ANTIBODIES FOR USE IN THERAPY

FIELD OF THE INVENTION

The present invention relates to a method for reducing or preventing progression of a tumor or treating cancer by administration of a binding agent comprising a first binding region binding to human CD137 and a second binding region binding to human PD-L1.

BACKGROUND OF THE INVENTION

CD137 (4-1BB, TNFRSF9) is a member of the tumor necrosis factor (TNF) receptor (TNFR) family. CD137 is a co-stimulatory molecule on $CD8^+$ and $CD4^+$ T cells, regulatory T cells (Tregs), natural killer (NK) and NKT cells, B cells and neutrophils. On T cells, CD137 is not constitutively expressed, but induced upon T-cell receptor (TCR)- activation. Stimulation via its natural ligand 4-1BBL or agonist antibodies leads to signaling using TNFR-associated factor (TRAF)-2 and TRAF-1 as adaptors. Early signaling by CD137 involves K-63 poly-ubiquitination reactions that ultimately result in activation of the nuclear factor (NF)-κB and mitogen-activated protein (MAP)-kinase pathways. Signaling leads to increased T cell co-stimulation, proliferation, cytokine production, maturation and prolonged $CD8^+$ T-cell survival. Agonistic antibodies against CD137 have been shown to promote anti-tumor control by T cells in various pre-clinical models (Murillo et al. 2008 Clin. Cancer Res. 14(21): 6895-6906). Antibodies stimulating CD137 can induce survival and proliferation of T cells, thereby enhancing the anti-tumor immune response. Antibodies stimulating CD137 have been disclosed in the prior art, and include urelumab, a human IgG4 antibody (WO2005035584) and utomilumab, a human IgG2 antibody (Fisher et al. 2012 Cancer Immunol. Immunother. 61: 1721-1733).

Programmed death ligand 1 (PD-L1, PDL1, CD274, B7H1) is a 33 kDa, single-pass type I membrane protein. Three isoforms of PD-L1 have been described, based on alternative splicing. PD-L1 belongs to the immunoglobulin (Ig) superfamily and contains one Ig-like C2-type domain and one Ig-like V-type domain. Freshly isolated T and B cells express negligible amounts of PD-L1 and a fraction (about 16%) of $CD14^+$ monocytes constitutively express PD-L1. However, interferon-γ (IFNγ) is known to upregulate PD-L1 on tumor cells.

PD-L1 obstructs anti-tumor immunity by 1) tolerizing tumor-reactive T cells by binding to its receptor, programmed cell death protein 1 (PD-1) (CD279) on activated T cells; 2) rendering tumor cells resistant to $CD8^+$ T cell and Fas ligand–mediated lysis by PD-1 signaling through tumor cell-expressed PD-L1; 3) tolerizing T cells by reverse signaling through T cell–expressed CD80 (B7.1); and 4) promoting the development and maintenance of induced T regulatory cells. PD-L1 is expressed in

many human cancers, including melanoma, ovarian, lung and colon cancer (Latchman et al., 2004 Proc Natl Acad Sci USA 101, 10691-6).

PD-L1 blocking antibodies have shown clinical activity in several cancers known to overexpress PD-L1 (incl. melanoma, NSCLC). For example, atezolizumab is a humanized IgG1 monoclonal antibody against PD-L1. It is currently in clinical trials as an immunotherapy for several indications including various types of solid tumors (see e.g. Rittmeyer et al., 2017 Lancet 389:255-265) and is approved for non-small-cell lung cancer and bladder cancer indications. Avelumab, a PD-L1 antibody, (Kaufman et al Lancet Oncol. 2016;17(10):1374-1385) has been approved by the FDA for the treatment of adults and pediatric patients 12 years and older with metastatic Merkel cell carcinoma, and is currently in clinical trials in several cancer indiciations, including bladder cancer, gastric cancer, head and neck cancer, mesothelioma, NSCLC, ovarian cancer and renal cancer. Durvalumab, a PD-L1 antibody, is approved for locally advanced or metastatic urothelial carcinoma indications and is in clinical development in multiple solid tumors and blood cancers (see e.g. Massard et al., 2016 J Clin Oncol. 34(26):3119-25). Further anti-PD-L1 antibodies have been described e.g. in WO2004004771.

Horton et al (J Immunother Cancer. 2015; 3(Suppl 2): O10) discloses combination of an agonistic 4-1BB antibody with a neutralizing PD-L1 antibody. WO 2019/025545 provides binding agents, such as bispecific antibodies, binding human PD-L1 and binding human CD137.

However, despite these advances in the art there is a considerable need for improved therapies targeting PD-L1 and CD137.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for reducing or preventing progression of a tumor or treating cancer in a subject, comprising administering to said subject, in at least one treatment cycle, a binding agent in a suitable amount, comprising a first binding region binding to human CD137, and a second binding region binding to human PD-L1.

The amount of binding agent administered in each dose and/or in each treatment cycle may be

a) about 0.3-5 mg/kg body weight or about 25-400 mg in total; and/or
b) about $2.1 \times 10^{-9} – 3.4 \times 10^{-8}$ mol/kg body weight or about $1.7 \times 10^{-7} – 2.7 \times 10^{-6}$ mol in total.

It is a further object of the invention to provide a composition comprising a binding agent comprising a first binding region binding to human CD137 and a second binding region binding to human PD-L1,

wherein the amount of binding agent in the composition is about 25-400 mg or about 1.7 x 10^{-7} – 2.7 x 10^{-6} mol.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Simultaneous binding of GEN1046 to PD-L1- and CD137-expressing K562 cells induces doublet formation with a bell-shaped dose-response curve. Equal numbers of CellTrace™ Far Red labelled K562 cells transgenic for CD137 (K562_h4-1BB) were co-incubated with CellTrace™ Violet labelled K562 cells transgenic for PD-L1 (K562_hPD-L1) in the presence of 0.001-100 µg/mL i) GEN1046 or ii) a combination of control antibodies PD-L1-547-FEALxb12-FEAR and b12-FEALxCD137-009-HC7LC2-FEAR for 15 minutes. Samples were analyzed by flow cytometry and the percent CellTrace™ Far Red/ CellTrace™ Violet double-positive doublets (A) plotted as a function of GEN1046 concentration (B). Data shown are mean ± standard deviation of n=3 technical replicates (perhaps the symbol needs to be smaller to show the SD).

Figure 2: Schematic representation of the anticipated mode of action of CD137xPD-L1 bispecific antibodies. (A) PD-L1 is expressed on antigen-presenting cells (APCs) as well as on tumor cells. PD-L1 binding to T cells expressing the negative regulatory molecule PD-1 effectively overrides T cell activation signals and eventually leads to T cell inhibition. (B) Upon addition of a CD137xPD-L1 bispecific antibody, the inhibitory PD-1:PD-L1 interaction is blocked via the PD-L1-specific arm and at the same time, the bispecific antibody, through the cell-cell interaction provides agonistic signaling to CD137 expressed on the T cells resulting in strong T cell costimulation.

Figure 3: Relative luminescence units (RLU) as a function of antibody concentration in a luciferase-based CD137-activation reporter assay performed in the presence of PD-L1 expressing tumor cell lines. Endogenously PD-L1 expressing human ovarian cancer cell line ES-2 (A) and breast cancer cell line MDA-MB-231 (B) were co-cultivated with NFkB-Luc2P/4-1BB Jurkat reporter cells in the presence of 0.00128-100 µg/mL i) GEN1046 or ii) b12-FEAL control antibody for 6 hours. Luciferase expression induction was determined by incubation with a luciferase substrate and measurement of relative luminescence units. Data shown are mean ± standard deviation of n=3 technical replicates.

