activity (e.g. binding affinity). In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antigen binding molecule to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antigen binding molecule complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

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

In certain aspects, the bispecific antibodies provided herein are altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation variants of the molecules may be conveniently obtained by altering the amino acid sequence such that one or more glycosylation sites is created or removed, e.g. the carbohydrates attached to the Fc domain may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in the bispecific antibodies of the invention may be made in order to create variants with certain

improved properties. In one aspect, variants of bispecific antibodies are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such fucosylation variants may have improved ADCC function, see e.g. US Patent Publication Nos. US 2003/0157108 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Further variants of the bispecific antibodies of the invention include those with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function., see for example WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function and are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

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In certain aspects, it may be desirable to create **cysteine engineered variants** of the bispecific antibodies of the invention, e.g., "thioMAbs," in which one or more residues of the molecule are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the molecule. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antigen binding molecules may be generated as described, e.g., in U.S. Patent No. 7,521,541.

In certain aspects, the bispecific antibodies provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. 25 The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either 30 homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can 35 be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the

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particular properties or functions of the antibody to be improved, whether the bispecific antibody derivative will be used in a therapy under defined conditions, etc.

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

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

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The term "polynucleotide" refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA). The term "nucleic acid molecule" refers to any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By "isolated" nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide

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sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

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The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette of the invention comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

The term "vector" or "expression vector" is synonymous with "expression construct" and refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated in a target cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

The terms "host cell", "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages.

Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or

selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the bispecific antigen binding molecules of the present invention. In particular, the host cell is a prokaryotic or eukaryotic host cell. Host cells include cultured cells, e.g. mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

An "effective amount" of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

A "therapeutically effective amount" of an agent, e.g. a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

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

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

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

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

As used herein, "**treatment**" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect

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pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, the molecules of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "cancer" as used herein includes lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalveolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. In one embodiment, the term cancer refers to a CD20 expressing cancer.

The term "**expression of CD20**" is intended to indicate an significant level of expression CD20 in a cell, preferably on the cell surface of a T- or B- cell, more preferably a B-cell, from a tumor or cancer, respectively, preferably a non-solid tumor. Patients having a "CD20 expressing cancer" can be determined by standard assays known in the art. For example CD20 antigen expression can be measured using immunohistochemical (IHC) detection, FACS or via PCR-based detection of the corresponding mRNA.

The term "CD20 expressing cancer" as used herein refers to all cancers in which the cancer cells show an expression of the CD20 antigen. Preferably CD20 expressing cancer as used herein refers to lymphomas (preferably B-Cell Non-Hodgkin's lymphomas (NHL)) and lymphocytic leukemias. Such lymphomas and lymphocytic leukemias include e.g. a) follicular lymphomas, b) Small Non-Cleaved Cell Lymphomas/ Burkitt's lymphoma (including endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma and Non-Burkitt's lymphoma), c) marginal zone lymphomas (including extranodal marginal zone B cell lymphoma (Mucosa-associated lymphatic tissue lymphomas, MALT), nodal marginal zone B cell lymphoma and splenic marginal zone lymphoma), d) Mantle cell lymphoma (MCL), e) Large Cell Lymphoma (including B-cell diffuse large cell lymphoma (DLCL), Diffuse Mixed Cell Lymphoma,

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Immunoblastic Lymphoma, Primary Mediastinal B-Cell Lymphoma, Angiocentric Lymphoma-Pulmonary B-Cell Lymphoma), f) hairy cell leukemia, g) lymphocytic lymphoma, waldenstrom's macroglobulinemia, h) acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL), B-cell prolymphocytic leukemia, i) plasma cell neoplasms, plasma cell myeloma, multiple myeloma, plasmacytoma, j) Hodgkin's disease.

In one aspect, the CD20 expressing cancer is a B-Cell Non-Hodgkin's lymphoma (NHL). In another aspect, the CD20 expressing cancer is selected from the group consisting of Mantle cell lymphoma (MCL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), B-cell diffuse large cell lymphoma (DLCL), Burkitt's lymphoma, hairy cell leukemia, follicular lymphoma, multiple myeloma, marginal zone lymphoma, post transplant lymphoproliferative disorder (PTLD), HIV associated lymphoma, waldenstrom's macroglobulinemia, or primary CNS lymphoma.

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By "B cell proliferative disorder" is meant a disease wherein the number of B cells in a patient is increased as compared to the number of B cells in a healthy subject, and particularly wherein the increase in the number of B cells is the cause or hallmark of the disease. A "CD20-positive B cell proliferative disorder" is a B cell proliferative disorder wherein B-cells, particularly malignant B-cells (in addition to normal B-cells), express CD20. Exemplary B cell proliferation disorders include Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), as well as some types of Multiple myeloma (MM) and Hodgkin lymphoma (HL).

The term "a method of treating", "a method of treatment" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in a patient, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a patient, is nevertheless deemed to induce an overall beneficial course of action.

The terms "**combination**", "**co-administration**" or "**co-administering**" refer to the administration of an anti-CD20/anti-CD3 bispecific antibody, and an anti-PD1/anti-LAG3 bispecific antibody as two separate formulations (or as one single formulation). The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. The

anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are coadministered either simultaneously or sequentially (e.g. intravenous (i.v.) through a continuous infusion (one for the anti-CD20/anti-CD3 bispecific antibody and one for the anti-PD1/anti-LAG3 bispecific antibody). When both therapeutic agents are co-administered sequentially, the dose is administered either on the same day in two separate administrations, or one of the agents is administered on day 1 and the second is co-administered on day 2 to day 7, preferably on day 2 to 4. Thus the term "sequentially" means within 7 days after the dose of the first component (anti-CD20/anti-CD3 bispecific antibody or the anti-PD1/anti-LAG3 bispecific antibody), preferably within 4 days after the dose of the first component; and the term "simultaneously" means at the same time. The terms "co-administration" with respect to the maintenance doses of the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody mean that the maintenance doses can be either co-administered simultaneously, if the treatment cycle is appropriate for both drugs, e.g. every week. Or the anti-PD1/anti-LAG3 bispecific antibody is administered e.g. every second week and the anti-CD20/anti-CD3 bispecific antibody is administered every third week. Or the maintenance doses are co-administered sequentially, either within one or within several days.

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Exemplary anti-CD20/anti-CD3 bispecific antibodies for use in the invention

The present invention relates to anti-CD20/anti-CD3 bispecific antibodies and their use in combination with anti-PD1/anti-LAG3 bispecific antibodies, in particular to their use in a method for treating or delaying progression of CD20-expressing cancer, more particularly for treating or delaying progression of B-cell proliferative disorders. The anti-CD20/anti-CD3 bispecific antibodies as used herein are bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CD20. They are thus targeting CD20-expressing B cells.

Thus, the anti-CD20/anti-CD3 bispecific antibody as used herein comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_HCD20) and a light chain variable region (V_LCD20).

In a particular aspect, the anti-CD20/anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46. More particularly, the anti-CD20/anti-CD3 bispecific comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID

NO:47 and/or a light chain variable region (V_LCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:48. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:48.

In one aspect, the antibody that specifically binds to CD3 is a full-length antibody. In one aspect, the antibody that specifically binds to CD3 is an antibody of the human IgG class, particularly an antibody of the human IgG₁ class. In one aspect, the antibody that specifically binds to CD3 is an antibody fragment, particularly a Fab molecule or a scFv molecule, more particularly a Fab molecule. In a particular aspect, the antibody that specifically binds to CD3 is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other). In one aspect, the antibody that specifically binds to CD3 is a humanized antibody.

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In another aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a second antigen 15 binding domain comprising a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. More particularly, the anti-CD20/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_HCD20) that is at least 90%, 95%, 96%, 20 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:56. In a further aspect, the anti-CD20/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_HCD20) 25 comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:56.

In another particular aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CD20. In particular, the anti-CD20/anti-CD3 bispecific antibody comprises a third antigen binding domain comprising a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51; and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. More particularly, the anti-CD20/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_HCD20) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:56. In a further aspect, the

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anti-CD20/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_HCD20) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:56.

In a further aspect, the anti-CD20/anti-CD3 bispecific antibody is bispecific antibody, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

In another aspect, the anti-CD20/anti-CD3 bispecific antibody is bispecific antibody, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain

The Fab molecules may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example, (G4S) (SEQ ID NO:71), (G₄S)₂ or GGGGSGGGGS (SEQ ID NO:72), (G4S)₃ (SEQ ID NO:73) and (G₄S)₄ (SEQ ID NO:74), more particularly (G₄S)₂ or GGGGSGGGGS (SEQ ID NO:72). A particularly suitable peptide linker for fusing the Fab light chains of the first and the second Fab molecule to each other is (G₄S)₂. Another suitable linker comprises the sequence (G₄S)₄ (G₄S)₄ (SEQ ID NO:74). Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In particular, the anti-CD20/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G (according to EU numbering).

In a particular aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 57, a

polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 58, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 59, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 60. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO: 57, a polypeptide sequence of SEQ ID NO: 58, a polypeptide sequence of SEQ ID NO: 59 and a polypeptide sequence of SEQ ID NO: 60 (CD20 TCB).

In a particular aspect, the anti-CD20/anti-CD3 bispecific antibody is glofitamab.

Glofitamab (Proposed INN: List 121 WHO Drug Information, Vol. 33, No. 2, 2019, also known as CD20-TCB, RO7082859, or RG6026) is a novel T-cell-engaging bispecific full-length 10 antibody with a 2:1 molecular configuration for bivalent binding to CD20 on B cells and monovalent binding to CD3, particularly the CD3 epsilon chain (CD3e), on T cells. Its CD3binding region is fused to one of the CD20-binding regions in a head-to-tail fashion via a flexible linker. This structure endows glofitamab with superior in vitro potency versus other CD20-CD3 bispecific antibodies with a 1:1 configuration, and leads to profound antitumor efficacy in 15 preclinical DLBCL models. CD20 bivalency preserves this potency in the presence of competing anti-CD20 antibodies, providing the opportunity for pre- or co-treatment with these agents. Glofitamab comprises an engineered, heterodimeric Fc region with completely abolished binding to FcgRs and C1q. By simultaneously binding to human CD20-expressing tumor cells and to the CD3ɛ of the T-cell receptor (TCR) complex on T-cells, it induces tumor cell lysis, in addition to 20 T-cell activation, proliferation and cytokine release. Lysis of B-cells mediated by glofitamab is CD20-specific and does not occur in the absence of CD20 expression or in the absence of simultaneous binding (cross-linking) of T-cells to CD20-expressing cells. In addition to killing, T-cells undergo activation due to CD3 cross-linking, as detected by an increase in T-cell activation markers (CD25 and CD69), cytokine release (IFNy, TNF\alpha, IL-2, IL-6, IL-10), 25 cytotoxic granule release (Granzyme B) and T-cell proliferation.

In another aspect, the anti-CD20/anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:83, CDR-H2 sequence of SEQ ID NO:84, and CDR-H3 sequence of SEQ ID NO:85; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:86, CDR-L2 sequence of SEQ ID NO:87, and CDR-L3 sequence of SEQ ID NO:88. More particularly, the anti-CD20/anti-CD3 bispecific comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:89 and/or a light chain variable region (V_LCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:90. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_HCD3) comprising the

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amino acid sequence of SEQ ID NO:89 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:90.

In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a second antigen binding domain comprising a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:91, CDR-H2 sequence of SEQ ID NO:92, and CDR-H3 sequence of SEQ ID NO:93, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:94, CDR-L2 sequence of SEQ ID NO:95, and CDR-L3 sequence of SEQ ID NO:96. More particularly, the anti-CD20/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_HCD20) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:97 and/or a light chain variable region (V_LCD20) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:98. In a further aspect, the anti-CD20/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_HCD20) comprising the amino acid sequence of SEQ ID NO:97 and/or a light chain variable region (V_LCD20) comprising the amino acid sequence of SEQ ID NO:98.

In a particular aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 99, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 100, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 101, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 102. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO: 99, a polypeptide sequence of SEQ ID NO: 100, a polypeptide sequence of SEQ ID NO: 101 and a polypeptide sequence of SEQ ID NO: 102.

In a particular aspect, the anti-CD20/anti-CD3 bispecific antibody is mosunetuzumab.Mosunetuzumab (RO7030816; also known as BTCT4465A) is a humanized full-length antiCD20/CD3 T-cell dependent bispecific (TDB) antibody of the human IgG1 class comprising an amino acid substitution N297G (according to EU Numbering) in the fragment crystallizable (Fc) region. This substitution results in a non-glycosylated heavy chain that has minimal binding to Fc gamma (FC-γ) receptors and, consequently, reduces Fc effector functions. The mechanism of action of mosunetuzumab involves engaging T-cells via CD3 with CD20-expressing cells, leading to T-cell activation and T-cell mediated cytolysis of the CD20-expressing cells. On the basis of its structure as a full-length antibody and nonclinical data, the pharmacokinetic (PK) properties of mosunetuzumab enable intermittent dosing in the clinical setting, similar to other monoclonal antibodies.

Particular bispecific antibodies are described in PCT publication no. WO 2016/020309 A1 or in WO 2015/095392 A1.

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In a further aspect, the anti-CD20/anti-CD3 bispecific antibody may also comprise a bispecific T cell engager (BiTE®). In a further aspect, the anti-CD20/anti-CD3 bispecific antibody is XmAb®13676. In another aspect, the bispecific antibody is REGN1979. In another aspect, the the bispecific antibody is FBTA05 (Lymphomun).

5 Exemplary bispecific anti-PD1/anti-LAG3 antibodies for use in the invention

For the combination provided herein, used are novel bispecific antibodies comprising a first antigen binding domain that specifically binds to to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), with particularly advantageous properties such as producibility, stability, binding affinity, biological activity, specific targeting of certain T cells, targeting efficiency and reduced toxicity. Particular bispecific anti-PD1/anti-LAG3 antibodies for use herein are described in WO 2018/185043 A1.

In certain aspects, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that shows reduced internalization upon binding to the T cell surface. The internalization represents an important sink for the molecule which can be degraded within a few hours while the targeted receptors are rapidly re-expressed on the cell-surface ready to inhibit TCR-signalling. In further aspects, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that preferentially binds to conventional T cells rather than to Tregs. This is advantageous because targeting LAG-3 on Tregs with blocking antibodies could be detrimental by increasing their suppressive function and eventually mask the positive blocking effect on other T cells. In a further aspect, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that is able to rescue T cell effector functions from Treg suppression. In another aspect, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that is able to induce Granzyme B secretion by CD4 T cells, when co-cultured with the tumor cell line ARH77 as shown in the assay provided herein. In a further aspect, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that shows increased tumor-specific T cell effector functions and/or enhances the cytotoxic effect of T cells. In another aspect, a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is provided that shows increased tumor eradication in vivo.

In one aspect, the invention provides a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein said first antigen binding domain specifically binding to PD1 comprises

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- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
- 10 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, the bispecific antibody comprises a Fc domain that is an IgG, particularly an IgG1 Fc domain or an IgG4 Fc domain and wherein the Fc domain has reduced or even abolished effector function. In particular, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcy receptor.

In a further aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the bispecific antibody comprises a Fc domain that is an IgG, particularly an IgG1 Fc domain or an IgG4 Fc domain and wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor.

In another aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the second antigen binding domain that specifically binds to LAG3 comprises

- (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
- 30 (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
- 35 (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,

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- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In a further aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the first antigen binding domain specifically binding to PD1 comprises the VH domain comprising the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO:10.

In another aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the second antigen binding domain specifically binding to LAG3 comprises

- (a) a VH domain comprising the amino acid sequence of SEQ ID NO:17 and a VL domain comprising the amino acid sequence of SEQ ID NO:18, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.
- In a further aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the second antigen binding domain specifically binding to LAG3 comprises
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 27 and a VL domain comprising the amino acid sequence of SEQ ID NO: 28, or
 - (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 29 and a VL domain comprising the amino acid sequence of SEQ ID NO: 30, or
 - (c) a VH domain comprising the amino acid sequence of SEQ ID NO: 31 and a VL domain comprising the amino acid sequence of SEQ ID NO: 32, or
- 30 (d) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and a VL domain comprising the amino acid sequence of SEQ ID NO: 34.

In another aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically

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binds to LAG3, wherein the second antigen binding domain specifically binding to LAG3 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 81 and a VL domain comprising the amino acid sequence of SEQ ID NO: 82.

In a particular aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein

the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,

and the second antigen binding domain specifically binding to LAG3 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18 or a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.

In one aspect, the bispecific antibody of the invention comprises a first antigen binding domain specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10 and a second antigen binding domain specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

In a further aspect, the bispecific antibody of the invention comprises a first antigen binding domain specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10 and a second antigen binding domain specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.

In a further aspect, the bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is a human, humanized or chimeric antibody. In particular, it is a humanized or chimeric antibody.

In one aspect, the bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 is bivalent. This means that the bispecific antibody comprises one antigen binding domain that

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specifically binds to PD1 and one antigen binding domain that specifically binds to LAG3 (1+1 format).

In one aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the bispecific antibody comprises an Fc domain, a first Fab fragment comprising the antigen binding domain that specifically binds to PD1 and a second Fab fragment comprising the antigen binding domain that specifically binds to LAG3. In a particular aspect, in one of the Fab fragments the variable domains VL and VH are replaced by each other so that the VH domain is part of the light chain and the VL domain is part of the heavy chain. In a particular aspect, in the first Fab fragment comprising the antigen binding domain that specifically binds to PD1 the variable domains VL and VH are replaced by each other.

In a particular aspect, provided is a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3, wherein the bispecific antibody comprises

- (a) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:38, or
 - (b) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 39, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:40.