Figure 4: Comparison of GEN1046 with control antibodies PD-L1-547-FEALxb12-FEAL or IgG1-b12-FEAL in a polyclonal T-cell proliferation assay. CFSE-labeled PBMCs were incubated with sub-optimal concentration of anti-CD3 antibody (0.03 µg/mL), and cultured in the presence of 0.0032-10 µg/mL i) GEN1046 ii) PD-L1-547-FEALxb12-FEAR or iii) b12-FEAL control antibody for four days. T-cell proliferation of total T cells (A) and CCR7+CD45RO+ central memory and CCR7-CD45RO+ effector memory T-cell subsets in total T cells (B) was measured by flow cytometry. Data are shown from one

representative donor as the mean expansion index of two replicates, as calculated using FlowJo v10.4 software. Error bars (SD) indicate the variation within the experiment (two replicates, using cells from one donor).

Figure 5: Release of the PD-1/PD-L1-mediated T-cell inhibition and additional co-stimulation of $CD8^+$ T-cell proliferation by GEN1046 in an antigen-specific T-cell assay with active PD-1/PD-L1 axis. $CD8^+$ T cells were electroporated with RNA encoding the alpha and beta chains of the CLDN6-specific TCR (10 μg each) either with RNA encoding PD-1 (0.4 - 10 μg) or without (w/o PD-1), labeled with CFSE and co-cultured with immature DC that were electroporated with 0.3 μg (A) or 1 μg (B) RNA encoding CLDN6. Electroporated $CD8^+$ T cells and iDC were co-cultured in the presence of GEN1046 (0.00015 – 1 μg/mL) or b12-FEAL (1 μg/mL) for 4 days. T-cell proliferation was assessed by analyzing CFSE dilution in $CD8^+$ T cells using flow cytometry and the T-cell expansion index (e.g. how much the total T cell population has expanded by proliferation) was automatically calculated by FlowJo (version 10.3). Data shown are mean expansion index ± SD of triplicate wells from one donor out of four donors included in two experiments.

Figure 6: Effect of GEN1046 on secretion of pro-inflammatory cytokines (IFNγ, TNFα, IL-13 and IL-8) in an antigen-specific T-cell assay with or without PD-1 electroporation into T cells. $CD8^+$ T cells were electroporated with RNA encoding the alpha and beta chains of the CLDN6-specific TCR (10 μg each) either with RNA encoding PD-1 (2 μg) or without (w/o PD-1), labeled with CFSE and co-cultured with immature DC that were electroporated with 1 μg RNA encoding CLDN6. Electroporated $CD8^+$ T cells and iDC were co-cultured in the presence of GEN1046 (0.00015 – 1 μg/mL) or b12-FEAL (1 μg/mL). Cytokine levels of supernatants were determined 48 hours after antibody addition by multiplex sandwich immunoassay using the MSD V-Plex Human Proinflammatory panel 1 (10-Plex) kit. Data shown are mean concentration ± SD of sextuplicate wells from one representative donor of two donors included in the experiment.

Figure 7: Ex vivo expansion of tumor infiltrating lymphocytes (TIL) from a human non-small-cell lung cancer tissue resection by CD137-009-FEALxPD-L1-547-FEAR. Tumor pieces from the resected tissue were cultured with 10 U/mL IL-2 and the indicated concentration of CD137-009-FEALxPD-L1-547-FEAR. After 10 days of culture, cells were harvested and analyzed by flow cytometry. (A) TIL count per 1,000 beads, (B) CD3+CD8+ T cell count per 1,000 beads, (C) CD3+CD4+ T cell count per 1,000 beads, (D) CD3-CD56+ NK cell count per 1,000 beads. Data shown are mean cell counts ± SD of five individual wells, with two tumor pieces per well as starting material. *$p<0.05$ using ordinary one-way ANOVA with Dunnett's multiple comparisons test.

Figure 8: Schematic outline of clinical trial design.

Figure 9: Dose escalation; best percent change from baseline in tumor size, all patients. Data cut-off: September 29, 2020. Post-baseline scans were not conducted for five patients. [a]Minimum duration of response (5 weeks) per RECIST v1.1 not reached. [b]PR was not confirmed on a subsequent scan.
NE, non-evaluable; NSCLC, non-small cell lung cancer; PD, progressive disease; PD-(L)1, programmed death (ligand) 1; PR, partial response; SD, stable disease; SoD, sum of diameters; uPR, unconfirmed partial response.

Figure 10: Dose escalation; Best change from baseline in tumor size, patients with NSCLC. Data cut-off: September 29, 2020.
[a]PR was not confirmed by a subsequent scan.
bPD-L1 expression was assessed in archival tumor specimens.
BOR, best overall response; CR, complete response; ICI, immune checkpoint inhibitor; NA, not available; PD, progressive disease; PD-(L)1, programmed death (ligand) 1; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; SoD, sum of diameters; TPS, tumor proportion score; uPR, unconfirmed partial response.

Figure 11: Expansion cohort 1; A) Best change from baseline in tumor size, B) Target lesion SoD change from baseline. Data cut-off: October 12, 2020.
*Denotes patients with ongoing treatment.
[a]PR was not confirmed by a subsequent scan. [b]PD-L1 expression was assessed in tumor biopsies obtained prior to initiation of GEN1046 treatment (22C3 pharmDx assay, HistoGeneX, Belgium). Includes all patients who had at least one post-baseline tumor assessment (schedule is every 6 weeks), and thus could be assessed for clinical benefit; 6 of 12 patients are still on treatment. Of the remaining 12 patients not shown, three patients had clinical progression prior to first response assessment, and nine patients were still receiving treatment and had not had a first response assessment.
BOR and time point response assessed using RECIST 1.1; NA: Assessment succeeding first PD. BOR, best overall response; ICI, immune checkpoint inhibitor; NA, not available; NE, non-evaluable; NSCLC, non-small cell lung cancer; PD, progressive disease; PD-(L)1, programmed death (ligand) 1; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; SoD, sum of diameters; TPS, tumor proportion score; uPR, unconfirmed partial response.

Figure 12: Model Predicted Maximal Trimer Formation and Receptor Occupancy for PD-L1 at 100 mg dose administered once every third week (1Q3W).

Figure 13: A-D Pharmacodynamic assessments, including changes in circulating levels of interferon-gamma (IFN-γ) and interferon-gamma–inducible protein 10 IP-10 (A-B), proliferating effector

memory CD8 T cells and total CD8 T cells (C-D), were conducted using blood samples from patients with advanced solid tumors enrolled in the dose escalation phase of an open-label, multi-center safety trial of GEN1046 (NCT03917381; Data cut off: Jan 19th 2021).

A-B. Circulating levels of IFN-γand IP-10 were measured in serum samples at baseline, and at multiple timepoints post administration of GEN1046 in cycle 1 and cycle 2 (days 1 [2h and between 4-6h post-administration], 2, 3, 8, and 15). IFN-γ and IP-10 levels in serum samples were determined by Meso Scale Discovery (MSD) multiplex immune assay. Data shown are the maximal fold-change from baseline measured during cycle 1. Statistical analysis was performed using the Wilcoxon-Mann-Whitney test.