More particularly, the bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38.

Fc domain modifications reducing Fc receptor binding and/or effector function

In certain aspects, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein the bispecific antibody comprises a Fc domain comprising one or more amino acid modifications

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that reduce binding to an Fc receptor, in particular towards Fc γ receptor, and reduce or abolish effector function.

In certain aspects, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

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The following section describes preferred aspects of the bispecific antigen binding molecules of the invention comprising Fc domain modifications reducing Fc receptor binding and/or effector function. In one aspect, the invention relates to an anti-PD1/anti-LAG3 bispecific antibody, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor. In particular, the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

The Fc domain confers favorable pharmacokinetic properties to the bispecific antibodies of the invention, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the bispecific antibodies of the invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular embodiments the Fc domain of the the bispecific antibodies of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG Fc domain, in particular an IgG1 Fc domain or an IgG4 Fc domain. More particularly, the Fc domain is an IgG1 FC domain.

In one such aspect the Fc domain (or the bispecific antigen binding molecule of the invention comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG1 Fc domain (or the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG1 Fc domain (or the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc domain). In one aspect, the Fc domain (or the bispecific antigen binding molecule of the invention comprising said Fc domain) does not substantially bind to an Fc receptor and/or induce effector function. In a particular aspect the Fc receptor is an Fcγ receptor. In one aspect, the Fc receptor is an activating Fc receptor. In a specific aspect, the Fc receptor is an activating human Fcγ

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receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one aspect, the Fc receptor is an inhibitory Fc receptor. In a specific aspect, the Fc receptor is an inhibitory human Fcγ receptor, more specifically human FcγRIIB. In one aspect the effector function is one or more of CDC, ADCC, ADCP, and cytokine secretion. In a particular aspect, the effector function is ADCC. In one aspect, the Fc domain domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG1 Fc domain. Substantially similar binding to FcRn is achieved when the Fc domain (or the the bispecific antigen binding molecule of the invention comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG1 Fc domain (or the the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc domain) to FcRn.

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In a particular aspect, the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In a particular aspect, the Fc domain of the bispecific antigen binding molecule of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one aspect, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In another aspect, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In one aspect, the bispecific antigen binding molecule of the invention comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to bispecific antibodies of the invention comprising a non-engineered Fc domain. In a particular aspect, the Fc receptor is an Fcy receptor. In other aspects, the Fc receptor is a human Fc receptor. In one aspect, the Fc receptor is an inhibitory Fc receptor. In a specific aspect, the Fc receptor is an inhibitory human Fcy receptor, more specifically human FcyRIIB. In some aspects the Fc receptor is an activating Fc receptor. In a specific aspect, the Fc receptor is an activating human Fcy receptor, more specifically human FcyRIIIa, FcyRI or FcyRIIa, most specifically human FcyRIIIa. Preferably, binding to each of these receptors is reduced. In some aspects, binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one aspect, binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the bispecific antigen binding molecule of the invention comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the bispecific antigen binding molecule of the invention comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or the the bispecific antigen binding molecule of the invention comprising said Fc domain, may

exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain of the bispecific antigen binding molecule of the invention is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming.

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Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581). Certain antibody variants with improved or diminished binding to FcRs are described. (e.g. U.S. Patent No. 6,737,056; WO 2004/056312, and Shields, R.L. et al., J. Biol. Chem. 276 (2001) 6591-6604).

In one aspect of the invention, the Fc domain comprises an amino acid substitution at a position of E233, L234, L235, N297, P331 and P329. In some aspects, the Fc domain comprises the amino acid substitutions L234A and L235A ("LALA"). In one such embodiment, the Fc domain is an IgG1 Fc domain, particularly a human IgG1 Fc domain. In one aspect, the Fc domain comprises an amino acid substitution at position P329. In a more specific aspect, the amino acid substitution is P329A or P329G, particularly P329G. In one embodiment the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution selected from the group consisting of E233P, L234A, L235A, L235E, N297A, N297D or P331S. In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA"). The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcy receptor binding of a human IgG1 Fc domain, as described in PCT Patent Application No. WO 2012/130831 A1. Said document also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions such antibody is an IgG1 with mutations L234A and L235A or with mutations L234A, L235A and P329G (numbering according to EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991).

In one aspect, the anti-PD1/anti-LAG3 bispecific antibody comprises (all positions according to EU index of Kabat) (i) a homodimeric Fc-region of the human IgG1 subclass

optionally with the mutations P329G, L234A and L235A, or (ii) a homodimeric Fc-region of the human IgG4 subclass optionally with the mutations P329G, S228P and L235E, or (iii) a homodimeric Fc-region of the human IgG1 subclass optionally with the mutations P329G, L234A, L235A, I253A, H310A, and H435A, or optionally with the mutations P329G, L234A, L235A, H310A, H433A, and Y436A, or (iv) a heterodimeric Fc-region wherein one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or wherein one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V, and S354C, or wherein one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations 10 T366S, L368A, Y407V and Y349C, or (v) a heterodimeric Fc-region of the human IgG1 subclass wherein both Fc-region polypeptides comprise the mutations P329G, L234A and L235A and one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or wherein one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide 15 comprises the mutations T366S, L368A, Y407V, and S354C, or wherein one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V and Y349C.

In one aspect, the Fc domain is an IgG4 Fc domain. In a more specific embodiment, the Fc 20 domain is an IgG4 Fc domain comprising an amino acid substitution at position S228 (Kabat numbering), particularly the amino acid substitution S228P. In a more specific embodiment, the Fc domain is an IgG4 Fc domain comprising amino acid substitutions L235E and S228P and P329G. This amino acid substitution reduces in vivo Fab arm exchange of IgG4 antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). Thus, in one aspect, provided is a bispecific antibody, comprising (all positions according to EU index of Kabat) a 25 heterodimeric Fc-region of the human IgG4 subclass wherein both Fc-region polypeptides comprise the mutations P329G, S228P and L235E and one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or wherein one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V, and 30 S354C, or wherein one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V and Y349C.

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer, R.L. et al., J. Immunol. 117 (1976) 587-593, and Kim, J.K. et al., J. Immunol. 24 (1994) 2429-2434), are described in US 2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include

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those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan, A.R. and Winter, G., Nature 322 (1988) 738-740; US 5,648,260; US 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is described herein. Alternatively, binding affinity of Fc domains or cell activating bispecific antigen binding molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcyIIIa receptor. Effector function of an Fc domain, or bispecific antibodies of the invention comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM nonradioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

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The following section describes preferred aspects of the bispecific antibodies of the invention comprising Fc domain modifications reducing Fc receptor binding and/or effector function. In one aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein the Fc domain comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor, in particular towards Fcγ receptor. In another aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein the Fc domain comprises one or more amino acid substitution that reduces effector function. In particular aspect, the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

Fc domain modifications promoting heterodimerization

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The bispecific antigen binding molecules as described herein comprise different antigen binding domains, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains.

5 Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific antibodies of the invention in recombinant production, it will thus be advantageous to introduce in the Fc domain of the bispecific antigen binding molecules described herein a modification promoting the association of the desired polypeptides.

Accordingly, in particular aspects provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one aspect said modification is in the CH3 domain of the Fc domain.

In a specific aspect said modification is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain. Thus, the invention relates to a bispecific antibody comprising a first antigen binding domain that specifically binds to PD1 and a second antigen-binding site that specifically binds to LAG3, wherein the first subunit of the Fc domain comprises knobs and the second subunit of the Fc domain comprises holes according to the knobs into holes method. In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

Accordingly, in one aspect, in the CH3 domain of the first subunit of the Fc domain of the bispecific antigen binding molecules of the invention an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific aspect, in the CH3 domain of the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In one aspect, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A).

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In yet a further aspect, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). Introduction of these two cysteine residues leads to the formation of a disulfide bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter (2001), J Immunol Methods 248, 7-15). In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

But also other knobs-in-holes technologies as described by EP 1 870 459, can be used alternatively or additionally. In one embodiment the multispecific antibody comprises the mutations R409D and K370E in the CH3 domain of the "knobs chain" and the mutations D399K and E357K in the CH3 domain of the "hole-chain" (numbering according to Kabat EU index).

In one aspect, the bispecific antibody comprises a T366W mutation in the CH3 domain of the "knobs chain" and the mutations T366S, L368A and Y407V in the CH3 domain of the "hole chain" and additionally the mutations R409D and K370E in the CH3 domain of the "knobs chain" and the mutations D399K and E357K in the CH3 domain of the "hole chain" (numbering according to the Kabat EU index).

In one aspect, the bispecific antibody comprises the mutations Y349C and T366W in one of the two CH3 domains and the mutations S354C, T366S, L368A and Y407V in the other of the

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two CH3 domains, or the multispecific antibody comprises the mutations Y349C and T366W in one of the two CH3 domains and the mutations S354C, T366S, L368A and Y407V in the other of the two CH3 domains and additionally the mutations R409D and K370E in the CH3 domain of the "knobs chain" and the mutations D399K and E357K in the CH3 domain of the "hole chain" (numbering according to the Kabat EU index).

In an alternative aspect, a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

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Apart from the "knob-into-hole technology" other techniques for modifying the CH3 domains of the heavy chains of a multispecific antibody to enforce heterodimerization are known in the art. These technologies, especially the ones described in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954 and WO 2013/096291 are contemplated herein as alternatives to the "knob-into-hole technology" in combination with a bispecific antibody.

In one aspect, in the bispecific antibody the approach described in EP 1870459 is used to support heterodimerization of the first heavy chain and the second heavy chain of the multispecific antibody. This approach is based on the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3-domain-interface between both, the first and the second heavy chain.

Accordingly, in this aspect in the tertiary structure of the multispecific antibody the CH3 domain of the first heavy chain and the CH3 domain of the second heavy chain form an interface that is located between the respective antibody CH3 domains, wherein the respective amino acid sequences of the CH3 domain of the first heavy chain and the amino acid sequence of the CH3 domain of the second heavy chain each comprise a set of amino acids that is located within said interface in the tertiary structure of the antibody, wherein from the set of amino acids that is located in the interface in the CH3 domain of one heavy chain a first amino acid is substituted by a positively charged amino acid and from the set of amino acids that is located in the interface in the CH3 domain of the other heavy chain a second amino acid is substituted by a negatively charged amino acid. The bispecific antibody according to this aspect is herein also referred to as "CH3(+/-)-engineered bispecific antibody" (wherein the abbreviation "+/-" stands for the oppositely charged amino acids that were introduced in the respective CH3 domains).

In one aspect, in the CH3(+/-)-engineered bispecific antibody the positively charged amino acid is selected from K, R and H, and the negatively charged amino acid is selected from E or D.

In one aspect, in the CH3(+/-)-engineered bispecific antibody the positively charged amino acid is selected from K and R, and the negatively charged amino acid is selected from E or D.

In one aspect, in the CH3(+/-)-engineered bispecific antibody the positively charged amino acid is K, and the negatively charged amino acid is E.

In one aspect, in the CH3(+/-)-engineered bispecific antibody in the CH3 domain of one heavy chain the amino acid R at position 409 is substituted by D and the amino acid K at position is substituted by E, and in the CH3 domain of the other heavy chain the amino acid D at position 399 is substituted by K and the amino acid E at position 357 is substituted by K (numbering according to Kabat EU index).

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In one aspect, the approach described in WO 2013/157953 is used to support heterodimerization of the first heavy chain and the second heavy chain of the multispecific antibody. In one embodiment in the CH3 domain of one heavy chain the amino acid T at position 366 is substituted by K, and in the CH3 domain of the other heavy chain the amino acid L at position 351 is substituted by D (numbering according to Kabat EU index). In another embodiment in the CH3 domain of one heavy chain the amino acid T at position 366 is substituted by K and the amino acid L at position 351 is substituted by K, and in the CH3 domain of the other heavy chain the amino acid L at position 351 is substituted by D (numbering according to Kabat EU index).

In another aspect, in the CH3 domain of one heavy chain the amino acid T at position 366 is substituted by K and the amino acid L at position 351 is substituted by K, and in the CH3 domain of the other heavy chain the amino acid L at position 351 is substituted by D (numbering according to Kabat EU index). Additionally at least one of the following substitutions is comprised in the CH3 domain of the other heavy chain: the amino acid Y at position 349 is substituted by E, the amino acid Y at position 349 is substituted by D and the amino acid L at position 368 is substituted by E (numbering according to Kabat EU index). In one embodiment the amino acid L at position 368 is substituted by E (numbering according to Kabat EU index).

In one aspect, the approach described in WO 2012/058768 is used to support

heterodimerization of the first heavy chain and the second heavy chain of the multispecific antibody. In one aspect, in the CH3 domain of one heavy chain the amino acid L at position 351 is substituted by Y and the amino acid Y at position 407 is substituted by A, and in the CH3 domain of the other heavy chain the amino acid T at position 366 is substituted by A and the amino acid K at position 409 is substituted by F (numbering according to Kabat EU index). In

another embodiment, in addition to the aforementioned substitutions, in the CH3 domain of the other heavy chain at least one of the amino acids at positions 411 (originally T), 399 (originally D), 400 (originally S), 405 (originally F), 390 (originally N) and 392 (originally K) is substituted (numbering according to Kabat EU index). Preferred substitutions are:

- substituting the amino acid T at position 411 by an amino acid selected from N, R, Q, K, D, E and W (numbering according to Kabat EU index),

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- substituting the amino acid D at position 399 by an amino acid selected from R, W, Y, and K (numbering according to Kabat EU index),
- substituting the amino acid S at position 400 by an amino acid selected from E, D, R and 10 K (numbering according to Kabat EU index),
 - substituting the amino acid F at position 405 by an amino acid selected from I, M, T, S, V and W (numbering according to Kabat EU index;
 - substituting the amino acid N at position 390 by an amino acid selected from R, K and D (numbering according to Kabat EU index; and
- substituting the amino acid K at position 392 by an amino acid selected from V, M, R, L, F and E (numbering according to Kabat EU index).

In another aspect, the bispecific antibody is engineered according to WO 2012/058768), i.e. in the CH3 domain of one heavy chain the amino acid L at position 351 is substituted by Y and the amino acid Y at position 407 is substituted by A, and in the CH3 domain of the other heavy chain the amino acid T at position 366 is substituted by V and the amino acid K at position 409 is substituted by F (numbering according to Kabat EU index). In another embodiment of the multispecific antibody, in the CH3 domain of one heavy chain the amino acid Y at position 407 is substituted by A, and in the CH3 domain of the other heavy chain the amino acid T at position 366 is substituted by A and the amino acid K at position 409 is substituted by F (numbering according to Kabat EU index). In the last aforementioned embodiment, in the CH3 domain of the other heavy chain the amino acid K at position 392 is substituted by E, the amino acid T at position 411 is substituted by E, the amino acid D at position 399 is substituted by R and the amino acid S at position 400 is substituted by R (numbering according to Kabat EU index).

In one aspect, the approach described in WO 2011/143545 is used to support

heterodimerization of the first heavy chain and the second heavy chain of the multispecific antibody. In one aspect, amino acid modifications in the CH3 domains of both heavy chains are introduced at positions 368 and/or 409 (numbering according to Kabat EU index).

In one aspect, the approach described in WO 2011/090762 is used to support heterodimerization of the first heavy chain and the second heavy chain of the bispecific antibody. WO 2011/090762 relates to amino acid modifications according to the "knob-into-hole" (KiH) technology. In one embodiment in the CH3 domain of one heavy chain the amino acid T at position 366 is substituted by W, and in the CH3 domain of the other heavy chain the amino acid Y at position 407 is substituted by A (numbering according to Kabat EU index). In another embodiment in the CH3 domain of one heavy chain the amino acid T at position 366 is substituted by Y, and in the CH3 domain of the other heavy chain the amino acid Y at position 407 is substituted by T (numbering according to Kabat EU index).

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In one aspect, the approach described in WO 2009/089004 is used to support heterodimerization of the first heavy chain and the second heavy chain of the bispecific antibody. In one embodiment in the CH3 domain of one heavy chain the amino acid K or N at position 392 is substituted by a negatively charged amino acid (in one embodiment by E or D, in one preferred embodiment by D), and in the CH3 domain of the other heavy chain the amino acid D at position 399 the amino acid E or D at position 356 or the amino acid E at position 357 is substituted by a positively charged amino acid (in one embodiment K or R, in one preferred embodiment by K, in one preferred embodiment the amino acids at positions 399 or 356 are substituted by K) (numbering according to Kabat EU index). In one further embodiment, in addition to the aforementioned substitutions, in the CH3 domain of the one heavy chain the amino acid K or R at position 409 is substituted by a negatively charged amino acid (in one embodiment by E or D, in one preferred embodiment by D) (numbering according to Kabat EU index). In one even further aspect, in addition to or alternatively to the aforementioned substitutions, in the CH3 domain of the one heavy chain the amino acid K at position 439 and/or the amino acid K at position 370 is substituted independently from each other by a negatively charged amino acid (in one embodiment by E or D, in one preferred embodiment by D) (numbering according to Kabat EU index).

In one aspect, the approach described in WO 2007/147901 is used to support heterodimerization of the first heavy chain and the second heavy chain of the multispecific antibody. In one embodiment in the CH3 domain of one heavy chain the amino acid K at position 253 is substituted by E, the amino acid D at position 282 is substituted by K and the amino acid K at position 322 is substituted by D, and in the CH3 domain of the other heavy chain the amino acid D at position 239 is substituted by K, the amino acid E at position 240 is substituted by K and the amino acid K at position 292 is substituted by D (numbering according to Kabat EU index).