C-D. Immunophenotyping of peripheral blood was conducted in whole blood collected at baseline and at multiple timepoints post administration of GEN1046 in cycle 1 and cycle 2 (days 2, 3, 8 and 15). The frequency of proliferating ($Ki67^+$) total CD8 T cells and effector memory CD8 T cells ($CD8^+CD45RA^-CCR7^-$ T cells) were assessed in whole blood samples by flow cytometry. Data shown are the maximal fold-change from baseline measured during cycle 1. Statistical analysis was performed using the Wilcoxon-Mann-Whitney test.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term “binding agent” in the context of the present invention refers to any agent capable of binding to desired antigens. In certain embodiments of the invention, the binding agent is an antibody, antibody fragment, or construct thereof. The binding agent may also comprise synthetic, modified or non-naturally occurring moieties, in particular non-peptide moieties. Such moieties may, for example, link desired antigen-binding functionalities or regions such as antibodies or antibody fragments. In one embodiment, the binding agent is a synthetic construct comprising antigen-binding CDRs or variable regions.

The term “immunoglobulin” refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as V_H or VH) and a heavy chain constant region (abbreviated herein as C_H or CH). The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The hinge region is the region between the CH1 and CH2 domains of the heavy chain and is highly

flexible. Disulphide bonds in the hinge region are part of the interactions between two heavy chains in an IgG molecule. Each light chain typically is comprised of a light chain variable region (abbreviated herein as V_L or VL) and a light chain constant region (abbreviated herein as C_L or CL). The light chain constant region typically is comprised of one domain, CL. The VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901-917 (1987)). Unless otherwise stated or contradicted by context, CDR sequences herein are identified according to IMGT rules using DomainGapAlign (Lefranc MP., Nucleic Acids Research 1999;27:209-212 and Ehrenmann F., Kaas Q. and Lefranc M.-P. Nucleic Acids Res., 38, D301-307 (2010); see also internet http address www.imgt.org/). Unless otherwise stated or contradicted by context, reference to amino acid positions in the constant regions in the present invention is according to the EU-numbering (Edelman et al., Proc Natl Acad Sci U S A. 1969 May;63(1):78-85; Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition. 1991 NIH Publication No. 91-3242). For example, SEQ ID NO:93 herein sets forth amino acids positions 118-447 according to EU numbering, of the IgG1m(f) heavy chain constant region.

The term “amino acid” and “amino acid residue” may herein be used interchangeably, and are not to be understood limiting. Amino acids are organic compounds containing amine ($-NH_2$) and carboxyl (-COOH) functional groups, along with a side chain (R group) specific to each amino acid. In the context of the present invention, amino acids may be classified based on structure and chemical characteristics. Thus, classes of amino acids may be reflected in one or both of the following tables:

Table 1: Main classification based on structure and general chemical characterization of R group

Class	Amino acid
Acidic Residues	D and E
Basic Residues	K, R, and H
Hydrophilic Uncharged Residues	S, T, N, and Q
Aliphatic Uncharged Residues	G, A, V, L, and I
Non-polar Uncharged Residues	C, M, and P
Aromatic Residues	F, Y, and W

Table 2: Alternative Physical and Functional Classifications of Amino Acid Residues

Class	Amino acid
Hydroxyl group containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	Q, T, K, S, G, P, D, E, and R

Substitution of one amino acid for another may be classified as a conservative or non-conservative substitution. In the context of the invention, a "conservative substitution" is a substitution of one amino acid with another amino acid having similar structural and/or chemical characteristics, such substitution of one amino acid residue for another amino acid residue of the same class as defined in any of the two tables above: for example, leucine may be substituted with isoleucine as they are both aliphatic, branched hydrophobes. Similarly, aspartic acid may be substituted with glutamic acid since they are both small, negatively charged residues.

The term "amino acid corresponding to position..." as used herein refers to an amino acid position number in a human IgG1 heavy chain. Corresponding amino acid positions in other immunoglobulins may be found by alignment with human IgG1. Thus, an amino acid or segment in one sequence that "corresponds to" an amino acid or segment in another sequence is one that aligns with the other amino acid or segment using a standard sequence alignment program such as ALIGN, ClustalW or similar, typically at default settings and has at least 50%, at least 80%, at least 90%, or at least 95% identity to a human IgG1 heavy chain. It is considered well-known in the art how to align a sequence or segment in a sequence and thereby determine the corresponding position in a sequence to an amino acid position according to the present invention.

The term "antibody" (Ab) in the context of the present invention refers to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions with a half-life of

significant periods of time, such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen and/or time sufficient for the antibody to recruit an effector activity). The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The term “antigen-binding region”, wherein used herein, refers to the region which interacts with the antigen and comprises both a VH region and a VL region. The term antibody when used herein comprises not only monospecific antibodies, but also multispecific antibodies which comprise multiple, such as two or more, e.g. three or more, different antigen-binding regions. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. As indicated above, the term antibody herein, unless otherwise stated or clearly contradicted by context, includes fragments of an antibody that are antigen-binding fragments, *i.e.*, retain the ability to specifically bind to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of antigen-binding fragments encompassed within the term "antibody" include (i) a Fab’ or Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains, or a monovalent antibody as described in WO2007059782 (Genmab); (ii) $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the VH and CH1 domains; (iv) a Fv fragment consisting essentially of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature 341, 544-546 (1989)), which consists essentially of a VH domain and also called domain antibodies (Holt et al; Trends Biotechnol. 2003 Nov;21(11):484-90); (vi) camelid or Nanobody molecules (Revets et al; Expert Opin Biol Ther. 2005 Jan;5(1):111-24) and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of

antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. These and other useful antibody fragments in the context of the present invention, as well as bispecific formats of such fragments, are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype. As used herein, the term "isotype" refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) that is encoded by heavy chain constant region genes. When a particular isotype, e.g. IgG1, is mentioned herein, the term is not limited to a specific isotype sequence, e.g. a particular IgG1 sequence, but is used to indicate that the antibody is closer in sequence to that isotype, e.g. IgG1, than to other isotypes. Thus, e.g. an IgG1 antibody of the invention may be a sequence variant of a naturally occurring IgG1 antibody, including variations in the constant regions.

The term “bispecific antibody” or “bs” in the context of the present invention refers to an antibody having two different antigen-binding regions defined by different antibody sequences. In some embodiments, said different antigen-binding regions bind different epitopes on the same antigen. However, in preferred embodiments, said different antigen-binding regions bind different target antigens. A bispecific antibody can be of any format, including any of the bispecific antibody formats described herein below.

The term “full-length” when used in the context of an antibody indicates that the antibody is not a fragment, but contains all of the domains of the particular isotype normally found for that isotype in nature, e.g. the VH, CH1, CH2, CH3, hinge, VL and CL domains for an IgG1 antibody. In some embodiments, the term “full-length” when used herein in the context of an antibody, refers to an antibody (e.g., a parent or variant antibody) comprising one or two pairs of heavy and light chains, each containing all heavy and light chain constant and variable domains that are normally found in a heavy chain-light chain pair of a wild-type antibody of that isotype. In a full-length antibody, the heavy and light chain constant and variable domains may contain amino acid substitutions that improve the functional properties of the antibody when compared to the full-length parent or wild type antibody. These include substitutions for the purpose of reducing antibody effector function and substitutions to facilitate assembly of multispecific antibodies, such as bispecific antibodies. A full-length antibody according to the present invention may be produced by a method comprising the steps of (i) cloning the CDR sequences into a suitable vector comprising complete heavy chain

sequences and complete light chain sequence, and (ii) expressing the complete heavy and light chain sequences in suitable expression systems. It is within the knowledge of the skilled person to produce a full-length antibody when starting out from either CDR sequences or full variable region sequences.