The C-terminus of the heavy chain of the bispecific antibody as reported herein can be a complete C-terminus ending with the amino acid residues PGK. The C-terminus of the heavy

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chain can be a shortened C-terminus in which one or two of the C terminal amino acid residues have been removed. In one preferred aspect, the C-terminus of the heavy chain is a shortened C-terminus ending PG.

In one aspect of all aspects as reported herein, a bispecific antibody comprising a heavy chain including a C-terminal CH3 domain as specified herein, comprises the C-terminal glycinelysine dipeptide (G446 and K447, numbering according to Kabat EU index). In one embodiment of all aspects as reported herein, a bispecific antibody comprising a heavy chain including a C-terminal CH3 domain, as specified herein, comprises a C-terminal glycine residue (G446, numbering according to Kabat EU index).

Modifications in the Fab domains

In one aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein in one of the Fab fragments either the variable domains VH and VL or the constant domains CH1 and CL are exchanged. The bispecific antibodies are prepared according to the Crossmab technology.

Multispecific antibodies with a domain replacement/exchange in one binding arm

(CrossMabVH-VL or CrossMabCH-CL) are described in detail in WO2009/080252,

WO2009/080253 and Schaefer, W. et al, PNAS, 108 (2011) 11187-1191. They clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange).

In a particular aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein in one of the Fab fragments the variable domains VL and VH are replaced by each other so that the VH domain is part of the light chain and the VL domain is part of the heavy chain. In a particular aspect, the bispecific antibody is one, wherein in the first Fab fragment comprising the antigen binding domain that specifically binds to PD1 the variable domains VL and VH are replaced by each other.

In another aspect, and to further improve correct pairing, the anti-PD1/anti-LAG3 bispecific antibody can contain different charged amino acid substitutions (so-called "charged residues"). These modifications are introduced in the crossed or non-crossed CH1 and CL domains. Such modifiactions are described e.g. in WO2015/150447, WO2016/020309 and PCT/EP2016/073408.

In a particular aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein in one of the Fab fragments in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU

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Index), and in the constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index). In a particular aspect, the bispecific antibody is one, wherein in the second Fab fragment comprising the antigen binding domain that specifically binds to TIM3 the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU Index), and in the constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

In a particular aspect, provided is an anti-PD1/anti-LAG3 bispecific antibody, wherein in one of CL domains the amino acid at position 123 (EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (EU numbering) has been substituted by lysine (K) and wherein in one of the CH1 domains the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E). In a particular aspect, the bispecific antibody is one, wherein in the Fab fragment comprising the antigen binding domain that specifically binds to LAG3 the amino acid at position 123 (EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (EU numbering) has been substituted by lysine (K) and wherein in one of the CH1 domains the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E).

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In a further aspect, the bispecific antibody is a bivalent antibody comprising

- a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
- b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the variable domains VL and VH of the second light chain and the second heavy chain are replaced by each other.
- The antibody under a) does not contain a modification as reported under b) and the heavy chain and the light chain under a) are isolated chains.

In the antibody under b) within the light chain the variable light chain domain VL is replaced by the variable heavy chain domain VH of said antibody, and within the heavy chain the variable heavy chain domain VH is replaced by the variable light chain domain VL of said antibody.

In one aspect, (i) in the constant domain CL of the first light chain under a) the amino acid at position 124 (numbering according to Kabat) is substituted by a positively charged amino acid, and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) is substituted by a negatively charged amino acid, or (ii) in the constant domain CL of the second

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light chain under b) the amino acid at position 124 (numbering according to Kabat) is substituted by a positively charged amino acid, and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) is substituted by a negatively charged amino acid.

In another aspect, (i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index), or (ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

In one aspect, in the constant domain CL of the second heavy chain the amino acids at position 124 and 123 are substituted by K (numbering according to Kabat EU index).

In one aspect, in the constant domain CL of the second heavy chain the amino acid at position 123 is substituted by R and the amino acid as position 124 is substituted by K (numbering according to Kabat EU index).

In one aspect, in the constant domain CH1 of the second light chain the amino acids at position 147 and 213 are substituted by E (numbering according to EU index of Kabat).

In one aspect, in the constant domain CL of the first light chain the amino acids at position 124 and 123 are substituted by K, and in the constant domain CH1 of the first heavy chain the amino acids at position 147 and 213 are substituted by E (numbering according to Kabat EU index).

In one aspect, in the constant domain CL of the first light chain the amino acid at position 123 is substituted by R and the amino acid at position 124 is substituted by K, and in the constant domain CH1 of the first heavy chain the amino acids at position 147 and 213 are both substituted by E (numbering according to Kabat EU index).

In one aspect, in the constant domain CL of the second heavy chain the amino acids at position 124 and 123 are substituted by K, and wherein in the constant domain CH1 of the

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second light chain the amino acids at position 147 and 213 are substituted by E, and in the variable domain VL of the first light chain the amino acid at position 38 is substituted by K, in the variable domain VH of the first heavy chain the amino acid at position 39 is substituted by E, in the variable domain VL of the second heavy chain the amino acid at position 38 is substituted by K, and in the variable domain VH of the second light chain the amino acid at position 39 is substituted by E (numbering according to Kabat EU index).

In one aspect, the bispecific antibody is a bivalent antibody comprising

- a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
- b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the variable domains VL and VH of the second light chain and the second heavy chain are replaced by each other, and wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other.

The antibody under a) does not contain a modification as reported under b) and the heavy chain and the light chain und a) are isolated chains. In the antibody under b) within the light chain the variable light chain domain VL is replaced by the variable heavy chain domain VH of said antibody, and the constant light chain domain CL is replaced by the constant heavy chain domain CH1 of said antibody; and within the heavy chain the variable heavy chain domain VH is replaced by the variable light chain domain VL of said antibody, and the constant heavy chain domain CH1 is replaced by the constant light chain domain CL of said antibody.

In one aspect, the bispecific antibody is a bivalent antibody comprising

- a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
- b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other.

The antibody under a) does not contain a modification as reported under b) and the heavy chain and the light chain under a) are isolated chains. In the antibody under b) within the light chain the constant light chain domain CL is replaced by the constant heavy chain domain CH1of said antibody; and within the heavy chain the constant heavy chain domain CH1 is replaced by the constant light chain domain CL of said antibody.

In one aspect, the bispecific antibody is a bispecific antibody comprising

a) a full length antibody specifically binding to a first antigen and consisting of two antibody heavy chains and two antibody light chains, and

b) one, two, three or four single chain Fab fragments specifically binding to a second antigen,

wherein said single chain Fab fragments under b) are fused to said full length antibody under a) via a peptide linker at the C- or N- terminus of the heavy or light chain of said full length antibody.

In one aspect, one or two identical single chain Fab fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C terminus of the heavy or light chains of said full length antibody.

In one aspect, one or two identical single chain Fab (scFab) fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C terminus of the heavy chains of said full length antibody.

In one aspect, one or two identical single chain Fab (scFab) fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C terminus of the light chains of said full length antibody.

In one aspect, two identical single chain Fab (scFab) fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C-terminus of each heavy or light chain of said full length antibody.

In one aspect, two identical single chain Fab (scFab) fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C-terminus of each heavy chain of said full length antibody.

In one aspect, two identical single chain Fab (scFab) fragments binding to a second antigen are fused to the full length antibody via a peptide linker at the C-terminus of each light chain of said full length antibody.

In one aspect, the bispecific antibody is a trivalent antibody comprising

- a) a full length antibody specifically binding to a first antigen and consisting of two antibody heavy chains and two antibody light chains,
 - b) a first polypeptide consisting of

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- ba) an antibody heavy chain variable domain (VH), or
- bb) an antibody heavy chain variable domain (VH) and an antibody constant domain 1 (CH1).

wherein said first polypeptide is fused with the N-terminus of its VH domain via a peptidic linker to the C-terminus of one of the two heavy chains of said full length antibody,

c) a second polypeptide consisting of

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- ca) an antibody light chain variable domain (VL), or
- cb) an antibody light chain variable domain (VL) and an antibody light chain constant domain (CL),

wherein said second polypeptide is fused with the N-terminus of the VL domain via a peptide linker to the C-terminus of the other of the two heavy chains of said full length antibody, and

wherein the antibody heavy chain variable domain (VH) of the first polypeptide and the antibody light chain variable domain (VL) of the second polypeptide together form an antigen binding domain specifically binding to a second antigen.

In one aspect, the antibody heavy chain variable domain (VH) of the polypeptide under b) and the antibody light chain variable domain (VL) of the polypeptide under c) are linked and stabilized via an interchain disulfide bridge by introduction of a disulfide bond between the following positions:

- (i) heavy chain variable domain position 44 to light chain variable domain position 100, or
- (ii) heavy chain variable domain position 105 to light chain variable domain position 43, or
- (iii) heavy chain variable domain position 101 to light chain variable domain position 100 (numbering always according to Kabat EU index).

Techniques to introduce unnatural disulfide bridges for stabilization are described e.g. in WO 94/029350, Rajagopal, V., et al., Prot. Eng. (1997) 1453-1459; Kobayashi, H., et al., Nucl. Med. Biol. 25 (1998) 387-393; and Schmidt, M., et al., Oncogene 18 (1999) 1711-1721. In one embodiment the optional disulfide bond between the variable domains of the polypeptides under b) and c) is between heavy chain variable domain position 44 and light chain variable domain position 100. In one embodiment the optional disulfide bond between the variable domains of the polypeptides under b) and c) is between heavy chain variable domain position 105 and light chain variable domain position 43 (numbering always according to Kabat). In one embodiment a trivalent, bispecific antibody without said optional disulfide stabilization between the variable domains VH and VL of the single chain Fab fragments is preferred.

In one aspect, the bispecific antibody is a trispecific or tetraspecific antibody, comprising

- a) a first light chain and a first heavy chain of a full length antibody which specifically binds to a first antigen, and
 - b) a second (modified) light chain and a second (modified) heavy chain of a full length antibody which specifically binds to a second antigen, wherein the variable domains VL and VH are replaced by each other, and/or wherein the constant domains CL and CH1 are replaced by each other, and

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c) wherein one to four antigen binding domains which specifically bind to one or two further antigens (i.e. to a third and/or fourth antigen) are fused via a peptide linker to the C- or N-terminus of the light chains or heavy chains of a) and/or b).

The antibody under a) does not contain a modification as reported under b) and the heavy chain and the light chain und a) are isolated chains.

In one aspect, the trispecific or tetraspecific antibody comprises under c) one or two antigen binding domains which specifically bind to one or two further antigens.

In one aspect, the antigen binding domains are selected from the group of a scFv fragment and a scFab fragment.

In one aspect, the antigen binding domains are scFv fragments.

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In one aspect, the antigen binding domains are scFab fragments.

In one aspect, the antigen binding domains are fused to the C-terminus of the heavy chains of a) and/or b).

In one aspect, the trispecific or tetraspecific antibody comprises under c) one or two antigen binding domains which specifically bind to one further antigen.

In one aspect, the trispecific or tetraspecific antibody comprises under c) two identical antigen binding domains which specifically bind to a third antigen. In one preferred embodiment such two identical antigen binding domains are fused both via the same peptidic linker to the C-terminus of the heavy chains of a) and b). In one preferred embodiment the two identical antigen binding domains are either a scFv fragment or a scFab fragment.

In one aspect, the trispecific or tetraspecific antibody comprises under c) two antigen binding domains which specifically bind to a third and a fourth antigen. In one embodiment said two antigen binding domains are fused both via the same peptide connector to the C-terminus of the heavy chains of a) and b). In one preferred embodiment said two antigen binding domains are either a scFv fragment or a scFab fragment.

In one aspect, the bispecific antibody is a bispecific, tetravalent antibody comprising

- a) two light chains and two heavy chains of an antibody, which specifically bind to a first antigen (and comprise two Fab fragments),
- b) two additional Fab fragments of an antibody, which specifically bind to a second antigen, wherein said additional Fab fragments are fused both via a peptidic linker either to the C- or N-termini of the heavy chains of a), and

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wherein in the Fab fragments the following modifications were performed

- (i) in both Fab fragments of a), or in both Fab fragments of b), the variable domains VL and VH are replaced by each other, and/or the constant domains CL and CH1 are replaced by each other, or
- (ii) in both Fab fragments of a) the variable domains VL and VH are replaced by each other, and the constant domains CL and CH1 are replaced by each other, and in both Fab fragments of b) the variable domains VL and VH are replaced by each other, or the constant domains CL and CH1 are replaced by each other, or
- (iii) in both Fab fragments of a) the variable domains VL and VH are replaced by each other, or the constant domains CL and CH1 are replaced by each other, and in both Fab fragments of b) the variable domains VL and VH are replaced by each other, and the constant domains CL and CH1 are replaced by each other, or
- (iv) in both Fab fragments of a) the variable domains VL and VH are replaced by each other, and in both Fab fragments of b) the constant domains CL and CH1 are replaced by each other, or
- (v) in both Fab fragments of a) the constant domains CL and CH1 are replaced by each other, and in both Fab fragments of b) the variable domains VL and VH are replaced by each other.

In one aspect, said additional Fab fragments are fused both via a peptidic linker either to the C-termini of the heavy chains of a), or to the N-termini of the heavy chains of a).

In one aspect, said additional Fab fragments are fused both via a peptidic linker either to the C-termini of the heavy chains of a).

In one aspect, said additional Fab fragments are fused both via a peptide linker to the N-termini of the heavy chains of a).

In one aspect, in the Fab fragments the following modifications are performed: in both Fab fragments of a), or in both Fab fragments of b), the variable domains VL and VH are replaced by each other, and/or the constant domains CL and CH1 are replaced by each other.

In one aspect, the bispecific antibody is a tetravalent antibody comprising:

- a) a (modified) heavy chain of a first antibody, which specifically binds to a first antigen and comprises a first VH-CH1 domain pair, wherein to the C terminus of said heavy chain the N-terminus of a second VH-CH1 domain pair of said first antibody is fused via a peptide linker,
 - b) two light chains of said first antibody of a),
- c) a (modified) heavy chain of a second antibody, which specifically binds to a second antigen and comprises a first VH-CL domain pair, wherein to the C-terminus of said heavy chain

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the N-terminus of a second VH-CL domain pair of said second antibody is fused via a peptide linker, and

- d) two (modified) light chains of said second antibody of c), each comprising a CL-CH1 domain pair.
- 5 In one aspect, the bispecific antibody comprises
 - a) the heavy chain and the light chain of a first full length antibody that specifically binds to a first antigen, and
 - b) the heavy chain and the light chain of a second full length antibody that specifically binds to a second antigen, wherein the N-terminus of the heavy chain is connected to the C-terminus of the light chain via a peptide linker.

The antibody under a) does not contain a modification as reported under b) and the heavy chain and the light chain are isolated chains.

In one aspect, the bispecific antibody comprises

- a) a full length antibody specifically binding to a first antigen and consisting of two antibody heavy chains and two antibody light chains, and
- b) an Fv fragment specifically binding to a second antigen comprising a VH2 domain and a VL2 domain, wherein both domains are connected to each other via a disulfide bridge,

wherein only either the VH2 domain or the VL2 domain is fused via a peptide linker to the heavy or light chain of the full length antibody specifically binding to a first antigen.

In the bispecific antibody the heavy chains and the light chains under a) are isolated chains.

In one aspect, the other of the VH2 domain or the VL2 domain is not fused via a peptide linker to the heavy or light chain of the full length antibody specifically binding to a first antigen.

In all aspects as reported herein the first light chain comprises a VL domain and a CL domain and the first heavy chain comprises a VH domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain.

In one aspect, the bispecific antibody is a trivalent antibody comprising

- a) two Fab fragments that specifically binds to a first antigen,
- b) one CrossFab fragment that specifically binds to a second antigen in which the CH1 and the CL domain are exchanged for each other,
- 30 c) one Fc-region comprising a first Fc-region heavy chain and a second Fc region heavy chain,

wherein the C-terminus of CH1 domains of the two Fab fragments are connected to the N-terminus of the heavy chain Fc-region polypeptides, and wherein the C-terminus of the CL

domain of the CrossFab fragment is connected to the N-terminus of the VH domain of one of the Fab fragments.

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In one aspect, the bispecific antibody is a trivalent antibody comprising

- a) two Fab fragments that specifically binds to a first antigen,
- b) one CrossFab fragment that specifically binds to a second antigen in which the CH1 and the CL domain are exchanged for each other,
 - c) one Fc-region comprising a first Fc-region heavy chain and a second Fc region heavy chain,

wherein the C-terminus of CH1 domain of the first Fab fragment is connected to the N-terminus of one of the heavy chain Fc-region polypeptides and the C-terminus of the CL-domain of the CrossFab fragment is connected to the N-terminus of the other heavy chain Fc-region polypeptide, and wherein the C-terminus of the CH1 domain of the second Fab fragment is connected to the N-terminus of the VH domain of the first Fab fragment or to the N-terminus of the VH domain of the CrossFab fragment.

In one aspect, the bispecific antibody comprises

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- a) a full length antibody specifically binding to a first antigen and consisting of two antibody heavy chains and two antibody light chains, and
- b) a Fab fragment specifically binding to a second antigen comprising a VH2 domain and a VL2 domain comprising a heavy chain fragment and a light chain fragment, wherein within the light chain fragment the variable light chain domain VL2 is replaced by the variable heavy chain domain VH2 of said antibody, and within the heavy chain fragment the variable heavy chain domain VH2 is replaced by the variable light chain domain VL2 of said antibody

wherein the heavy chain Fab fragment is inserted between the CH1 domain of one of the heavy chains of the full length antibody and the respective Fc-region of the full length antibody, and the N-terminus of the light chain Fab fragment is conjugated to the C-terminus of the light chain of the full length antibody that is paired with the heavy chain of the full length antibody into which the heavy chain Fab fragment has been inserted.

In one aspect, the bispecific antibody comprises

- a) a full length antibody specifically binding to a first antigen and consisting of two antibody heavy chains and two antibody light chains, and
- b) a Fab fragment specifically binding to a second antigen comprising a VH2 domain and a VL2 domain comprising a heavy chain fragment and a light chain fragment, wherein within the light chain fragment the variable light chain domain VL2 is replaced by the variable heavy chain domain VH2 of said antibody, and within the heavy chain fragment the variable heavy chain domain VH2 is replaced by the variable light chain domain VL2 of said antibody and wherein the C-terminus of the heavy chain fragment of the Fab fragment is conjugated to the N-terminus

of one of the heavy chains of the full length antibody and the C-terminus of the light chain fragment of the Fab fragment is conjugated to the N-terminus of the light chain of the full length antibody that pairs with the heavy chain of the full length antibody to which the heavy chain fragment of the Fab fragment is conjugated.

5 **Polynucleotides**

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Provided are furthermore isolated polynucleotides encoding a bispecific antibody as described herein or a fragment thereof.

The term "nucleic acid molecule" or "polynucleotide" includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g., complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples of non-naturally occurring nucleotides include modified nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention in vitro and/or in vivo, e.g., in a host or patient. Such DNA (e.g., cDNA) or RNA (e.g., mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody in vivo (see e.g., Stadler ert al, Nature Medicine 2017, published online 12 June 2017, doi:10.1038/nm.4356 or EP 2 101 823 B1).

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

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The isolated polynucleotides encoding bispecific antibodies of the invention may be expressed as a single polynucleotide that encodes the entire antigen binding molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional antigen binding molecule. For example, the light chain portion of an immunoglobulin may be encoded by a separate polynucleotide from the heavy chain portion of the immunoglobulin. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the immunoglobulin.

In some aspects, the isolated polynucleotide encodes a polypeptide comprised in the bispecific antibody according to the invention as described herein.

In one aspect, the isolated polynucleotides are provided encoding an anti-PD1/anti-LAG3 bispecific antibody, wherein said first antigen binding domain specifically binding to PD1 comprises a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

Preparation of bispecific antibodies for use in the invention

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Antibodies may be produced using recombinant methods and compositions, e.g., as described in US 4,816,567. For these methods one or more isolated nucleic acid(s) encoding an antibody are provided.

In case of a native antibody or native antibody fragment two nucleic acids are required, one for the light chain or a fragment thereof and one for the heavy chain or a fragment thereof. Such nucleic acid(s) encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chain(s) of the antibody). These nucleic acids can be on the same expression vector or on different expression vectors. In case of certain bispecific antibodies with heterodimeric heavy chains four nucleic acids are required, one for the first light chain, one for the first heavy chain comprising the first hetreomonomeric Fc-region polypeptide, one for the second light chain, and one for the second heavy chain comprising the second heteromonomeric Fc-region polypeptide. The four nucleic acids can be comprised in one or more nucleic acid molecules or expression vectors. For example, such nucleic acid(s) encode an amino acid sequence comprising the first VL and/or an amino acid sequence comprising the first VH including the first heteromonomeric Fc-region and/or an amino acid sequence comprising the second VL and/or an amino acid sequence

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comprising the second VH including the second heteromonomeric Fc-region of the antibody (e.g., the first and/or second light and/or the first and/or second heavy chains of the antibody). These nucleic acids can be on the same expression vector or on different expression vectors, normally these nucleic acids are located on two or three expression vectors, i.e. one vector can comprise more than one of these nucleic acids. Examples of these bispecific antibodies are CrossMabs and T-cell bispecifics (see, e.g. Schaefer, W. et al, PNAS, 108 (2011) 11187-1191). For example, one of the heteromonomeric heavy chain comprises the so-called "knob mutations" (T366W and optionally one of S354C or Y349C) and the other comprises the so-called "hole mutations" (T366S, L368A and Y407V and optionally Y349C or S354C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73).

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In one aspect, isolated nucleic acid encoding a bispecific antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antigen binding domains that specifically bind to PD1 and LAG3, respectively (e.g., in the light and/or heavy chains of the antibody). In a further aspect, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further aspect, a host cell comprising such nucleic acid is provided. In one such aspect, a host cell comprises (e.g., has been transformed with): (1) a first vector comprising a first pair of nucleic acids that encode amino acid sequences one of them comprising the first VL and the other comprising the first VH of the antibody and a second vector comprising a second pair of nucleic acids that encode amino acid sequences one of them comprising the second VL and the other comprising the second VH of the antibody, or (2) a first vector comprising a first nucleic acid that encode an amino acid sequence comprising one of the variable domains (preferably a light chain variable domain), a second vector comprising a pair of nucleic acids that encode amino acid sequences one of them comprising a light chain variable domain and the other comprising the first heavy chain variable domain, and a third vector comprising a pair of nucleic acids that encode amino acid sequences one of them comprising the respective other light chain variable domain as in the second vector and the other comprising the second heavy chain variable domain, or (3) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the first VL of the antibody, a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the first VH of the antibody, a third vector comprising a nucleic acid that encodes an amino acid sequence comprising the second VL of the antibody, and a fourth vector comprising a nucleic acid that encodes an amino acid sequence comprising the second VH of the antibody. In one aspect, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one aspect, a method of making a bispecific antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions

suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of the anti-CD20/anti-CD3 bispecific antibodies or anti-PD1/anti-LAG3 bispecific antibodies described herein, nucleic acid encoding the bispecific antibodies, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

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Suitable host cells for cloning or expression of antibody-encoding vectors include

prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5,648,237, US 5,789,199, and US 5,840,523. (See also Charlton, K.A., In: Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2003), pp. 245-254, describing expression of antibody fragments in E. coli.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, T.U., Nat. Biotech. 22 (2004) 1409-1414; and Li, H. et al., Nat. Biotech. 24 (2006) 210-215.

Suitable host cells for the expression of glycosylated antibodies are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham, F.L. et al., J. Gen Virol. 36 (1977) 59-74); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, J.P., Biol. Reprod. 23 (1980) 243-252); monkey kidney cells (CV1); African green monkey kidney

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cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather, J.P. et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68; MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub, G. et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A.M., Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2004), pp. 255-268.

10 Assays

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

1. Affinity assays

The affinity of the bispecific antigen binding molecules, antibodies and antibody fragments provided herein for the corresponding antigens can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a Biacore® instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. A specific illustrative and exemplary embodiment for measuring binding affinity has been described in Examples 2, 8 or 11 of WO WO 2018/185043. According to one aspect, K_D is measured by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25 °C.

2. Binding assays and other assays

In one aspect, the bispecific antibodies of the invention are tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc. The binding of the anti-PD1/anti-LAG3 bispecific antibodies provided herein to the corresponding recombinant antigen or to antigen-expressing cells may be evaluated by ELISA as described in Examples 8 or 11 of WO 2018/185043. In a further aspect, fresh peripheral blood mononuclear cells (PBMCs) can be used in binding assays to show binding to different peripheral blood mononuclear cells (PBMC) such as monocytes, NK cells and T cells.

In another aspect, a cellular dimerization assay was used to demonstrate the dimerization or at last binding/interaction of two different receptors PD1 and LAG3, which are cytosolically fused with two fragments of an enzyme, upon ligation or cross-linking with a bispecific antibody

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against both targets. Hereby only one receptor alone shows no enzymatic activity. For this specific interaction, the cytosolic C-terminal ends of both receptors were individually fused to heterologous subunits of a reporter enzym. A single enzyme subunit alone showed no reporter activity. However, simultaneous binding to both receptors was expected to lead to local cytocolic accumulation of both receptors, complementation of the two heterologous enzyme subunits, and finally to result in the formation of a specific and functional enzyme that hydrolyzes a substrate thereby generating a chemiluminescent signal (Example 11 of WO 2018/185043).

3. Activity assays

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In one aspect, assays are provided for identifying an anti-PD1/anti-LAG3 bispecific antibody having biological activity. Biological activity may include, e.g., the ability to enhance the activation and/or proliferation of different immune cells, especially T-cells, secretion of immune-modulating cytokines such IFNγ or TNF-alpha, blocking the PD1 pathway, blocking the LAG3 pathway, killing of tumor cells. Antibodies having such biological activity in vivo and/or in vitro are also provided. In certain aspects, an antibody of the invention is tested for such biological activity. In one aspect, provided is an immune cell assay which measures the activation of lymphocytes from one individual (donor X) to lymphocytes from another individual (donor Y). The mixed lymphocyte reaction (MLR) can demonstrate the effect of blocking the PD1 pathway to lymphocyte effector cells. T cells in the assay were tested for activation and their IFN-gamma secretion in the presence or absence of bispecific antibodies of the invention. The assay is described in more detail in Example 9 of WO 2018/185043.

Pharmaceutical Compositions, Formulations and Routes of Administation

In a further aspect, the invention provides pharmaceutical compositions comprising the anti-CD20/anti-CD3 antibodies and anti-PD1/anti-LAG3 antibodies provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises an anti-CD20/anti-CD3 antibody and an anti-PD1/anti-LAG3 antibody provided herein and at least one pharmaceutically acceptable excipient. In another embodiment, a pharmaceutical composition comprises an antibody provided herein and at least one additional therapeutic agent, e.g., as described below.

Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more bispecific antibodies dissolved or dispersed in a pharmaceutically acceptable excipient. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one antibody and optionally an additional

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active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. In particular, the compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable excipient" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g. antibacterial agents, antifungal agents), isotonic agents, salts, stabilizers and combinations thereof, as would be known to one of ordinary skill in the art.

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Parenteral compositions include those designed for administration by injection, e.g. subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the antigen binding molecules of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the fusion proteins may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the fusion proteins of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less that 0.5 ng/mg protein. Suitable pharmaceutically acceptable excipients include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as

EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl cleats or triglycerides, or liposomes.

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Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

Exemplary pharmaceutically acceptable excipients herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

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

In addition to the compositions described previously, the bispecific antibodies may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

Thus, for example, the fusion proteins may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the bispecific antigen binding molecules of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

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The bispecific antibodies disclosed herein may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g. those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

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

In one aspect, there is provided a pharmaceutical composition comprising an anti-CD20/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier, and a second medicament comprising an anti-PD1/anti-LAG3 antibody as described herein. In one aspect, the pharmaceutical composition is for use in the treatment of a CD20-expressing cancer. In a particular aspect, the pharmaceutical composition is for use in the treatment of B-cell proliferative disorders, in particular a disease selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).

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

Administration of the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody

Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody (both called substance herein) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The methods described herein are particularly useful, however, in relation to therapeutic agents administered by parenteral, particularly intravenous, infusion.

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Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein. In one aspect, the therapeutic agent is administered parenterally, particularly intravenously. In a particular aspect, the substance is administered by intravenous infusion. In another aspect, the substance is administered subcutaneously.

Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of therapeutic agent present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody (when used in their combination or with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of anti-CD20/anti-CD3 bispecific antibody, the severity and course of the disease, whether both agents are administered for preventive or therapeutic purposes, previous

therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. Each substance is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of the substance can be an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the bispecific antibody would be in the range from about 0.005 mg/kg to about 10 mg/kg. In other examples, a dose may also comprise from about 1 µg/kg body weight, about 5 µg/kg body weight, about 10 μg/kg body weight, about 50 μg/kg body weight, about 100 μg/kg body weight, about 200 μg/kg body weight, about 350 μg/kg body weight, about 500 μg/kg body weight, about 1 mg/kg body weight, about 5 mg/kg body weight, about 10 mg/kg body weight, about 50 mg/kg body weight, about 100 mg/kg body weight, about 200 mg/kg body weight, about 350 mg/kg body weight, about 500 mg/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 μg/kg body weight to about 500 mg/kg body weight etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

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In one aspect, the administration of both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody is a single administration. In certain aspects, the administration of the therapeutic agent is two or more administrations. In one such aspect, the substances are administered every week, every two weeks, or every three weeks, particularly every two weeks. In one aspect, the substance is administered in a therapeutically effective amount. In one aspect the substance is administered at a dose of about 10 μ g/kg, about 100 μ g/kg, about 200 μ g/kg, about 300 μ g/kg, about 400 μ g/kg, about 500 μ g/kg, about 600 μ g/kg, about 700 μ g/kg, about 800 μ g/kg, about 900 μ g/kg or about 1000 μ g/kg. In one embodiment, the anti-CD20/anti-CD3 bispecific antibody is administered at a dose which is higher than the dose of the anti-CD20/anti-CD3 bispecific antibody in a corresponding treatment regimen without the administration of the

anti-PD1/anti-LAG3 antibody. In one aspect, the administration of the anti-CD20/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the the anti-CD20/anti-CD3 bispecific antibody, and one or more subsequent administrations of a second dose of the anti-CD20/anti-CD3 bispecific antibody, wherein the second dose is higher than the first dose. In one aspect, the administration of the anti-CD20/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the anti-CD20/anti-CD3 bispecific antibody, and one or more subsequent administrations of a second dose of the anti-CD20/anti-CD3 bispecific antibody, wherein the first dose is not lower than the second dose.

In one aspect, the administration of the anti-CD20/anti-CD3 bispecific antibody in the treatment regimen according to the invention is the first administration of the anti-CD20/anti-CD3 bispecific antibody to the subject (at least within the same course of treatment). In one aspect, no administration of the anti-PD1/anti-LAG3 antibody is made to the subject prior to the administration of the anti-CD20/anti-CD3 bispecific antibody. In another aspect, the anti-PD1/anti-LAG3 antibody is administered prior to the administration of the anti-CD20/anti-CD3 bispecific antibody.

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In another aspect, the anti-CD20/anti-CD3 bispecific antibody is for use in combination with the anti-PD1/anti-LAG3 antibody, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab.

Activation of T cells can lead to severe cytokine release syndrome (CRS). In a phase 1 study conducted by TeGenero (Suntharalingam et al., N Engl J Med (2006) 355,1018-1028), all 6 healthy volunteers experienced near fatal, severe cytokine release syndrome (CRS) rapidly post-infusion of an inappropriately-dosed, T-cell stimulating super-agonist anti-CD28 monoclonal antibody. The cytokine release associated with administration of a T-cell activating therapeutic agent, such as the anti-CD20/anti-CD3 bispecific antibody, to a subject can be significantly reduced by pre-treatment of said subject with a Type II anti-CD20 antibody, such as obinutuzumab, the use of GAZYVA® pre-treatment (Gpt) should aid in the rapid depletion of B cells, both in the peripheral blood and in secondary lymphoid organs, such that the risk of highly relevant adverse events (AEs) from strong systemic T cell activation by T-cell activating therapeutic agents (e.g. CRS) is reduced, while supporting exposure levels of T-cell activating therapeutic agents that are high enough from the start of dosing to mediate tumour cell elimination. To date, the safety profile of obinutuzumab (including cytokine release) has been assessed and managed in hundreds of patients in ongoing obinutuzumab clinical trials. Finally, in addition to supporting the safety profile of T-cell activating therapeutic agents such as the anti-

CD20/anti-CD3 bispecific antibody, Gpt should also help prevent the formation of anti-drug antibodies (ADAs) to these unique molecules.

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In the present invention, the combination of the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody can be used in combination with one or more further agents in a therapy. For instance, at least one additional therapeutic agent may be co-administered. In certain aspects, an additional therapeutic agent is an immunotherapeutic agent.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the therapeutic agent can occur prior to, simultaneously, and/or following, administration of an additional therapeutic agent or agents. In one embodiment, administration of the therapeutic agent and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

Therapeutic methods and compositions

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15 CD20 is expressed on most B-cells (pan-B-cell marker) with the exception of stem cells and plasma cells, and are frequently expressed on most human B-cell malignancies (tumor associated antigen), such as lymphoma and leukemias except for multiple myeloma, e.g. in non-Hodgkin lymphoma and acute lymphoblastic leukemia.

In one aspect, there is provided a method for treating or delaying progression of CD20-20 expressing cancer in a subject comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 antibody.

In one such aspect, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent. In further embodiments, herein is provided a method for depleting B-cells comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 antibody. An "individual" or a "subject" according to any of the above aspects is preferably a human.

In further aspects, a composition for use in cancer immunotherapy is provided comprising an anti-CD20/anti-CD3 antibody and an anti-PD1/anti-LAG3 antibody. In certain embodiments, a composition comprising an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 antibody for use in a method of cancer immunotherapy is provided.

In a further aspect, herein is provided the use of a composition comprising an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 antibody in the manufacture or preparation of a medicament. In one aspect, the medicament is for treatment of a

CD20-expressing cancer. In one aspect, the medicament is for treatment of a B-cell proliferative disorder. In a further aspect, the medicament is for use in a method of treating a B-cell proliferative disorder comprising administering to an individual having a B-cell proliferative disorder an effective amount of the medicament. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In a further aspect, the medicament is for depleting B-cells. B-cell proliferative disorders are selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL). In one particular aspect, the B-cell cancer is non-Hodgkin lymphoma or acute lymphoblastic leukemia.

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In a further aspect, herein is provided a method for treating a B-cell cancer. In one embodiment, the method comprises administering to an individual having such B-cell cancer an effective amount of an effective amount of an anti-PD1/anti-LAG3 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human. In particular, the B-cell cancer is a B-cell lymphoma or a B-cell leukemia. In one aspect, the B-cell cancer is non-Hodgkin lymphoma or acute lymphoblastic leukemia.

The combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the anti-PD1/anti-LAG3 bispecific antibody as reported herein can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one aspect, administration of the effective amount of an anti-CD20/anti-CD3 bispecific antibody, of the effective amount of an anti-PD1/anti-LAG3 antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody as reported herein (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or

multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

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Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody as reported herein would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibodies need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibodies present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

A skilled artisan readily recognizes that in many cases the bispecific molecule may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some aspects, an amount of the bispecific antibody that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount".

Both the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 antibody as defined herein is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of the bispecific antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 10 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the anti-CD20/anti-CD3 bispecific antibody would be in the range from about 0.05 μg/kg to about 1000 μg/kg. For the the anti-PD1/anti-LAG3 antibody, a dose may also comprise from about 0.01 mg/kg body weight, about 0.05 mg/kg body weight, about 2 mg/kg body weight, about 4 mg/kg body weight, about 10 mg/kg body weight, about 20 mg/kg body weight, about 30 mg/kg body weight, about 40 mg/kg body weight, about 45 mg/kg body weight, about 50 mg/kg body weight, about 100 mg/kg body weight, about 200 mg/kg body weight, about 300 mg/kg body weight, about 400 mg/kg body weight, about 500 mg/kg body weight, about 600 mg/kg body weight, about 800 mg/kg body weight, about 1000 mg/kg body weight, top about 1200 mg/kg body weight or more -90-

per administration, and any range derivable therein. In examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 0.05 µg/kg body weight to about 500 mg/kg body weight etc., can be administered, based on the numbers described above. In one aspect, the anti-CD20/anti-CD3 bispecific antibody may be administered to the patient in a dose of from about 0.01 mg, from 2.5 mg, to about 10 mg or about 20 mg or about 30 mg. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the fusion protein). An initial lower loading dose, followed by one or more higher doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. In one aspect, the anti-PD1/anti-LAG3 antibody may be administered to the patient in a dose of from about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg. about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg or about 1500 mg.

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The bispecific antibodies comprising a first antigen binding domain that specifically binds to PD1 and a second antigen binding domain that specifically binds to LAG3 as defined herein will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the bispecific antibodies of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount.

Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from in vivo data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the bispecific antibody which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

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In cases of local administration or selective uptake, the effective local concentration of the bispecific antibody may not be related to plasma concentration. One skilled in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

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A therapeutically effective dose of the bispecific antibodies described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of a fusion protein can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD₅₀ (the dose lethal to 50% of a population) and the ED₅₀ (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD₅₀/ED₅₀. Bispecific antibodies that exhibit large therapeutic indices are preferred. In one embodiment, the bispecific antibody according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with bispecific antibodies of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of fusion protein used, the type of disorder or treatment, and other factors discussed above. The bispecific antibodies are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the bispecific antibody can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

H. Articles of Manufacture

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In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper that is pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PD1/anti-LAG3 antibody as defined herein before.

The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an anti-CD20/anti-CD3 bispecific antibody and (b) a second container with a composition contained therein, wherein the composition comprises an anti-PD1/anti-LAG3 antibody. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Table C (Sequences):

SEQ	Name	Sequence
ID NO:		
1	heavy chain HVR-H1, PD1-0103	GFSFSSY
2	heavy chain HVR-H2, PD1-0103	GGR
3	heavy chain HVR-H3, PD1-0103	TGRVYFALD
4	light chain HVR-L1, PD1-0103	SESVDTSDNSF

5	light chain HVR-L2, PD1-0103	RSS
6	light chain HVR-L3, PD1-0103	NYDVPW
7	heavy chain variable domain VH, PD1-0103	EVILVESGGGLVKPGGSLKLSCAASGFSFSSYTM SWVRQTPEKRLDWVATISGGGRDIYYPDSVKGRF TISRDNAKNTLYLEMSSLMSEDTALYYCVLLTGR VYFALDSWGQGTSVTVSS
8	light chain variable domain VL, PD1-0103	KIVLTQSPASLPVSLGQRATISCRASESVDTSDN SFIHWYQQRPGQSPKLLIYRSSTLESGVPARFSG SGSRTDFTLTIDPVEADDVATYYCQQNYDVPWTF GGGTKLEIK
9	humanized variant -heavy chain variable domain VH of PD1- 0103_01 (PD1 0376)	EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYTM SWVRQAPGKGLEWVATISGGGRDIYYPDSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCVLLTGR VYFALDSWGQGTLVTVSS
10	humanized variant -light chain variable domain VL of PD1- 0103_01 (PD1 0376)	DIVMTQSPDSLAVSLGERATINCKASESVDTSDN SFIHWYQQKPGQSPKLLIYRSSTLESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQNYDVPWTF GQGTKVEIK
11	heavy chain HVR-H1, aLAG3(0414)	DYTMN
12	heavy chain HVR-H2, aLAG3(0414)	VISWDGGGTY YTDSVKG
13	heavy chain HVR-H3, aLAG3(0414)	GLTDTTLYGS DY
14	light chain HVR-L1, aLAG3(0414)	RASQSISSYL N
15	light chain HVR-L2, aLAG3(0414)	AASTLQS
16	light chain HVR-L3, aLAG3(0414)	QQTYSSPLT
17	heavy chain variable domain VH, aLAG3(0414)	EVQLLESGGGLVQPGGSLRLSCAASGFIFDDYTM NWVRQAPGKGLEWVAVISWDGGGTYYTDSVKGRF TISRDDFKNTLYLQMNSLRAEDTAVYYCAKGLTD TTLYGSDYWGQGTLVTVSS
18	light chain variable domain VL, aLAG3(0414)	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLN WYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQTYSSPLTFGGGT KVEIK
19	heavy chain HVR-H1, aLAG3(0416)	DYAMS
20	heavy chain HVR-H2, aLAG3(0416)	GIDNSGYYTY YTDSVKG
21	heavy chain HVR-H3, aLAG3(0416)	THSGLIVNDA FDI
22	light chain HVR-L1, aLAG3(0416)	RASQSISSYL N
23	light chain HVR-L2, aLAG3(0416)	DASSLES
24	light chain HVR-L3, aLAG3(0416)	QQSYSTPLT
25	heavy chain variable domain VH, aLAG3(0416)	EVQLVESGGGLVQPGGSLRLACAASGFTFSDYAM SWVRQAPGKGLEWVSGIDNSGYYTYYTDSVKGRF TISRDDVKNTLYLQMNSLRAEDTAVYLCTKTHSG LIVNDAFDIWGQGTMVTVSS
26	light chain variable domain VL, aLAG3(0416)	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLN WYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSG TDATLTISSLQPEDFATYYCQQSYSTPLTFGGGT KVEIK

27	heavy chain variable domain VH,	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYW
	BMS-986016	NWIRQPPGKGLEWIGEINHRGSTNSNPSLKSRVT
		LSLDTSKNQFSLKLRSVTAADTAVYYCAFGYSDY
		EYNWFDPWGQGTLVTVSS
28	light chain variable domain VL	EIVLTQSPATLSLSPGERATLSCRASQSISSYLA
	BMS-986016	WYQQKPGQAPRLLIYDASNRATGIPARFSGSGSG
		TDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQGT NLEIK
29	heavy chain variable domain VH,	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYW
29	MDX25F7 (25F7)	NWIRQPPGKGLEWIGEINHNGNTNSNPSLKSRVT
	NIDA23F1 (23F1)	LSLDTSKNQFSLKLRSVTAADTAVYYCAFGYSDY
		EYNWFDPWGQGTLVTVSS
30	light chain variable domain VL,	EIVLTQSPATLSLSPGERATLSCRASQSISSYLA
	MDX25F7 (25F7)	WYQQKPGQAPRLLIYDASNRATGIPARFSGSGSG
		TDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQGT
		NLEIK
31	heavy chain variable domain VH,	QVQLVQSGAEVKKPGASVKVSCKASGFTLTNYGM
	humanized BAP050 (LAG525)	NWVRQARGQRLEWIGWINTDTGEPTYADDFKGRF
		VFSLDTSVSTAYLQISSLKAEDTAVYYCARNPPY
32	light shain yenishle damain VI	YYGTNNAEAMDYWGQGTTVTVSS DIQMTQSPSSLSASVGDRVTITCSSSQDISNYLN
32	light chain variable domain VL,	WYLQKPGQSPQLLIYYTSTLHLGVPSRFSGSGSG
	humanized BAP050 (LAG525)	TEFTLTISSLQPDDFATYYCQQYYNLPWTFGQGT
		KVEIK
33	heavy chain variable domain VH,	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGM
	MDX26H10 (26H10)	HWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRF
		TISRDNSKNTLYLQMNSLRAEDTAVYYCAREWAV
		ASWDYGMDVWGQGTTVTVSS
34	light chain variable domain VL,	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYL
	MDX26H10 (26H10)	AWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGS
		GTDFTLTISRLEPEDFAVYYCQQYGSSPFTFG PGTKVDIK
35		DIVMTQSPDSLAVSLGERATINCKASESVDTSDN
33	heavy chain 1 of 1+1 PD1/LAG3	SFIHWYQQKPGQSPKLLIYRSSTLESGVPDRFSG
	0927 based on PD1(0376)	SGSGTDFTLTISSLQAEDVAVYYCQQNYDVPWTF
		GQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGTA
		ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
		QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
		TKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFL
		FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
		QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPR
		EPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIA
		VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
36		EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYTM
30	light chain 1 of 1+1 PD1/LAG3	SWVRQAPGKGLEWVATISGGGRDIYYPDSVKGRF
	0927 based on PD1(0376)	TISRDNSKNTLYLQMNSLRAEDTAVYYCVLLTGR
	, ,	VYFALDSWGQGTLVTVSSASVAAPSVFIFPPSDE
		QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
		NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
		YACEVTHQGLSSPVTKSFNRGEC

37	1 2 2 21 1 77 1 77 1 72	EVQLLESGGGLVQPGGSLRLSCAASGFIFDDYTM
31	heavy chain 2 of 1+1 PD1/LAG3	NWVRQAPGKGLEWVAVISWDGGGTYYTDSVKGRF
	0927 based on aLAG3(0414)	TISRDDFKNTLYLQMNSLRAEDTAVYYCAKGLTD
		TTLYGSDYWGQGTLVTVSSASTKGPSVFPLAPSS
		KSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSG
		VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
		NVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEA
		AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
		EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
		VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI
		SKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK
		GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
		FLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSP
38	1: 1, 1 : 0 : 01 : 1 : DD1// A : C2	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLN
30	light chain 2 of 1+1 PD1/LAG3	WYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSG
	0927 based on aLAG3(0414)	TDFTLTISSLQPEDFATYYCQQTYSSPLTFGGGT
		KVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLL
		NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
		STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
		VTKSFNRGEC
39	heavy chain 2 of 1+1 PD1/LAG3	EVQLVESGGGLVQPGGSLRLACAASGFTFSDYAM
	0799 based on aLAG3(0416)	SWVRQAPGKGLEWVSGIDNSGYYTYYTDSVKGRF
	0799 based on aLAG3(0410)	TISRDDVKNTLYLQMNSLRAEDTAVYLCTKTHSG
		LIVNDAFDIWGQGTMVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTS
		GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
		CNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPE
		AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
		HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
		VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT
		ISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV
		KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
		FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		QKSLSLSP
40	light chain 2 of 1+1 PD1/LAG3	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLN
	0799 based on aLAG3(0416)	WYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSG
		TDATLTISSLQPEDFATYYCQQSYSTPLTFGGGT KVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLL
		NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
		STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
		VTKSFNRGEC
41	CD3-HCDR1	TYAMN
42	CD3-HCDR2	RIRSKYNNYATYYADSVKG
43	CD3-HCDR3	HGNFGNSYVSWFAY
44	CD3-LCDR1	GSSTGAVTTSNYAN
45	CD3-LCDR2	GTNKRAP
46	CD3-LCDR3	ALWYSNLWV
47	CD3 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAM
		NWVRQAPGKGLEWVSRIRSKYNNYATYYADSVKG
		RFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHG
		NFGNSYVSWFAYWGQGTLVTVSS
48	CD3 VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNY
		ANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSL

		LGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGG
		GTKLTVL
49	CD20-HCDR1	YSWIN
50	CD20-HCDR2	RIFPGDGDTDYNGKFKG
51	CD20-HCDR3	NVFDGYWLVY
52	CD20-LCDR1	RSSKSLLHSNGITYLY
53	CD20-LCDR2	QMSNLVS
54	CD20-LCDR3	AQNLELPYT
55	CD20 VH	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWI NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS
56	CD20 VL	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNG ITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYT FGGGTKVEIK
57	CD20 VH-CH1(EE)-CD3 VL-CH1-Fc (knob, P329G LALA)	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWI NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDEKVEPKSCDGGGGSGGGSQAVVT QEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQ EKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKA ALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLT VLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL PPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSP
58	CD20 VH-CH1(EE)-Fc (hole, P329G LALA)	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWI NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL VSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSP
59	CD20 VL-CL(RK)	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNG ITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYT FGGGTKVEIKRTVAAPSVFIFPPSDRKLKSGTAS

	T	THIRT I MININD BE THE CONTROLLED OF CONTROL
		VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
		QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
(0	CD2 LH CI	GLSSPVTKSFNRGEC
60	CD3 VH-CL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAM
		NWVRQAPGKGLEWVSRIRSKYNNYATYYADSVKG
		RFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHG
		NFGNSYVSWFAYWGQGTLVTVSSASVAAPSVFIF
		PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
		ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
61	CD20	UniProt accession No. P11836
62	Obinutuzumab heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWI
		NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRV
		TITADKSTSTAYMELSSLRSEDTAVYYCARNVFD
		GYWLVYWGQGTLVTVSSASTKGPSVFPLAPSSKS
		TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
		TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
		GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
		PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
		VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
		AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
		YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
		YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
		LSLSP
63	Obinutuzumab light chain	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNG
03	Oomutuzumao ngnt cham	ITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFS
		GSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYT
		FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
		VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
		QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
		GLSSPVTKSFNRGEC
64	murine anti-CD20 B-Ly1	GPELVKPGASVKISCKASGYAFSYSWMNWVKLRP
	VH	GQGLEWIGRIFPGDGDTDYNGKFKGKATLTADKS
	111	SNTAYMQLTSLTSVDSAVYLCARNVFDGYWLVYW
		GQGTLVTVSA
65	murine anti-CD20 B-Ly1	NPVTLGTSASISCRSSKSLLHSNGITYLYWYLQK
	VL	PGQSPQLLIYQMSNLVSGVPDRFSSSGSGTDFTL
	-	RISRVEAEDVGVYYCAQNLELPYTFGGGTKLEIK
		R
66	human CD3ε	UniProt accession no. P07766
67	cynomolgus CD3ε	NCBI GenBank no. BAB71849.1
68	human PD-1	UniProt accession no. Q15116
69	exemplary human LAG3 sequence	VPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVT
= =.	(without signal sequence)	WQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRP
	(anout organico)	RRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRG
		DFSLWLRPARRADAGEYRAAVHLRDRALSCRLRL
		RLGQASMTASPPGSLRASDWVILNCSFSRPDRPA
		SVHWFRNRGQGRVPVRESPHHHLAESFLFLPQVS
		PMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPT
		PLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTP
		PGGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCH
		IHLQEQQLNATVTLAIITVTPKSFGSPGSLGKLL
		CEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQE

		,
		AQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQ
		RSGRAPGALPAGHLLLFLILGVLSLLLLVTGAFG
		FHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQ
		EPEPEPEPEPEPEPEQL
70	human LAG3 Extracellular Domain	VPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVT
, ,	(ECD)	WQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRP
	(LCD)	RRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRG
		DFSLWLRPARRADAGEYRAAVHLRDRALSCRLRL
		RLGQASMTASPPGSLRASDWVILNCSFSRPDRPA
		SVHWFRNRGQGRVPVRESPHHHLAESFLFLPQVS
		PMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPT
		PLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTP
		PGGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCH
		IHLQEQQLNATVTLAIITVTPKSFGSPGSLGKLL
		CEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQE
		AQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQ
		RSGRAPGALPAGHL
71	Peptide linker G ₄ S	GGGGS
72	Peptide linker (G ₄ S) ₂	GGGGSGGGS
73	Peptide linker (G ₄ S) ₃	GGGGSGGGGGS
74	Peptide linker (G ₄ S) ₄	GGGGSGGGSGGGS
75	Pembrolizumab heavy chain	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYM
		YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRV
		TLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYR
		FDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSR
		STSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
		HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCN
		VDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGP
		SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
		VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
		TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
		GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP
		SDIAVEWESNGOPENNYKTTPPVLDSDGSFFLYS
		RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
		LSLG
76	Pembrolizumab light chain	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGY
70	1 emoronzumao ngin cham	SYLHWYQQKPGQAPRLLIYLASYLESGVPARFSG
		SGSGTDFTLTISSLEPEDFAVYYCOHSRDLPLTF
		GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
		VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
		DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
		LSSPVTKSFNRGEC
77	Nivolumab heavy chain	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGM
		HWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF
		TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY
		WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTA
		ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
		QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSN
		TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPP
		KPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
		DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
		LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
		VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW

		ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
		RWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
78	Nivolumab light chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLA
		WYQQKPGQAPRLLIYDASNRATGIPARFSGSGSG TDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGT
		KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
		NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
		STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
		VTKSFNRGEC
79	Anti-Lag3 heavy chain	QMQLVQSGPEVKKPGTSVKVSCKASGYTFTDYNV
		DWVRQARGQRLEWIGDINPNDGGTIYAQKFQERV
		TITVDKSTSTAYMELSSLRSEDTAVYYCARNYRW
		FGAMDHWGQGTTVTVSSASTKGPSVFPLAPSSKS
		TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
		NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
		GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
		PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
		VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK
		AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
		YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
		YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSP
80	Anti-Lag3 light chain	DIVMTQTPLSLSVTPGQPASISCKASQSLDYEGD
80	Anti-Lago fight chain	SDMNWYLQKPGQPPQLLIYGASNLESGVPDRFSG
		SGSGTDFTLKISRVEAEDVGVYYCQQSTEDPRTF
		GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
		VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
		DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
		LSSPVTKSFNRGEC
81	Anti-Lag3 heavy chain variable	QMQLVQSGPEVKKPGTSVKVSCKASGYTFTDYNV
	domain VH	DWVRQARGQRLEWIGDINPNDGGTIYAQKFQERV TITVDKSTSTAYMELSSLRSEDTAVYYCARNYRW
		FGAMDHWGQGTTVTVSS
82	Anti-Lag3 light chain variable	DIVMTQTPLSLSVTPGQPASISCKASQSLDYEGD
	domain VL	SDMNWYLQKPGQPPQLLIYGASNLESGVPDRFSG
		SGSGTDFTLKISRVEAEDVGVYYCQQSTEDPRTF
		GGGTKVEIK
83	CD3 (40G5c)-HCDR1	NYYIH
84	CD3 (40G5c)-HCDR2	WIYPGDGNTKYNEKFKG
85	CD3 (40G5c)-HCDR3	DSYSNYYFDY
86	CD3 (40G5c)-LCDR1	KSSQSLLNSRTRKNYLA
87	CD3 (40G5c)-LCDR2	WASTRES
88	CD3 (40G5c)-LCDR3	TQSFILRT
89	CD3 (40G5c) VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYI HWVRQAPGQGLEWIGWIYPGDGNTKYNEKFKGRA
		TLTADTSTSTAYLELSSLRSEDTAVYYCARDSYS
		NYYFDYWGQGTLVTVSS
90	CD3 (40G5c) VL	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRT
		RKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRF
		SGSGSGTDFTLTISSLQAEDVAVYYCTQSFILRT
		FGQGTKVEIK
91	CD20 (2H7.v16)-HCDR1	GYTFTSYNMH
92	CD20 (2H7.v16)-HCDR2	AIYPGNGDTSYNQKFKG

93	CD20 (2H7.v16)-HCDR3	VVYYSNSYWYFD V
94	CD20 (2H7.v16)-HCDR3	RASSSVSYMH
95	CD20 (2H7.v16)-LCDR1	APSNLAS
96	, ,	QQWSFNPPT
97	CD20 (2H7.v16)-LCDR3	
97	CD20 (2H7.v16) VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNM HWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRF TISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY SNSYWYFDVWGQGTLVTVSS
98	CD20 (2H7.v16) VL	DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHW YQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTK VEIK
99	CD3 (40G5c) light chain	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRT RKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRF SGSGSGTDFTLTISSLQAEDVAVYYCTQSFILRT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
100	CD3 (40G5c) heavy chain	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYI HWVRQAPGQGLEWIGWIYPGDGNTKYNEKFKGRA TLTADTSTSTAYLELSSLRSEDTAVYYCARDSYS NYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL VSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSP
101	CD20 (2H7.v16) light chain	DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHW YQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
102	CD20 (2H7.v16) heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNM HWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRF TISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY SNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSP

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103	KIEELE (part of LAG intracellular	KIEELE
	domain)	

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered and referred to according to the numbering systems according to Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) as defined above.

Aspects of the Invention

In the following, some of the aspects of the invention are listed.

- 1. An anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20-expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with an anti-PD1/anti-LAG3 bispecific antibody.
- 2. The anti-CD20/anti-CD3 bispecific antibody for use in a method of paragraph (para) 1, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.
- 3. The anti-CD20/anti-CD3 bispecific antibody for use in a method of paras 1 or 2, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fc domain that is an IgG Fc domain, particularly an IgG1 Fc domain or an IgG4 Fc domain, and wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcy receptor.
- 4. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 3, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fc domain of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).
- 5. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 4, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein a first antigen binding domain specifically binding to PD1 comprises a VH domain comprising
- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and

- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- 5 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
 - 6. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 4, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising
 - (a) a VH domain comprising
- 10 (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
 - (b) a VH domain comprising

- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
- 20 (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.
- 7. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 6, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen-binding domain specifically binding to PD1 comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO: 10.
- 8. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to
 7, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domaincomprising the amino acid sequence of SEQ ID NO: 26.

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- 9. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 5 or 7, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 27 and a VL domain comprising the amino acid sequence of SEQ ID NO: 28, or
 - (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 29 and a VL domain comprising the amino acid sequence of SEQ ID NO: 30, or
 - (c) a VH domain comprising the amino acid sequence of SEQ ID NO: 31 and a VL domain comprising the amino acid sequence of SEQ ID NO: 32, or
- (d) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and a VL domain comprising the amino acid sequence of SEQ ID NO: 34.
- 10. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 9, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises
 - a first antigen binding domain specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,
 - and a second antigen binding domain specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.
- 11. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 10, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fab fragment specifically binding to PD1 and a Fab fragment specifically binding to LAG3.
 - 12. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 11, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fab fragment specifically binding to PD1, wherein the variable domains VL and VH are replaced by each other so that VL is part of the heavy chain and VH is part of the light chain.
 - 13. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 12, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises monovalent binding to PD-1 and monovalent binding to LAG3.
- 30 14. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 13, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises
 - (a) a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38, or

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- (b) a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 39, and a second light chain comprising an amino acid sequence of SEQ ID NO:40.
- 15. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 14, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38.
- 16. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 15, wherein the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_LCD20) and a light chain variable region (V_LCD20).
- 17. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 16, wherein the first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46.
 - 18. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 17, wherein the first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:48.
- 19. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 18, wherein the second antigen binding domain comprises a heavy chain variable region (V_HCD20) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCD20) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.
 - 20. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 19, wherein the second antigen binding domain comprises a heavy chain variable region ($V_{L}CD20$) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region ($V_{L}CD20$) comprising the amino acid sequence of SEQ ID NO:56.

- 21. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 20, wherein the anti-CD20/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CD20.
- 22. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 21, wherein the anti-CD20/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.
 - 23. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 22, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with anti-PD1/anti-LAG3 bispecific antibody and wherein the combination is for administration at intervals from about about one week to three weeks.

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- 24. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of paras 1 to 23, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab.
- 25. A pharmaceutical composition comprising a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody for use in the combined, sequential or simultaneous, treatment of a disease, in particular CD20 expressing cancer.
- 26. A pharmaceutical composition comprising an anti-CD20/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier, and a second medicament comprising an anti-PD1/anti-LAG3 bispecific antibody.
 - 27. The pharmaceutical composition of para 26 for use in the treatment of a CD20 expressing cancer, in particular a hematological cancer selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).
- 28. Use of a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-30 PD1/anti-LAG3 bispecific antibody in the manufacture of a medicament for treating a CD20 expressing cancer.
 - 29. A method for treating a CD20 expressing cancer in a subject comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 bispecific antibody.

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- 30. The method of para 29, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.
- 31. The method of paras 29 or 30, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered intravenously or subcutaneously.
 - 32. The method of any one of paras 29 to 31, wherein the anti-CD20/anti-CD3 bispecific antibody is administered concurrently with, prior to, or subsequently to the anti-PD1/anti-LAG3 bispecific antibody.

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EXAMPLES

Recombinant DNA techniques

Standard methods were used to manipulate DNA as described in Sambrook et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions. General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242.

DNA sequencing

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DNA sequences were determined by double strand sequencing.

Gene synthesis

Desired gene segments were either generated by PCR using appropriate templates or were synthesized by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. In cases where no exact gene sequence was available, oligonucleotide primers were designed based on sequences from closest homologues and the genes were isolated by RT-PCR from RNA originating from the appropriate tissue. The gene segments flanked by singular restriction endonuclease cleavage sites were cloned into standard cloning / sequencing vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells.

Cell culture techniques

Standard cell culture techniques were used as described in Current Protocols in Cell Biology (2000), Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-Schwartz, J. and Yamada, K.M. (eds.), John Wiley & Sons, Inc.

Protein purification

Proteins were purified from filtered cell culture supernatants referring to standard protocols. In brief, antibodies were applied to a Protein A Sepharose column (GE healthcare) and washed with PBS. Elution of antibodies was achieved at pH 2.8 followed by immediate

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neutralization of the sample. Aggregated protein was separated from monomeric antibodies by size exclusion chromatography (Superdex 200, GE Healthcare) in PBS or in 20 mM Histidine, 150 mM NaCl pH 6.0. Monomeric antibody fractions were pooled, concentrated (if required) using e.g., a MILLIPORE Amicon Ultra (30 MWCO) centrifugal concentrator, frozen and stored at -20°C or -80°C. Part of the samples were provided for subsequent protein analytics and analytical characterization e.g. by SDS-PAGE, size exclusion chromatography (SEC) or mass spectrometry.

SDS-PAGE

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The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instruction. In particular, 10% or 4-12% NuPAGE® Novex® Bis-TRIS Pre-Cast gels (pH 6.4) and a NuPAGE® MES (reduced gels, with NuPAGE® Antioxidant running buffer additive) or MOPS (non-reduced gels) running buffer was used.

Analytical size exclusion chromatography

Size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH₂PO₄/K₂HPO₄, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

Determination of binding and binding affinity of multispecific antibodies to the respective antigens using surface plasmon resonance (SPR) (BIACORE)

Binding of the generated antibodies to the respective antigens is investigated by surface plasmon resonance using a BIACORE instrument (GE Healthcare Biosciences AB, Uppsala, Sweden). Briefly, for affinity measurements Goat-Anti-Human IgG, JIR 109-005-098 antibodies are immobilized on a CM5 chip via amine coupling for presentation of the antibodies against the respective antigen. Binding is measured in HBS buffer (HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% Tween 20, ph 7.4), 25°C (or alternatively at 37°C). Antigen (R&D Systems or in house purified) was added in various concentrations in solution. Association was measured by an antigen injection of 80 seconds to 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 - 10 minutes and a KD value was estimated using a 1:1 Langmuir binding model. Negative control data (e.g. buffer curves) are subtracted from sample curves for correction of system intrinsic baseline drift and for noise signal reduction. The respective

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Biacore Evaluation Software is used for analysis of sensorgrams and for calculation of affinity data.

Example 1

Preparation, purification and characterization of T-cell bispecific (TCB) antibodies

5 TCB molecules have been prepared according to the methods described in WO 2016/020309 A1.

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The anti-CD20/anti-CD3 bispecific antibody (CD20 CD3 TCB or CD20 TCB) used in the experiments corresponds to molecule B as described in Example 1 of WO 2016/020309 A1. Molecule B is a "2+1 IgG CrossFab" antibody and is comprised of two different heavy chains and two different light chains. Point mutations in the CH3 domain ("knobs into holes") were introduced to promote the assembly of the two different heavy chains. The Pro329Gly, Leu234Ala and Leu235Ala mutations were introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in WO 2012/130831. Exchange of the VH and VL domains in the CD3 binding Fab and point mutations in the CH and CL domains in the CD20 binding Fab were made in order to promote the correct assembly of the two different light chains. 2+1 means that the molecule has two antigen binding domains specific for CD3.

CD20 TCB comprises the amino acid sequences of SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59 and SEQ ID NO:60. A schematic scheme of the bispecific antibody in 2+1 format is shown in **Figure 1B**.

The molecule is further characterized in Example 1 of WO 2016/020309 A1.

Example 2

Preparation, purification and characterization of bispecific anti-PD1/anti-LAG3 antibodies

Bispecific antibodies which bind to human PD1 and human LAG3 with VH/VL domain exchange/replacement (*CrossMAbVh-VL*) in one binding arm were generated as described in Example 10.1 of WO 2018/185043. The preparation of multispecific 1+1 *CrossMAbVh-Vl* antibodies is also described in WO 2009/080252. The bispecific antibodies were expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in **Table 1**. A schematic structure of the 1+1 *CrossMAbVh-Vl* bispecific antibodies is shown in Fig. 1A.

Table 1: Amino acid sequences of light chains (LC) and heavy chains (HC), with VH/VL domain exchange/replacement (1+1 CrossMAb^{Vh-Vl)}

1+1 Antibody	HC1	HC2	LC1	LC2
PD1/LAG3 0799 PD1(0376)/ aLAG3(0416)	SEQ ID NO:35	SEQ ID NO:39	SEQ ID NO:36	SEQ ID NO:40
PD1/LAG3 0927 PD1(0376)/ aLAG3(0414)	SEQ ID NO:35	SEQ ID NO:37	SEQ ID NO:36	SEQ ID NO:38

For all constructs knobs into holes heterodimerization technology was used with a typical knob (T366W) substitution in the first CH3 domain and the corresponding hole substitutions (T366S, L368A and Y410V) in the second CH3 domain (as well as two additional introduced cysteine residues S354C/Y349°C) (contained in the respective corresponding heavy chain (HC) sequences depicted above). The Pro329Gly, Leu234Ala and Leu235Ala mutations were introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in WO 2012/130831. In order to improve correct pairing, amino acid substitutions were additionally introduced in the CH and CL domain of the conventional Fab (charged variants).

The bispecific antibodies expressed as above were purified from the supernatant by a combination of Protein A affinity chromatography and size exclusion chromatography. The obtained products were characterized for identity by mass spectrometry and analytical properties such as purity by SDS-PAGE, monomer content and stability.

The parental PD1 antibody PD1(0376) IgG1 used for comparison comprises the VH domain comprising the amino acid sequence of SEQ ID NO:9 and the VL domain comprising the amino acid sequence of SEQ ID NO:10.

20 Example 3

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Effect of PD-1/LAG-3 Bispecific antibodies in combination with CD20 CD3 TCB on cytotoxic Granzyme B release by human CD4 T cells cocultured with a B cell-lymphoblatoid cell line (ARH77)

To investigate the combinability of the PD-1/LAG-3 bispecific antibodies with CD20-TCB, we developed an assay in which freshly purified CD4 T cells are co-cultured for 5 days in presence of an EBV-immortalized B-cell lymphoblastoid tumor cell line (ARH77). We chose the ARH77 cell line because of its intermediate expression levels of PD-1 ligand, PD-L1, and high levels of LAG-3 ligand MHC-II, allowing for the assessment of the contribution of LAG-3 blockade in addition to PD-1.

5 CD4 T cells were enriched via a microbead kit (Miltenyi Biotec) from 10⁸ PBMCs obtained from 5 healthy donors. Prior culture, CD4 T cells were labeled with 5 μM of Carboxy-Fluorescein-Succinimidyl Esther (CFSE). 10⁵ CD4 T cells were then plated in a 96 well plate together with the B cell line (5:1) in presence or absence of blocking anti-PD1 antibodies (either parental anti-PD-1, nivolumab or pembrolizumab) or the PD-1/LAG-3 bispecific antibody
10 PD1/LAG3 0927 (PD1-LAG3 BsAb) at concentrations between 10⁻⁷ and 10 μg/ml and a fixed concentration of CD20-TCB (66pM). Five days later, for the last five hours of incubation, we added Golgi-plug and Golgi-stop to block protein transportation and allow intracellular accumulation of the cytokines.

Interestingly, we observed a dose dependent effect of PD-1 blocking antibodies in combination with CD20-TCB on CD4 T cell secretion of Granzyme B (see **Figure 2**). However, equimolar PD1-LAG3 BsAb was more potent and efficacious (E_{max}) than the PD-1 blocking antibodies in increasing in a dose dependent fashion the Granzyme B secreted by CD4 T cells, making it a suitable combination partner for CD20-TCB. The corresponding EC50 values are shown in **Table 2** below:

Table 2: Granzyme B secreted by CD4 T cells in coculture with ARH77 when plated with PD1-LAG3 BsAb or blocking anti-PD1 antibodies in combination with CD20 CD3 TCB

Molecule	EC ₅₀
pembrolizumab	n.d.
nivolumab	4.039
Parental aPD-1 antibody PD1(0376)	0.3318
PD1-LAG3 BsAb (PD1/LAG3 0927)	0.2162

Example 4

Potent anti-tumor effect of the combination therapy of PD1/LAG3 bispecific antibodies and CD20 CD3 TCB in vivo in the WSU-DLCL2 graft model in humanized NSG mice

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The anti-tumour activity of PD-1/LAG-3 bispecific antibody PD1/LAG3 0927 (PD1-LAG3 BsAb) in combination with CD20 CD3 TCB (CD20 TCB) was assessed *in vivo* in HSC-NSG mice engrafted with the human diffuse large B cell lymphoma model WSU-DLCL2,

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injected s.c. The efficacy of this combination was compared to single treatment CD20 CD3 TCB and its combination with Nivolumab or Nivolumab plus anti-LAG3 reference antibody.

a) Experimental Material and Methods

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Preparation of WSU-DLCL2 cell line: WSU-DLCL2 cells (human diffuse large B cell lymphoma) were originally obtained from ECACC (European Collection of Cell Culture) and after expansion deposited in the Roche Glycart internal cell bank. Cells were cultured in RPMI containing 10% FCS and 1x Glutamax. The cells were cultured at 37 °C in a water-saturated atmosphere at 5 % CO₂. 1.5 x10⁶ cells (in vitro passage P13) per animal were injected s.c. per mouse in RPMI cell culture medium (Gibco) and GFR matrigel (1:1, total volume of 100ul) at a viability of 98.6%.

Production of fully humanized mice: Female NSG mice, in the age of 4-5 weeks at the start of the experiment (Jackson Laboratory) were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2011/128). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis. The NSG mice were injected i.p. with 15 mg/kg of Busulfan followed one day later by an i.v. injection of 1x10⁵ human hematopoietic stem cells isolated from cord blood. At week 14-16 after stem cell injection humanized immunodeficient mice (HSC-NSG) were bled sublingual and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups.