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

The term "humanized antibody" as used herein, refers to a genetically engineered non-human antibody, which contains human antibody constant domains and non-human variable domains modified to contain a high level of sequence homology to human variable domains. This can be achieved by grafting of the six non-human antibody complementarity-determining regions (CDRs), which together form the antigen binding site, onto a homologous human acceptor framework region (FR) (see WO92/22653 and EP0629240). In order to fully reconstitute the binding affinity and specificity of the parental antibody, the substitution of framework residues from the parental antibody (i.e. the non-human antibody) into the human framework regions (back-mutations) may be required. Structural homology modeling may help to identify the amino acid residues in the framework regions that are important for the binding properties of the antibody. Thus, a humanized antibody may comprise non-human CDR sequences, primarily human framework regions optionally comprising one or more amino acid back-mutations to the non-human amino acid sequence, and fully human constant regions. Optionally, additional amino acid modifications, which are not necessarily back-mutations, may be applied to obtain a humanized antibody with preferred characteristics, such as affinity and biochemical properties.

When used herein, unless contradicted by context, the term "Fc region" refers to an antibody region consisting of the two Fc sequences of the heavy chains of an immunoglobulin, wherein said Fc sequences comprise at least a hinge region, a CH2 domain, and a CH3 domain.

The term "Fc region" as used herein, refers to a region comprising, in the direction from the N- to C-terminal end of the antibody, at least a hinge region, a CH2 region and a CH3 region. An Fc region of the antibody may mediate the binding of the immunoglobulin to host tissues or factors, including

various cells of the immune system (such as effector cells) and components of the complement system.

The term “hinge region” as used herein refers to the hinge region of an immunoglobulin heavy chain. Thus, for example the hinge region of a human IgG1 antibody corresponds to amino acids 216-230 according to the Eu numbering as set forth in Kabat (Kabat, E.A. et al., Sequences of proteins of immunological interest. 5th Edition - US Department of Health and Human Services, NIH publication No. 91-3242, pp 662,680,689 (1991). However, the hinge region may also be any of the other subtypes as described herein.

The term “CH1 region” or “CH1 domain” as used herein refers to the CH1 region of an immunoglobulin heavy chain. Thus, for example the CH1 region of a human IgG1 antibody corresponds to amino acids 118-215 according to the Eu numbering as set forth in Kabat (*ibid*) . However, the CH1 region may also be any of the other subtypes as described herein.

The term “CH2 region” or “CH2 domain” as used herein refers to the CH2 region of an immunoglobulin heavy chain. Thus, for example the CH2 region of a human IgG1 antibody corresponds to amino acids 231-340 according to the Eu numbering as set forth in Kabat (*ibid*). However, the CH2 region may also be any of the other subtypes as described herein.

The term “CH3 region” or “CH3 domain” as used herein refers to the CH3 region of an immunoglobulin heavy chain. Thus, for example the CH3 region of a human IgG1 antibody corresponds to amino acids 341-447 according to the Eu numbering as set forth in Kabat (*ibid*). However, the CH3 region may also be any of the other subtypes as described herein.

As used herein, the terms “binding” or "capable of binding'' in the context of the binding of an antibody to a predetermined antigen or epitope typically is a binding with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less, when determined using Bio-Layer Interferometry (BLI) or, for instance, when determined using surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody as the analyte. The antibody binds to the predetermined antigen with an affinity corresponding to a K_D that is at least ten-fold lower, such as at least 100-fold lower, for instance at least 1,000-fold lower, such as at least 10,000-fold lower, for instance at least 100,000-fold lower than its K_D for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The amount with which the affinity is higher is dependent on the K_D of the antibody, so that when the K_D of the antibody is very low (that is, the antibody is highly specific), then the degree to which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000-fold.

The term "k_d" (sec^{-1}), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. Said value is also referred to as the k_{off} value.

The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

The term "PD-L1" when used herein, refers to the Programmed Death-Ligand 1 protein. PD-L1 is found in humans and other species, and thus, the term "PD-L1" is not limited to human PD-L1 unless contradicted by context. The human PD-L1 sequences can be found through Genbank accession no. NP_054862.1. The sequence of human PD-L1 is also shown in SEQ ID NO: 25, wherein amino acids 1-18 are predicted to be a signal peptide. The mature polypeptide sequence is provided in SEQ ID NO: 26.

The term "PD-1" when used herein, refers to the human Programmed Death-1 protein, also known as CD279 (UniProtKB Q15116).

The term "programmed cell death-1 (PD-1) pathway" or "PD-1 pathway" refers to the molecular signaling pathway comprising cell surface receptor PD-1 and its ligands PD-L1 and PD-L2. Activation of this pathway induces immune tolerance, while inhibition release T-cell suppression, which may lead to immune activation.

The term "CD137" as used herein, refers to the human Cluster of Differentiation 137 protein. CD137 (4-1BB), also referred to as TNFRSF9, is the receptor for the ligand TNFSF9/4-1BBL. CD137 is believed to be involved in T cell activation. Human CD137, has UniProt accession number Q07011. The sequence of human CD137 is also shown in SEQ ID NO: 23, wherein amino acids 1-23 are predicted to be a signal peptide. The mature sequence of human CD137 is provided in SEQ ID NO: 24.

"Treatment cycle" is herein defined as the time period, within the effects of separate dosages of the biding agent adds on due to its pharmacodynamics, or in other words the time period after which the subject,s body is essentially cleared from or being cleared from the administrated biding agent. Multiple small doses in a small time window; e.g. within 2-24 few hours, such as 2-12 hours or on the same day, might be equal to a larger single dose.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci 4, 11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using

a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch, J. Mol. Biol. 48, 444-453 (1970) algorithm.

In the context of the present invention, the following notations are, unless otherwise indicated, used to describe a mutation: i) substitution of an amino acid in a given position is written as e.g. K409R which means a substitution of a lysine in position 409 of the protein with an arginine; and ii) for specific variants the specific three or one letter codes are used, including the codes Xaa and X to indicate any amino acid residue. Thus, the substitution of lysine with arginine in position 409 is designated as: K409R, and the substitution of lysine with any amino acid residue in position 409 is designated as K409X. In case of deletion of lysine in position 409 it is indicated by K409*.

In the context of the present invention, "inhibition of PD-L1 binding to PD-1" refers to any detectably significant reduction in the binding of PD-L1 to PD-1 in the presence of an antibody capable of binding PD-L1. Typically, inhibition means an at least about 10% reduction, such as an at least about 15%, e.g. an at least about 20%, such as an at least 40% reduction in binding between PD-L1 and PD-1, caused by the presence of an anti-PD-L1 antibody. Inhibition of PD-L1 binding to PD-1 may be determined by any suitable technique. In one embodiment, inhibition is determined as described in Example 6 of WO 2019/025545.

The term "treatment" refers to the administration of an effective amount of a therapeutically active antibody of the present invention with the purpose of easing, ameliorating, arresting or eradicating (curing) symptoms or disease states.

The resistance to, failure to respond to and/or relapse from treatment with binding agent of the invention may be determined according to the Response Evaluation Criteria In Solid Tumors; version 1.1 (RECIST Criteria v1.1). The RECIST Criteria are set forth in the table below.

Table 3: Definition of Response (RECIST Criteria v1.1)

	Category	Criteria
Based on target lesions	Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis to < 10 mm.
	Partial Response (PR)	≥ 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
	Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest

	Category	Criteria
		sum of LDs since the treatment started.
	Progressive Disease (PD)	≥ 20% increase in the sum of the LDs of target lesions, taking as reference the smallest sum of the LDs recorded since the treatment started or the appearance of one or more new lesions.
Based on non-target lesions	CR	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
	SD	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.
	PD	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

The "best overall response" is the best response recorded from the start of the treatment until disease progression/recurrence (the smallest measurements recorded since the treatment started will be used as the reference for PD). Subjects with CR or PR are considered to be objective response. Subjects with CR, PR or SD are considered to be in disease control. Subjects with NE are counted as non-responders. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (the smallest measurements recorded since the treatment started will be used as the reference for PD). Subjects with CR, PR or SD are considered to be in disease control. Subjects with NE are counted as non-responders.