Efficacy Experiment: Fully humanized HSC-NSG mice were challenged subcutaneously with 1.5 x 10⁶ WSU-DLCL2 cells (human diffuse large B cell lymphoma, expressing CD20) at day 0 in the presence of matrigel at 1:1 ratio. Tumors were measured 3 times per week during the whole experiment by Caliper. At day 14 (tumor average around 350-400 mm³), the mice were randomized into seven groups (**Figure 3**) and the first therapy was given. A weekly scheduled therapy started: Group A received vehicle (phosphate-buffer saline, PBS), Group B received CD20 TCB (0.15 mg/kg once/week i.v.), Group C received CD20 TCB (0.15 mg/kg once/week i.v.) + Nivolumab (1.5 mg/kg once/week i.v.), Group D received CD20 TCB (0.15 mg/kg once/week i.v.) + Nivolumab (1.5 mg/kg once/week i.v.) + anti-LAG3 (BMS-986016, 1.5 mg/kg once/week i.v.), Group E received CD20 TCB (0.15 mg/kg once/week i.v.) + PD1-LAG3 BsAb (1.5 mg/kg once/week i.v.) and Group F received CD20 TCB (0.15 mg/kg once/week i.v.) + PD1-LAG3 BsAb (3 mg/kg once/week i.v.). The treatment was given by intra-peritoneal

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injection in 400 µl max. Tumor growth was measured 3 times weekly using a caliper and tumor volume was calculated as followed:

$$T_v$$
: (W²/2) x L (W: Width, L: Length)

The study was terminated at day 45.

The impact of the therapy was thus assessed by measuring the tumour size and displayed as tumour growth over time either as mean (**Figure 4**), or as as tumor growth over time for each single mouse (**Figures 5A** to **5F**). For statistical analysis the last observed tumour volume of each animal was used as endpoint and it was evaluated if it is below 800 mm³ or not. This endpoint was then subject to pairwise group comparisons based on a Chi² test (**Figure 6**).

10 <u>b) Results</u>

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In this setting, the treatment of WSU-DCLC2 bearing mice with CD20 TCB was found to mediate strong tumor growth inhibition starting at day 30 when compared to the vehicle (**Figure 4**). As it is known that activation through TCB induces PD1 expression as well as LAG3 expression on T cells, CD20 TCB was combined with either Nivolumab or Nivolumab plus an anti-LAG3 antibody in the attempt to further improve efficacy. However, such combination did not induce a tumour growth reduction to a statistically significant level when compared to the single treatment (**Figures 4** and **6**). In contrast, the treatment with PD1-LAG3 BsAb, both at 1.5 and 3 mg/kg, in combination with CD20 TCB resulted in a strong tumour protection with strong tumour regression by day 42. When the statistical analysis was applied considering the last observed tumor size and fixing the threshold at 800 mm³, a significant increase in anti-tumor efficacy were observed when animals were treated with PD1-LAG3 BsAb at 3mg/kg in combination with CD20 TCB compared to the treatment with CD20 TCB in combination with Nivolumab and anti-LAG3 antibody.

The tumor growth of each single animal is depicted in the spider plots in **Figures 5A** to **5F**.

The plots show that in vehicle all tumors except two progressed over the entire experimental window. When CD20-TCB was combined with Nivolumab or Nivolumab plus anti-LAG3, no major improvement of anti-tumor efficacy was observed. In contrast, the combination of CD20-TCB with PD1-LAG3 BsAb at 3 mg/kg and at 1.5 mg/kg showed consistent tumor control in most of the mice except one.

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Example 5

Potent anti-tumor effect of the combination therapy of PD1/LAG3 bispecific antibodies and CD20 CD3 TCB in vivo in the OCI-Ly18 graft model in humanized NSG mice

In order to evaluate the contribution of PD-1 and LAG-3 co-blockade in combination with CD20 CD3 bispecific antibodies, the combination with CD20 TCB (glofitamab) was assessed in OCI-Ly18 bearing huHSC-NSG mice. The OCI-Ly18 is a human DLBC lymphoma model less sensible to CD20-TCB treatment which, as monotherapy, failed in controlling tumor growth.

a) Experimental Material and Methods

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Production of fully humanized mice: Female NSG mice, age 4-5 weeks at start of the experiment (Jackson Laboratory) were maintained under specific-pathogen-free conditions with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2011/128). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis. The NSG mice were injected i.p. with 15 mg/kg of Busulfan followed one day later by an i.v. injection of 1x10⁵ human hematopoietic stem cells isolated from cord blood. At week 14-16 after stem cell injection mice were bled sublingual and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups.

Preparation of OCI-Ly18 cell line: *OCI-Ly18* cells (human diffuse large B cell lymphoma) were originally obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and after expansion deposited in the Glycart internal cell bank. *OCI-Ly18* cells were cultivated in RPMI 1640 medium (Gibco/Lubioscience # 42401-042) containing 10% fetal calf serum (FCS, Gibco) and 1% Glutamax (Invitrogen/Gibco # 35050- 038). The cells were cultured at 37 °C in a water-saturated atmosphere at 5 % CO₂.

Efficacy Experiment: Fully humanized HSC-NSG mice (20 mice per group) were challenged subcutaneously with 5 x 10⁶ OCI-Ly18 cells (human diffuse large B cell lymphoma) at day 0 in the presence of matrigel at 1:1 ratio. At day 11 (tumor average around 200 mm³) a first treatment with obinutuzumab was administered to eliminate peripheral B cells and avoid cytokine release syndrome. The pre-treatment with obinutuzumab (30 mg/kg) was followed by a weekly scheduled therapy of: vehicle (histidine buffer), CD20 TCB (0.5 mg/kg), PD1-LAG3 BsAb (3 mg/kg), pembrolizumab (1.5 mg/kg) and anti-LAG3 antibody (1.5 mg/kg; antibody comprising the amino acid sequences of SEQ ID NO:79 and SEQ ID NO:80) (i.v.) (see **Figure**

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7). Tumor growth was measured 2-3 times weekly using a caliper and tumor volume was calculated as followed:

$$T_v$$
: $(W^2/2) \times L$ (W: Width, L: Length)

The study was terminated on day 35.

5 <u>b) Results</u>

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In this experiment, monotherapy of CD20 TCB (0.5 mg/kg) led to a delay in tumor growth when compared to the vehicle group (**Figure 8**). However, the combination of CD20 TCB with PD1-LAG3 BsAb (3 mg/kg) provided tumor control and in some mice promoted tumor rejection (**Figure 9C**). Interestingly, the combination of CD20 TCB with Pembrolizumab (1.5 mg/kg) and anti-LAG3 antibody (1.5 mg/kg) did not differ from CD20 TCB monotherapy.

These data demonstrate that PD1-LAG3 BsAb improves the anti-tumor activity of CD20 TCB in the context of lymphoma xenograft models in a manner superior to standard-of-care anti PD-1 antibodies in combination with monospecific anti-LAG-3 antibodies administered at 1.5 mg/kg versus 3 mg/kg of PD1-LAG3 BsAb in order to match PD-1 and LAG-3 binding sites. These studies establish the contribution of LAG-3 inhibition by PD1-LAG3 BsAb over PD-1 inhibition and support its differentiated mechanism of action versus competitor anti-PD-1 anitbodies in combination with anti-LAG-3 antibodies.

Example 6

Anti-tumor effect after pre-treatment with obinutuzumab of the combination therapy of
PD1/LAG3 bispecific antibodies and CD20 CD3 TCB in vivo in the OCI-Ly18 graft model in
humanized NSG mice

In this additional experiment, pre-treatment with obinutuzumab, an anti-CD20 depleting antibody, to reduce the cytokine release syndrome (CRS) induced by the peripheral B cell engagement with T cells mediated by glofitamab was further evaluated (**Figure 10**).

25 *a) Experimental Material and Methods*

<u>Production of fully humanized mice</u>: Female NSG mice, age 4-5 weeks at start of the experiment (Jackson Laboratory) were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2011/128). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular

basis. The NSG mice were injected i.p. with 15 mg/kg of Busulfan followed one day later by an i.v. injection of 1x10⁵ human hematopoietic stem cells isolated from cord blood. At week 14-16 after stem cell injection mice were bled sublingual and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups.

Preparation of OCI-Ly18 cell line: *OCI-Ly18* cells (human diffuse large B cell lymphoma) were originally obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and after expansion deposited in the Glycart internal cell bank. *OCI-Ly18* cells were cultivated in RPMI 1640 medium (Gibco/Lubioscience # 42401-042) containing 10% fetal calf serum (FCS, Gibco) and 1% Glutamax (Invitrogen/Gibco # 35050-038). The cells were cultured at 37 °C in a water-saturated atmosphere at 5 % CO₂.

Efficacy Experiment: Fully humanized HSC-NSG mice (14 mice per group) were challenged subcutaneously with 5 x 10⁶ OCI-Ly18 cells (human diffuse large B cell lymphoma) at day 0 in the presence of matrigel at 1:1 ratio. At day 17 (tumor average around 400 mm³) a first treatment with obinutuzumab (30 mg/kg) was administered to eliminate peripheral B cells and avoid cytokine release syndrome. The obinutuzumab pre-treatment was followed by a weekly scheduled therapy of: vehicle (histidine buffer), CD20 TCB (0.5 mg/kg), PD1-LAG3 BsAb (3 mg/kg), all i.v. (see **Figure 10**). Tumor growth was measured 2-3 times weekly using a caliper and tumor volume was calculated as followed:

T_v:
$$(W^2/2) \times L$$
 (W: Width, L: Length)

The study was terminated on day 35.

b) Results

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In this experiment, monotherapy of CD20 TCB (0.5 mg/kg), with a pre-treatment with obitunuzumab (30 mg/kg), led to a partial tumor control when compared to the vehicle group (**Figure 11** and **Figures 12A** and **12B**). However, the combination of CD20 TCB with PD1-LAG3 BsAb (3 mg/kg) provided strong tumor control (**Figure 11**). In some mice it was observed tumor rejection (**Figure 12C**). These data demonstrate that PD1-LAG3 BsAb improves the antitumor activity of CD20 TCB in the context of lymphoma xenograft models also when a pre-treatment with obinutuzumab is used to reduced CRS.

This study established the contribution of PD-1 and LAG-3 inhibition by PD1-LAG3 BsAb over single treatment of CD20-TCB in a context of obinutuzumab pre-treatment.

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Claims

- 1. An anti-CD20/anti-CD3 bispecific antibody for use in a method of treating CD20-expressing cancer, wherein the anti-CD20/anti-CD3 bispecific antibody is used in combination with an anti-PD1/anti-LAG3 bispecific antibody, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- 10 (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- 15 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
 - 2. The anti-CD20/anti-CD3 bispecific antibody for use in a method of claim 1, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.
- 3. The anti-CD20/anti-CD3 bispecific antibody for use in a method of claims 1 or 2, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fc domain that is an IgG Fc domain, particularly an IgG1 Fc domain or an IgG4 Fc domain, and wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor.
- 4. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 3, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising
 - (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
- 35 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or

- (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and
- a VL domain comprising

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- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.
- 5. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 4, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen-binding domain specifically binding to PD1 comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO: 10.
- 6. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 5, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18, or
 - (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.
 - 7. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 3 or 5, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigenbinding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 27 and a VL domain comprising the amino acid sequence of SEQ ID NO: 28, or
 - (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 29 and a VL domain comprising the amino acid sequence of SEQ ID NO: 30, or
 - (c) a VH domain comprising the amino acid sequence of SEQ ID NO: 31 and a VL domain comprising the amino acid sequence of SEQ ID NO: 32, or
- 30 (d) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and a VL domain comprising the amino acid sequence of SEQ ID NO: 34.

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- 8. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 6, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises
 - a first antigen binding domain specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,

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- and a second antigen binding domain specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.
- 9. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 8, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a Fab fragment specifically binding to PD1 and a Fab fragment specifically binding to LAG3.
 - 10. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 6 or 8 or 9, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 35, a first light chain comprising an amino acid sequence of SEQ ID NO: 36, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 37, and a second light chain comprising an amino acid sequence of SEQ ID NO:38.
 - 11. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 10, wherein the anti-CD20/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3), and a second antigen binding domain comprising a heavy chain variable region (V_LCD20) and a light chain variable region (V_LCD20).
 - 12. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 11, wherein the first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46.
- 13. The anti-CD20/anti-CD3 bispecific antibody for use in a method of any one of claims 1 to 12, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab.

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- 14. A composition comprising an anti-PD1/anti-LAG3 bispecific antibody for use in the treatment of CD20 expressing cancer, wherein said treatment comprises administration of said composition comprising an anti-PD1/anti-LAG3 bispecific antibody in combination with a composition comprising an anti-CD20/anti-CD3 bispecific antibody, wherein the anti-PD1/anti-
- LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- 10 (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
- 15 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
 - 15. The composition of claim 13, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen-binding domain specifically binding to PD1 comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 9 and the VL domain comprising the amino acid sequence of SEQ ID NO: 10.
- 20 16. The composition of claims 14 or 15, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising
 - (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and 25 a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
- 30 (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
- 35 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,

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- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

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ID NO: 10,

- 17. The composition of claims 14 to 16, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen-binding domain specifically binding to LAG3 comprising
 - (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.
- 18. The composition of claims 14 to 17, wherein the anti-PD1/anti-LAG3 bispecific
 antibody comprises
 a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino
 acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ
- and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.
 - 19. The composition of claims 14 to 18, wherein the anti-CD20/anti-CD3 bispecific antibody is glofitamab.
- 20. The composition of claims 14 to 18, wherein the anti-CD20/anti-CD3 bispecific antibody is mosunetuzumab.
 - 21. A pharmaceutical composition comprising a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody for use in the combined, sequential or simultaneous, treatment of a disease, in particular CD20 expressing cancer.
- 22. The pharmaceutical composition of claim 21 for use in the treatment of a CD20 expressing cancer, in particular a hematological cancer selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).
- 30 23. Use of a combination of an anti-CD20/anti-CD3 bispecific antibody and an anti-PD1/anti-LAG3 bispecific antibody in the manufacture of a medicament for treating a CD20

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expressing cancer, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising

- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
- (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
- 10 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
 - 24. The use according to claim 23, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising
- 15 (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
- 20 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
 - (b) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- 30 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.
 - 25. The use according to claims 23 or 24, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises

a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ

35 ID NO: 10,

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and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.

- 26. A method for treating a CD20 expressing cancer in a subject comprising administering to the subject an effective amount of an anti-CD20/anti-CD3 antibody and an effective amount of an anti-PD1/anti-LAG3 bispecific antibody, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a first antigen binding domain that specifically binds to programmed cell death protein 1 (PD1) and a second antigen binding domain that specifically binds to Lymphocyte activation gene-3 (LAG3), wherein the first antigen binding domain specifically binding to PD1 comprises a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:3; and a VL domain comprising
- 15 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
 - 27. The method of claim 26, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises a second antigen binding domain that specifically binds to LAG3 comprising
- 20 (a) a VH domain comprising
 - (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:11,
 - (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:12, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:13; and a VL domain comprising
- 25 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:14,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:15, and
 - (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:16; or
 - (b) a VH domain comprising

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- (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:19,
- (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:20, and
 - (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:21; and a VL domain comprising
 - (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:22,
 - (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:23, and
- 35 (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

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- 28. The method of claims 26 or 27, wherein the anti-PD1/anti-LAG3 bispecific antibody comprises
- a first Fab fragment specifically binding to PD1 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10,
- and a second Fab fragment specifically binding to LAG3 comprising a VH domain comprising the amino acid sequence of SEQ ID NO: 17 and a VL domain comprising the amino acid sequence of SEQ ID NO: 18.
- 29. The method of any one of claims 26 to 28, the anti-CD20/anti-CD3 bispecific antibody 10 is glofitamab.
 - 30. The method of any one of claims 26 to 29, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.
- 31. The method of any one of claims 26 to 30, wherein the anti-CD20/anti-CD3 bispecific antibody and the anti-PD1/anti-LAG3 bispecific antibody are administered intravenously or subcutaneously.
 - 32. The method of any one of claims 26 to 31, wherein the anti-CD20/anti-CD3 bispecific antibody is administered concurrently with, prior to, or subsequently to the anti-PD1/anti-LAG3 bispecific antibody.