"Duration of response (DOR)" only applies to subjects whose confirmed best overall response is CR or PR and is defined as the time from the first documentation of objective tumor response (CR or PR) to the date of first PD or death due to underlying cancer.

"Progression-free survival (PFS)" is defined as the number of days from Day 1 in Cycle 1 to the first documented progression or death due to any cause.

"Overall survival (OS)" is defined as the number of days from Day 1 in Cycle 1 to death due to any cause. If a subject is not known to have died, then OS will be censored at the latest date the subject was known to be alive (on or before the cut-off date).

In the context of the present invention, the term "treatment regimen" refers to a structured treatment plan designed to improve and maintain health.

In a first aspect the present invention provides a method for reducing or preventing progression of a tumor or treating cancer in a subject, comprising administering to said subject, in at least one treatment cycle, a binding agent in a suitable amount, comprising a first binding region binding to human CD137, such as human CD137 having the sequence set forth in SEQ ID NO: 24, and a second binding region binding to human PD-L1, such as human PD-L1 having the sequence set forth in SEQ ID NO: 26.

Preferably, the amount of binding agent administered in each dose and/or treatment cycle leads to the proliferation, cytokine production, maturation and prolonged survival of T-cells and renders such T cells unsusceptible for inhibition by PD-L1.

The amount of binding agent administered in each dose and/or treatment cycle may in particular be in a range wherein more than 5%, preferably more than 10%, more preferably more than 15%, even more preferably more than 20%, even more preferably more than 25%, even more preferably more than 30%, even more preferably more than 35%, even more preferably more than 40%, even more preferably more than 45%, most preferably more than 50% of said binding agents bind to both, CD137 and PD-L1.

In currently preferred embodiments, the amount of binding agent administered in each dose and/or in each treatment cycle is

a) about 0.3-5 mg/kg body weight or about 25-400 mg in total; and/or
b) about 2.1 x 10^{-9} – 3.4 x 10^{-8} mol/kg body weight or about 1.7 x 10^{-7} – 2.7 x 10^{-6} mol in total.

According to these embodiments, the dose defined in mg/kg may be converted to flat dose, and vice versa, based on the median body weight of the subjects to whom the binding agent is administered being 80 kg

The amount of binding agent administered in each dose and/or in each treatment cycle may in particular be

about 0.3-4.0 mg/kg body weight or about 25-320 mg in total; and/or
about 2.1 x 10^{-9} – 2.7 x 10^{-8} mol/kg body weight or about 1.7 x 10^{-7} – 2.2 x 10^{-6} mol in total;
about 0.38-4.0 mg/kg body weight or about 30-320 mg in total; and/or
about 2.6 x 10^{-9} – 2.7 x 10^{-8} mol/kg body weight or about 2.4 x 10^{-7} – 2.2 x 10^{-6} mol in total;
about 0.5-3.3 mg/kg body weight or about 40-260 mg in total; and/or
about 3.4 x 10^{-9} – 2.2 x 10^{-8} mol/kg body weight or about 2.7 x 10^{-7} – 1.8 x 10^{-6} mol in total;
about 0.6-2.5 mg/kg body weight or about 50-200 mg in total; and/or

about $4.3 \times 10^{-9} – 1.7 \times 10^{-8}$ mol/kg body weight or about $3.4 \times 10^{-7} – 1.4 \times 10^{-6}$ mol in total;
about 0.8-1.8 mg/kg body weight or about 60-140 mg in total; and/or
about $5.1 \times 10^{-9} – 1.2 \times 10^{-8}$ mol/kg body weight or about $4.1 \times 10^{-7} – 9.5 \times 10^{-7}$ mol in total;
about 0.9-1.8 mg/kg body weight or about 70-140 mg in total; and/or
about $6.0 \times 10^{-9} – 1.2 \times 10^{-8}$ mol/kg body weight or about $4.8 \times 10^{-7} – 9.5 \times 10^{-7}$ mol in total;
about 1-1.5 mg/kg body weight or about 80-120 mg in total; and/or
about $6.8 \times 10^{-9} – 1.0 \times 10^{-8}$ mol/kg body weight or about $5.5 \times 10^{-7} – 8.2 \times 10^{-7}$ mol in total;
about 1.1-1.4 mg/kg body weight or about 90-110 mg in total; and/or
about $7.7 \times 10^{-9} – 9.4 \times 10^{-9}$ mol/kg body weight or about $6.1 \times 10^{-7} – 7.5 \times 10^{-7}$ mol in total;
about 1.2-1.3 mg/kg body weight or about 95-105 mg in total; and/or
about 6.8×10^{-9} - 8.9×10^{-9} mol/kg body weight or about $6.5 \times 10^{-7} – 7.2 \times 10^{-7}$ mol in total,
about 0,8-1.5 mg/kg body weight or about 65-120 mg in total; and/or
about $5.5 \times 10^{-9} – 1.0 \times 10^{-8}$ mol/kg body weight or about $4.4 \times 10^{-7} – 8.2 \times 10^{-7}$ mol in total;
about 0.9-1.3 mg/kg body weight or about 70-100 mg in total; and/or
about $6.0 \times 10^{-9} – 8.5 \times 10^{-9}$ mol/kg body weight or about $4.8 \times 10^{-7} – 6.8 \times 10^{-7}$ mol in total,
about 0.9-1.1 mg/kg body weight or about 75-90 mg in total; and/or
about $6.4 \times 10^{-9} – 7.7 \times 10^{-9}$ mol/kg body weight or about $5.1 \times 10^{-7} – 6.1 \times 10^{-7}$ mol in total.

Further, the amount of binding agent administered in each dose and/or in each treatment cycle may in particular be

0.3-4.0 mg/kg body weight or 25-320 mg in total; and/or
$2.1 \times 10^{-9} – 2.7 \times 10^{-8}$ mol/kg body weight or $1.7 \times 10^{-7} – 2.2 \times 10^{-6}$ mol in total;
0.38-4.0 mg/kg body weight or 30-320 mg in total; and/or
$2.6 \times 10^{-9} – 2.7 \times 10^{-8}$ mol/kg body weight or $2.4 \times 10^{-7} – 2.2 \times 10^{-6}$ mol in total;
0.5-3.3 mg/kg body weight or 40-260 mg in total; and/or
$3.4 \times 10^{-9} – 2.2 \times 10^{-8}$ mol/kg body weight or $2.7 \times 10^{-7} – 1.8 \times 10^{-6}$ mol in total;
0.6-2.5 mg/kg body weight or 50-200 mg in total; and/or
$4.3 \times 10^{-9} – 1.7 \times 10^{-8}$ mol/kg body weight or $3.4 \times 10^{-7} – 1.4 \times 10^{-6}$ mol in total;
0.8-1.8 mg/kg body weight or 60-140 mg in total; and/or
$5.1 \times 10^{-9} – 1.2 \times 10^{-8}$ mol/kg body weight or $4.1 \times 10^{-7} – 9.5 \times 10^{-7}$ mol in total;
0.9-1.8 mg/kg body weight or 70-140 mg in total; and/or
$6.0 \times 10^{-9} – 1.2 \times 10^{-8}$ mol/kg body weight or $4.8 \times 10^{-7} – 9.5 \times 10^{-7}$ mol in total;
1-1.5 mg/kg body weight or 80-120 mg in total; and/or
$6.8 \times 10^{-9} – 1.0 \times 10^{-8}$ mol/kg body weight or $5.5 \times 10^{-7} – 8.2 \times 10^{-7}$ mol in total;
1.1-1.4 mg/kg body weight or 90-110 mg in total; and/or

$7.7 \times 10^{-9} – 9.4 \times 10^{-9}$ mol/kg body weight or $6.1 \times 10^{-7} – 7.5 \times 10^{-7}$ mol in total;

1.2-1.3 mg/kg body weight or 95-105 mg in total; and/or

6.8×10^{-9} - 8.9×10^{-9} mol/kg body weight or $6.5 \times 10^{-7} – 7.2 \times 10^{-7}$ mol in total,

0,8-1.5 mg/kg body weight or 65-120 mg in total; and/or

$5.5 \times 10^{-9} – 1.0 \times 10^{-8}$ mol/kg body weight or $4.4 \times 10^{-7} – 8.2 \times 10^{-7}$ mol in total;

0.9-1.3 mg/kg body weight or 70-100 mg in total; and/or

$6.0 \times 10^{-9} – 8.5 \times 10^{-9}$ mol/kg body weight or $4.8 \times 10^{-7} – 6.8 \times 10^{-7}$ mol in total,

0.9-1.1 mg/kg body weight or 75-90 mg in total; and/or

$6.4 \times 10^{-9} – 7.7 \times 10^{-9}$ mol/kg body weight or $5.1 \times 10^{-7} – 6.1 \times 10^{-7}$ mol in total.

The amount of binding agent administered in each dose and/or in each treatment cycle is

a) about 1.1 mg/kg body weight or about 80 mg in total; and/or
b) about 6.8×10^{-9} mol/kg body weight or about 5.5×10^{-7} mol in total.

The amount of binding agent administered in each dose and/or in each treatment cycle is

a) 1.1 mg/kg body weight or 80 mg in total; and/or
b) 6.8×10^{-9} mol/kg body weight or 5.5×10^{-7} mol in total.

The amount of binding agent administered in each dose and/or in each treatment cycle is

a) about 1.0 mg/kg body weight or about 80 mg in total; and/or
b) about 6.8×10^{-9} mol/kg body weight or about 5.5×10^{-7} mol in total.

The amount of binding agent administered in each dose and/or in each treatment cycle is

a) 1.0 mg/kg body weight or 80 mg in total; and/or
b) 6.8×10^{-9} mol/kg body weight or 5.5×10^{-7} mol in total.

It is currently preferred that the amount of binding agent administered in each dose and/or in each treatment cycle is

a) about 1.25 mg/kg body weight or about 100 mg in total; and/or
b) about 8.5×10^{-9} mol/kg body weight or about 6.8×10^{-7} mol in total.

It is equally preferred that the amount of binding agent administered in each dose and/or in each treatment cycle is

a) 1.25 mg/kg body weight or 100 mg in total; and/or
b) 8.5×10^{-9} mol/kg body weight or 6.8×10^{-7} mol in total.

The binding agent may be one that activates human CD137 when bound thereto and inhibits the binding of human PD-L1 to human PD-1 when bound to PD-L1.

In the method according to the invention the binding agent may be one, wherein

a) the first binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 1, and a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 5;

and

b) the second antigen-binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 8, and a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 12.

In the method according to the invention the binding agent may be one, wherein

a) the first binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences set forth in: SEQ ID NO: 2, 3, and 4, respectively, and a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences set forth in: SEQ ID NO: 6, GAS, 7, respectively;

and

b) the second antigen-binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences set forth in: SEQ ID NO: 9, 10, 11 respectively, and a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences set forth in: SEQ ID NO: 13, DDN, 14, respectively.

Further, in the method according to the invention the binding agent may be one, wherein

a) The first binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 1 and a light chain variable region (VL) region comprising an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 5;

and

b) the second binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 8 and a light chain variable region (VL) region comprising an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 12.

In the method according to the invention the binding agent is one, wherein

a) The first binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region (VL) region comprising the amino acid sequence set forth in SEQ ID NO: 5;

and

b) the second binding region comprises, consists of or consist essentially of a heavy chain variable region (VH) comprising the amino acid sequence set forth in SEQ ID NO: 8 and a light chain variable region (VL) region comprising the amino acid sequence set forth in SEQ ID NO: 12.

The binding agent may in particular be an antibody, such as a multispecific antibody, or such as a bispecific antibody.

Also, the binding agent may be in the format of a full-length antibody or an antibody fragment.

It is further preferred that the antibody is a human antibody or a humanized antibody

Each variable region may comprise three complementarity determining regions (CDR1, CDR2, and CDR3) and four framework regions (FR1, FR2, FR3, and FR4).

The complementarity determining regions and the framework regions may be arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The binding agent may comprise

i) a polypeptide comprising, consisting of or consisting essentially of, said first heavy chain variable region (VH) and a first heavy chain constant region (CH), and

ii) a polypeptide comprising, consisting of or consisting essentially of, said second heavy chain variable region (VH) and a second heavy chain constant region (CH).

In the method according to the invention the binding agent may comprise, consist of or consist essentially of

i) a polypeptide comprising said first light chain variable region (VL) and further comprising a first light chain constant region (CL), and

ii) a polypeptide comprising said second light chain variable region (VL) and further comprising a second light chain constant region (CL).

The binding agent may be an antibody comprising a first binding arm and a second binding arm, wherein the first binding arm comprises, consists of or consist essentially of

i) a polypeptide comprising said first heavy chain variable region (VH) and said first heavy chain constant region (CH), and

ii) a polypeptide comprising said first light chain variable region (VL) and said first light chain constant region (CL);

and the second binding arm comprises, consists of or consist essentially of

iii) a polypeptide comprising said second heavy chain variable region (VH) and said second heavy chain constant region (CH), and

iv) a polypeptide comprising said second light chain variable region (VL) and said second light chain constant region (CL).

The binding agent may comprise, consist of or consist essentially of

i) a first heavy chain and light chain comprising said antigen-binding region capable of binding to CD137, and

ii) a second heavy chain and light chain comprising said antigen-binding region capable of binding PD-L1.

The binding agent may comprise, consist of or consist essentially of

i) a first heavy chain and light chain comprising said antigen-binding region capable of binding to CD137, the first heavy chain comprising a first heavy chain constant region and the first light chain comprising a first light chain constant region; and

ii) a second heavy chain and light chain comprising said antigen-binding region capable of binding PD-L1, the second heavy chain comprising a second heavy chain constant region and the second light chain comprising a second light chain constant region.

Each of the first and second heavy chain constant regions (CH) may comprise one or more of a constant heavy chain 1 (CH1) region, a hinge region, a constant heavy chain 2 (CH2) region and a constant heavy chain 3 (CH3) region, preferably at least a hinge region, a CH2 region and a CH3 region.

Each of the first and second heavy chain constant regions (CHs) may comprise a CH3 region and wherein the two CH3 regions comprise asymmetrical mutations.

In the said first heavy chain constant region (CH) at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain according to EU numbering may have been substituted, and in said second heavy chain constant region (CH) at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain according to EU numbering may have been substituted. In particular embodiments, the first and the second heavy chains are not substituted in the same positions.

The binding agent may be one, wherein (i) the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said first heavy chain constant region (CH), and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said second heavy chain constant region (CH), or (ii) the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said second heavy chain.

In the method according to the invention, the binding agent may be one which induces Fc-mediated effector function to a lesser extent compared to another antibody comprising the same first and second antigen binding regions and two heavy chain constant regions (CHs) comprising human IgG1 hinge, CH2 and CH3 regions.