Fig. 1A

anti-CD20 anti-CD3 톳 CH1* Fig. 1B 3 , CH1* CH1 Fc(Hole) P329G LALA anti-CD20

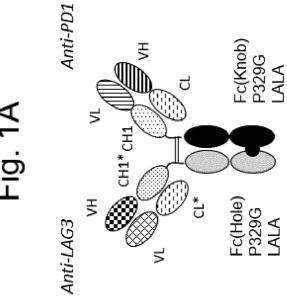


Fig. 2

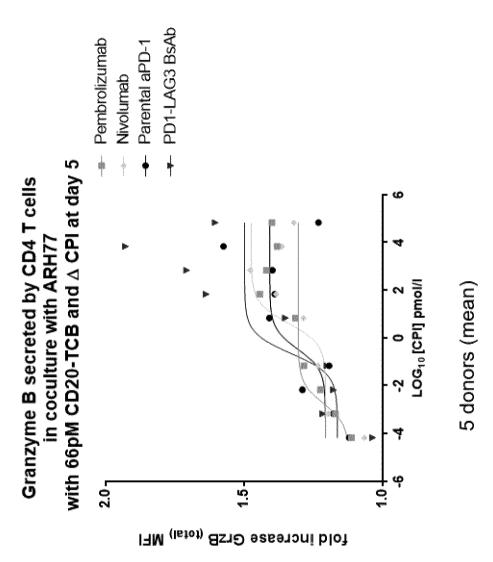
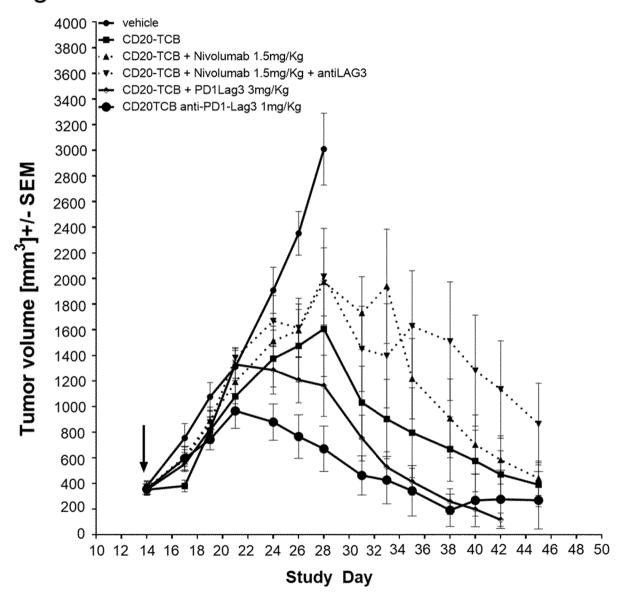
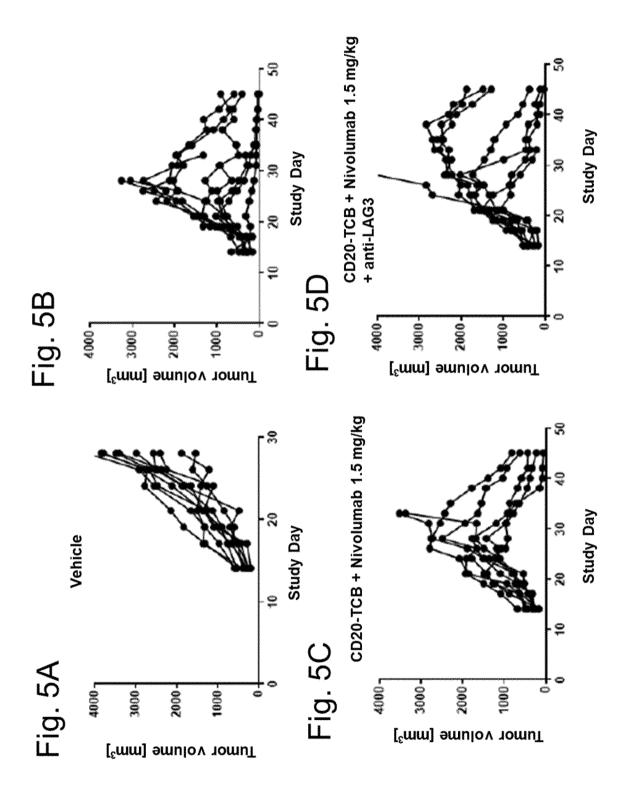


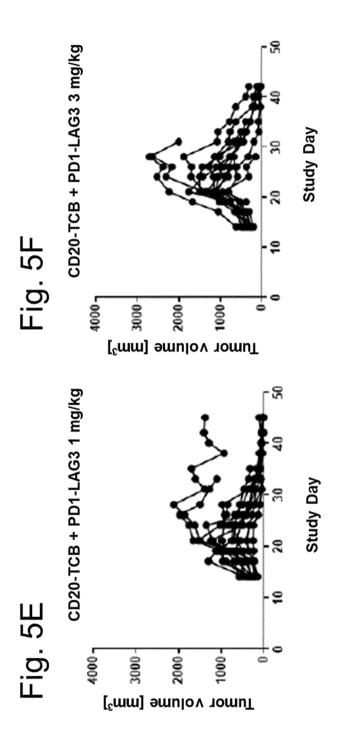
Fig.

Termination anti-Lag3 (BMS-986016) 1.5 Therapy Therapy Therapy d42 Therapy 3 mg/kg **d35** Nivolumab 1.5 mg/kg Nivolumab 1.5 mg/kg PD1-LAG3 1.5 mg/kg PD1-LAG3 3 mg/kg d28 Therapy 2 Therapy Therapy d21 Therapy 1 CD20-TCB CD20-TCB CD20-TCB CD20-TCB CD20-TCB Vehicle d14 Groups Human tumor cells injection s.c. (1.5 Mio WSU) V Ω

Fig. 4







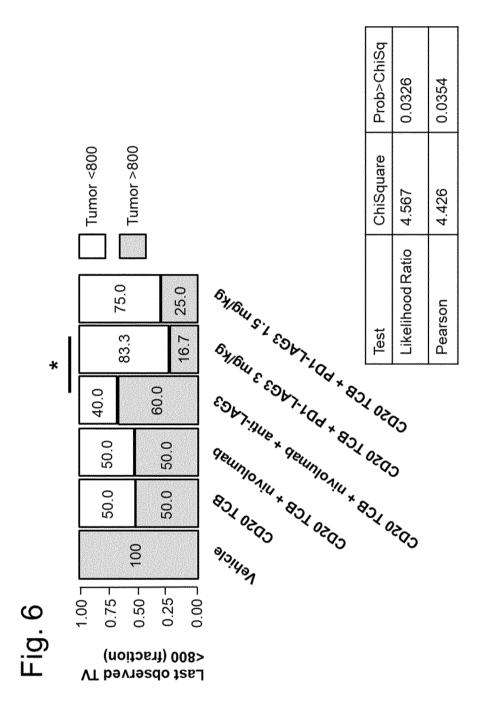
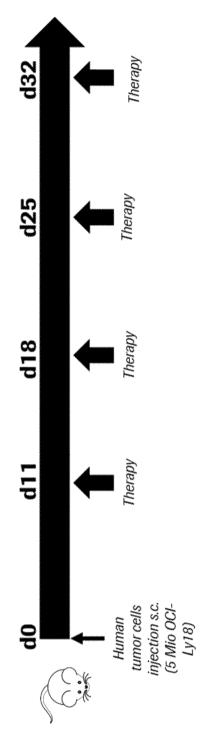
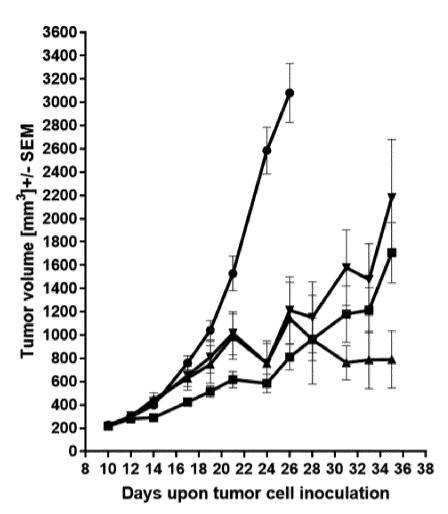


Fig. 7

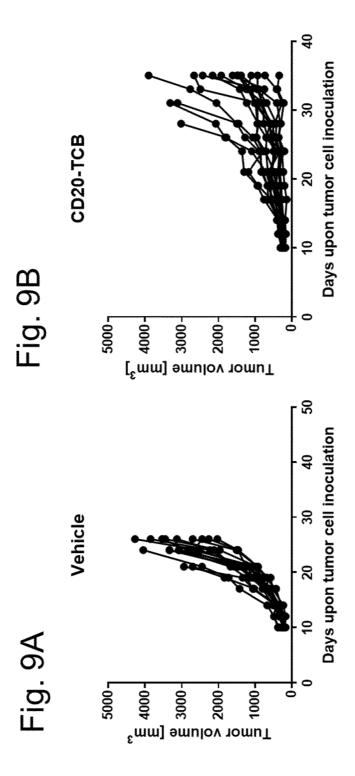


9√ 3			•	463
Therapy 3				Anti-LAG3
Therapy 2			PD1-LAG3	Pembrolizumab
Therapy 1	Vehicle	CD20-TCB	CD20-TCB	CD20-TCB
Pre-treatment	Vehicle	Obitunuzumab	Obitunuzumab	Obitunuzumab
Group	А	В	J	O

Fig. 8



- Vehicle
- CD20-TCB
- → CD20-TCB + Pembrolizumab +anti-Lag3



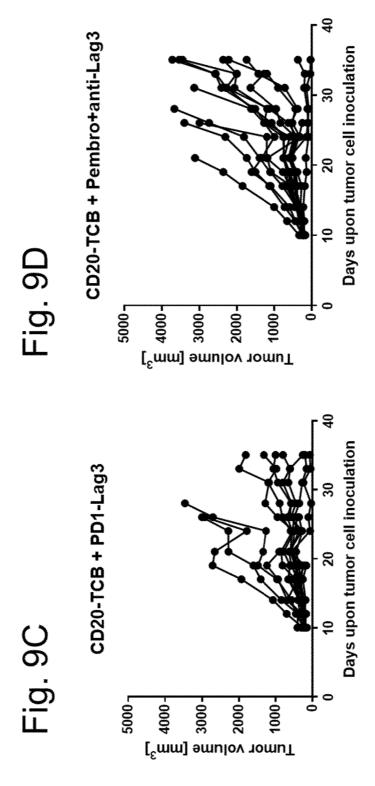
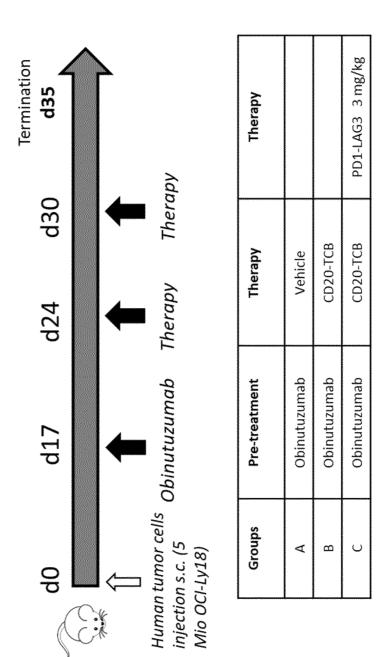
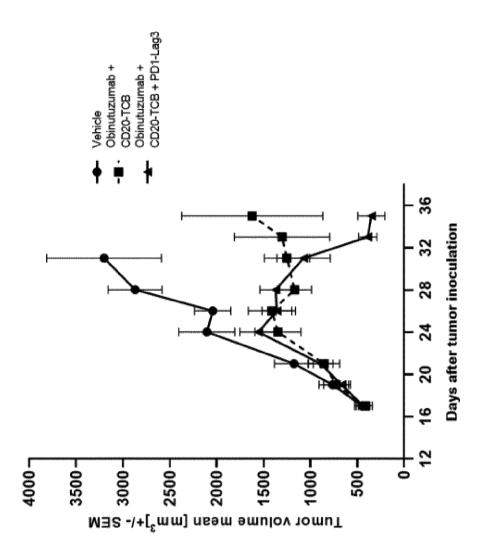
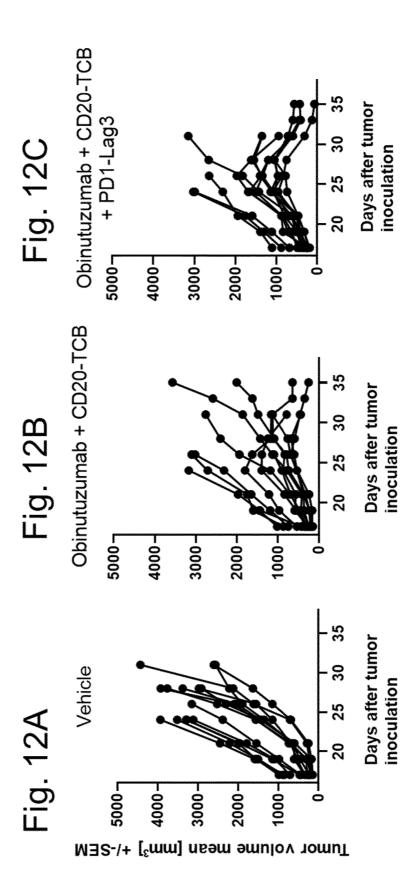


Fig. 10





⁼ig. 11



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/050040

	FICATION OF SUBJECT MATTER A61P35/00 C07K16/28 A61K39/	00		
ADD.				
According to	o International Patent Classification (IPC) or to both national classific	ation and IPC		
B. FIELDS	SEARCHED			
	ocumentation searched (classification system followed by classificati A61K C07K	ion symbols)		
Documenta	tion searched other than minimum documentation to the extent that s	such documents are included in the fields s	earched	
Electronic d	ata base consulted during the international search (name of data ba	use and, where practicable, search terms us	sed)	
EPO-In	ternal, BIOSIS, EMBASE, WPI Data			
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the rel	levant passages	Relevant to claim No.	
x	WO 2018/223004 A1 (XENCOR INC [US]) 6 December 2018 (2018-12-06) paragraph [0001] - paragraph [0016];		1-32	
	claims 5-8	• /		
	paragraph [0223]	001		
	paragraphs [0010], [0123], [02 [0281]; claim all	80],		
A	WO 2016/020309 A1 (HOFFMANN LA R	OCHE [CH];	1-32	
	HOFFMANN LA ROCHE [US]) 11 February 2016 (2016-02-11)			
	claim all; example all; sequence	all		
A	WO 2018/185043 A1 (HOFFMANN LA R HOFFMANN LA ROCHE [US])	OCHE [CH];	1-32	
	11 October 2018 (2018-10-11) claim all; example all; sequence	211		
	craim air; example air; sequence	all		
				
Furti	her documents are listed in the continuation of Box C.	See patent family annex.		
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cited to	ent which may throw doubts on priority claim(s) or which is o establish the publication date of another citation or other	step when the document is taken alor "Y" document of particular relevance;; the	ne	
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<u> </u>	ority date claimed actual completion of the international search	"&" document member of the same patent Date of mailing of the international sea		
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	Fax: (+31-70) 340-3016	Fellows, Edward		

International application No.

INTERNATIONAL SEARCH REPORT

PCT/EP2022/050040

Box	No. I	Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)
1.		gard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was out on the basis of a sequence listing:
	a. 🗦	forming part of the international application as filed:
		X in the form of an Annex C/ST.25 text file.
		on paper or in the form of an image file.
	b	furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
	c. [furnished subsequent to the international filing date for the purposes of international search only:
		in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
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2.		In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3.	Additio	nal comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/050040

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018223004 A1	06-12-2018	NONE	
WO 2016020309 A1	11-02-2016	AU 2015299163 A1	08-12-2016
		AU 2019250201 A1	07-11-2019
		BR 112016030462 A2	16-01-2018
		CA 2951599 A1	11-02-2016
		CL 2017000278 A1	03-11-2017
		CN 106661120 A	10-05-2017
		CR 20170032 A	04-04-2017
		DK 3177643 T3	15-07-2019
		EP 3177643 A1	14-06-2017
		EP 3608337 A1	12-02-2020
		ES 2734178 T3	04-12-2019
		HR P20191174 T1	04-10-2019
		HU E044814 T2	28-11-2019
		IL 249120 A	30-04-2020
		IL 271133 A	01-01-2022
		JP 6464255 B2	06-02-2019
		JP 2017525690 A	07-09-2017
		KR 20170020723 A	23-02-2017
		KR 20190077125 A	02-07-2019
		KR 20210132202 A	03-11-2021
		LT 3177643 T	12-08-2019
		MA 40510 A	14-06-2017
		MA 50584 A	16-09-2020
		MY 179611 A	11-11-2020
		NZ 727600 A	30-10-2020
		PE 20170263 A1	30-03-2017
		PE 20211533 A1	16-08-2021
		PH 12016502505 A1	10-04-2017
		PL 3177643 T3	30-09-2019
		PT 3177643 T	05-07-2019
		SG 10201803384T A	28-06-2018
		SG 11201700879W A	30-03-2017
		SI 3177643 T1	30-08-2019
		TW 201619196 A	01-06-2016
		UA 124254 C2	18-08-2021
		US 2016075785 A1	17-03-2016
		US 2018222980 A1	09-08-2018
		US 2018222981 A1	09-08-2018
		US 2020231673 A1	23-07-2020
		WO 2016020309 A1	11-02-2016
		ZA 201608205 B	27-05-2020
WO 2018185043 A1	 11-10-2018	AR 111361 A1	03-07-2019
		AU 2018247794 A1	22-08-2019
		BR 112019019821 A2	22-04-2020
		CA 3052532 A1	11-10-2018
		CL 2019002620 A1	10-01-2020
		CN 110506059 A	26-11-2019
		CO 2019009076 A2	30-08-2019
		CR 20190435 A	12-11-2019
		EP 3606955 A1	12-02-2020
		JP 6997212 B2	04-02-2022
		JP 2020516252 A	11-06-2020
		JP 2022033742 A	02-03-2022
		KR 20190126356 A	11-11-2019
		KR 20220003130 A	07-01-2022
Form PCT/ISA/210 (natent family annay) (April 2005)			J. JI 2022

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/050040

cited in search report date	MA PE	member(s) 49038 A 20191463 A1	date 12-02-2020
			12-02-2020
	PE	20101462 31	
		20191403 AI	16-10-2019
	PH	12019502282 A1	06-07-2020
	RU	2019134227 A	05-05-2021
	SG	11201909154S A	30-10-2019
	TW	201841941 A	01-12-2018
	TW	202030207 A	16-08-2020
	US	2018326054 A1	15-11-2018
	WO	2018185043 A1	11-10-2018