In particular, the method may use a binding agent, wherein said first and second heavy chain constant regions (CHs) are modified so that the antibody induces Fc-mediated effector function to a lesser extent compared to an antibody which is identical except for comprising non-modified first and second heavy chain constant regions (CHs). In particular, each or both of said non-modified first and second heavy chain constant regions (CHs) may comprise, consists of or consist essentially of the amino acid sequence set forth in SEQ ID NO: 15.

The Fc-mediated effector function may be determined by measuring binding of the binding agent to Fcγ receptors, binding to C1q, or induction of Fc-mediated cross-linking of Fcγ receptors. In particular, the Fc-mediated effector function may be determined by measuring binding of the binding agent to C1q.

The first and second heavy chain constant regions of the binding agent may have been modified so that binding of C1q to said antibody is reduced compared to a wild-type antibody, preferably reduced by at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, or 100%, wherein C1q binding is preferably determined by ELISA.

The binding agent used in the method provided herein may be one wherein, in at least one of said first and second heavy chain constant regions (CH), one or more amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain according to EU numbering, are not L, L, D, N, and P, respectively.

In the binding agent used according to the invention the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering may be F and E, respectively, in said first and second heavy chains.

In particular, the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering may be F, E, and A, respectively, in said first and second heavy chain constant regions (HCs).

The binding agent used in the method according to the invention may be one, wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering of both the first and second heavy chain constant regions are F and E, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.

The binding agent used in the method according to the invention may be one, wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering of both the first and second heavy chain constant regions are F, E, and A, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain constant region is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.

The binding agent used in the method according to the invention may be one, wherein the constant region of said first and/or second heavy chain comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 30 [IgG1-FC],
b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and
c) a sequence having at the most 10 substitutions, such as at the most 9 substitutions, at the most 8, at the most 7, at the most 6, at the most 5, at the most 4, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the constant region of said first or second heavy chain, such as the second heavy chain, comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 31 [IgG1-F405L],
b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and
c) a sequence having at the most 9 substitutions, such as at the most 8, at the most 7, at the most 6, at the most 5, at the most 4, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the constant region of said first or second heavy chain, such as the first heavy chain comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 17 or 32 [IgG1-F409R]
b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and
c) a sequence having at the most 10 substitutions, such as at the most 9 substitutions, at the most 8, at the most 7, at the most 6, at the most 5, at the most 4 substitutions, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the constant region of said first and/or second heavy chain comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 33 [IgG1-Fc_FEA],
b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and
c) a sequence having at the most 7 substitutions, such as at the most 6 substitutions, at the most 5, at the most 4, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the constant region of said first and/or second heavy chain, such as the second heavy chain, comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 19 or SEQ ID NO: 34 [IgG1-Fc_FEAL],

b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and

c) a sequence having at the most 6 substitutions, such as at the most 5 substitutions, at the most 4 substitutions, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the constant region of said first and/or second heavy chain, such as the first heavy chain, comprises or consists essentially of or consists of an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 20 or SEQ ID NO: 35 [IgG1-Fc_FEAR]

b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and

c) a sequence having at the most 6 substitutions, such as at the most 5 substitutions, at the most 4, at the most 3, at the most 2 or at the most 1 substitution compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may comprise a kappa (κ) light chain constant region.

The binding agent used in the method according to the invention may comprises comprise a lambda (λ) light chain constant region.

The binding agent used in the method according to the invention may be one, wherein said first light chain constant region is a kappa (κ) light chain constant region.

The binding agent used in the method according to the invention may be one, wherein said second light chain constant region is a lambda (λ) light chain constant region.

The binding agent used in the method according to the invention may be one, wherein said first light chain constant region is a lambda (λ) light chain constant region.

The binding agent used in the method according to the invention may be one, wherein second light chain constant region is a kappa (κ) light chain constant region.

The binding agent used in the method according to the invention may be one, wherein the kappa (κ) light chain comprises an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 21,

b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have been deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and

c) a sequence having at the most 10 substitutions, such as at the most 9 substitutions, at the most 8, at the most 7, at the most 6, at the most 5, at the most 4 substitutions, at the most 3, at the most 2 or at the most 1 substitution, compared to the amino acid sequence defined in a) or b).

The binding agent used in the method according to the invention may be one, wherein the lambda (λ) light chain comprises an amino acid sequence selected from the group consisting of

a) the sequence set forth in SEQ ID NO: 22,

b) a subsequence of the sequence in a), such as a subsequence wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive amino acids has/have been deleted, starting from the N-terminus or C-terminus of the sequence defined in a); and

c) a sequence having at the most 10 substitutions, such as at the most 9 substitutions, at the most 8, at the most 7, at the most 6, at the most 5, at the most 4 substitutions, at the most 3, at the most 2 or at the most 1 substitution, compared to the amino acid sequence defined in a) or b).

The binding agent may be of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

In particular, the binding agent may be a full-length IgG1 antibody.

In currently preferred embodiments, the antibody is of the IgG1m(f) allotype.

The subject to be treated according to the present invention is preferably a human subject.

The tumor or cancer is a solid tumor.

The tumor or cancer may be selected from the group consisting of melanoma, ovarian cancer, lung cancer (e.g. non-small cell lung cancer (NSCLC), colorectal cancer, head and neck cancer, gastric cancer, breast cancer, renal cancer, urothelial cancer, bladder cancer, esophageal cancer, pancreatic cancer, hepatic cancer, thymoma and thymic carcinoma, brain cancer, glioma, adrenocortical carcinoma, thyroid cancer, other skin cancers, sarcoma, multiple myeloma, leukemia, lymphoma, myelodysplastic syndromes, ovarian cancer, endometrial or cancer, prostate cancer, penile cancer, cervical cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Merkel cell carcinoma and mesothelioma.

In particular embodiments, the tumor or cancer is selected from the group consisting of lung cancer (e.g. non-small cell lung cancer (NSCLC), urothelial cancer (cancer of the bladder, ureter, urethra, or renal pelvis), endometrial cancer (EC), breast cancer (e.g. triple negative breast cancer (TNBC)), squamous cell carcinoma of the head and neck (SCCHN) (e.g. cancer of the oral cavity, pharynx or larynx) and cervical cancer.

The tumor or cancer may in particular be a lung cancer.

The lung cancer may be a non-small cell lung cancer (NSCLC), such as a squamous or a non-squamous NSCLC.

Lung cancer is the most common malignancy and the most common cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) accounts for 85-90% of all lung cancer cases (Jemal et al., 2011). The five-year survival rate for NSCLC is approximately 18% (SEER, 2018). Major histological subtypes of NSCLC include adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, carcinoid tumors, and other less common subtypes, with adenocarcinoma being the most common.

Standard of care for patients with advanced or metastatic NSCLC who have progressed on targeted therapy or are no longer candidates for targeted therapy typically includes platinum-based chemotherapy. Platinum combinations have generated an overall response rate (ORR) of approximately 25-35%, a time to progression (TTP) of 4 6 months, and median survival of 8-10 months.

Tumor gene mutations/alterations have been identified and have impact on therapy selection. Identification of specific mutations or alterations in genes within the tumor, such as anaplastic lymphoma kinase (ALK), epidermal growth factor receptor (EGFR), c-ROS oncogene 1 (ROS1), BRAF, KRAS, and program death ligand-1 (PD-L1), aids the selection of potentially efficacious targeted therapies, while avoiding the use of therapies unlikely to provide clinical benefit (NCCN, 2018c). Activating sensitizing EGFR mutations are predictive for response to the EGFR Tyrosine Kinase Inhibitors (TKIs) (e.g., gefitinib, erlotinib, afatinib, and osimertinib). Similarly, TKIs (e.g., alectinib, ceritinib, and crizotinib) are effective therapies for ALK and ROS1 mutations and are also approved as first-line therapy for the respective mutations. Checkpoint inhibitor antibodies (e.g., pembrolizumab and nivolumab) that block the PD 1 and PD-L1 interaction have also been shown as effective treatment alone or in combination with chemotherapy for the treatment of patients with advanced or metastatic NSCLC whose tumors express PD-L1.

Despite multiple treatment options, patients with stage IV NSCLC ultimately have a poor prognosis and lung cancer remains the leading cause of cancer death for both men and women. The treatment

rate diminishes with each line of therapy, as patients succumb to their cancer or experience deterioration of their health that makes further treatment impossible.

The lung cancer may be NSCLC, which does not have an epidermal growth factor (EGFR)-sensitizing mutation and/or anaplastic lymphoma (ALK) translocation / ROS1 rearrangement. EGFR sensitizing mutations refers to mutations that confer sensitivity to EGFR tyrosine kinase inhibitors (TKIs), such as approved tyrosine kinase inhibitors erlotinib, osimertinib, gefintinib, olmutinib, nazartinib and avitinib.

The epidermal growth factor receptor (EGFR) amino acid sequence is provided herein as SEQ ID NO: 27.

The sensitizing mutation in the epidermal growth factor receptor (EGFR) amino acid sequence may be selected from the group consisting of:

i) An in-frame deletion and optionally insertion of one or more amino acids at position 746-751, such as any of the deletions and insertions defined in table 4,

ii) Substitution of a single amino acid at any one of positions 709, 715, 719, 720, 768, 858 and 861 such as any of the deletions and insertions defined in table 5, and

iii) An in-frame duplication and/or insertion selected from the duplications/insertions defined in Table 6;

amino acid numbering referring to the numbering of amino acids in SEQ ID NO: 27.

Designation	Amino acid change
Δ1	E746-A750 del
Δ2	E746-A750 del
Δ3	L747-T751 del
Δ4	L747-E749 del P ins
Δ5	L747-T750 del P ins
Δ6	L747-S752 del S ins
Δ7	E746-T751 del V ins
Δ8	L747-S752 del
Δ9	E746-T751 del I ins
Δ10	E746-A750 del V ins
Δ11	L747-S752 del Q ins

Table 4: In-frame deletions within exon 19 of the human EGFR gene (Adapted from Shigematsu *et al.*, Clinical and Biological Features Associated With Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers, JNCI: Journal of the National Cancer Institute, Volume 97, Issue 5, 2 March 2005). del = deletion; ins = insertion.

Designation	Amino acid change
M1	L858R
M2	E709V
M3	I715S
M4	G719C
M5	G719S
M6	G719A
M7	S720F
M8	S768I
M9	L861Q

Table 5: Single nucleotide substitutions and resulting amino acid changes within exon 21 of the human EGFR gene (Adapted from Shigematsu *et al.*, Clinical and Biological Features Associated With Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers, JNCI: Journal of the National Cancer Institute, Volume 97, Issue 5, 2 March 2005).

Designation	Amino acid change
D1	ASV770-772 ins
D2	H774 ins
D3	G771 ins
D4	CV770-771 ins
D5	NP773-774 ins, H775Y
D6	PH774-775 ins
D7	NPH774-776 ins
D8	HV775-776 ins

Table 6: In-frame duplications and/or insertions within exon 20 of the human EGFR gene (Adapted from Shigematsu *et al.*, Clinical and Biological Features Associated With Epidermal Growth Factor

Receptor Gene Mutations in Lung Cancers, JNCI: Journal of the National Cancer Institute, Volume 97, Issue 5, 2 March 2005). ins = insertion.

The non-small cell lung cancer may be characterized by, and/or the subject receiving the treatment may have, at least one mutation in the EGFR amino acid sequence selected from L747S, D761Y, T790M, C797S, T854A, such as T790M, C797S, D761Y, and double mutations T790M/D761Y and T790/C797S; amino acid numbering referring to the numbering of amino acids in SEQ ID NO: 27.

The non-small cell lung cancer may be characterized by expression of an epidermal growth factor receptor (EGFR) selected form the group consisting of:

i. a wild-type human EGFR; e.g. a human EFGR that comprises the sequence set forth in SEQ ID NO: 27 or a mature polypeptide thereof; and
ii. a human EGFR which is a variant of the EGFR in item i and which, when compared with the EGFR in item I, does not have any sensitizing mutations.

The non-small cell lung cancer may be a cancer which is not characterized by a sensitizing epidermal growth factor receptor (EGFR) mutation selected from the group consisting of:

i) An in-frame deletion and optionally insertion of one or more amino acids at position 746-751, such as any of the deletions and insertions defined in table 4,
ii) Substitution of a single amino acid at any one of positions 709, 715, 719, 720, 768, 858 and 861 such as any of the deletions and insertions defined in table 5, and
iii) An In-frame duplication and/or insertion selected from the duplications/insertions defined in Table 6;

amino acid numbering referring to the numbering of amino acids in SEQ ID NO: 27. Likewise, the subject receiving treatment according to the invention may be a subject that does not have such a sensitizing EGFR mutation.

The non-small cell lung cancer may be a cancer, which is not characterized by a mutation in the EGFR amino acid sequence selected from L747S, D761Y, T790M, C797S, T854A, such as from T790M, C797S, D761Y, and double mutations T790M/D761Y and T790/C797S; amino acid numbering referring to the numbering of amino acids in SEQ ID NO: 27. Likewise, the subject receiving treatment according to the invention may be a subject that does not have any of the said mutations.

The non-small cell lung cancer and/or the subject receiving treatment according to the invention may be characterized by having a mutation in the gene coding for the ALK tyrosine kinase (ALK), which leads to rearrangement of the gene coding for ALK (UniProt Q9UM73) with a gene coding for a fusion partner, to form a fusion oncogene.

The non-small cell lung cancer may be characterized by, and/or the subject receiving treatment according to the invention may have a mutation in the gene coding the ALK, said mutation leading to rearrangement of the gene coding for ALK with the gene (EML4) coding for Echinoderm microtubule-associated protein-like 4 (EMAPL4) (UniProt Q9HC35) (and formation of an EML4-ALK fusion oncogene).

The non-small cell lung cancer may be characterized by, and/or the subject receiving treatment according to the invention may have a mutation in the gene coding for the ALK tyrosine kinase (ALK), leading to rearrangement of the gene coding for the ALK with a gene selected from the group consisting of

i. KIF5B coding for Kinesin-1 heavy chain (KINH) (UniProt P33176),
ii. KLC1 coding for Kinesin light chain 1 (KLC1) (UniProt Q07866),
iii. TFG coding for Protein TFG (UniProt Q92734),
iv. TPR coding for Nucleoprotein TPR (UniProt P12270),
v. HIP1 coding for Huntington-interacting protein 1 (HIP-1) (UniProtKB - O00291),
vi. STRN coding for Striatin (UniProtKB - O43815),
vii. DCTN1 coding for dynactin subunit 1 (UniProt Q14203),
viii. SQSTM1 coding for sequestosome-1 (UniProtKB - Q13501),
ix. NPM1 coding for nucleophosmin (UniProt P06748),
x. BCL11A coding for B-cell lymphoma/leukemia 11A (UniProt Q9H165), and
xi. BIRC6 coding for baculoviral IAP repeat-containing protein (UniProt Q13490);

and formation of the respective fusion oncogene selected from the group consisting of a KIF5B-ALK fusion oncogene, a KLC1-ALK fusion oncogene, a TFG-ALK fusion oncogene, a TPR-ALK fusion oncogene, an HIP1-ALK fusion oncogene, a STRN-ALK fusion oncogene, a DCTN1-ALK fusion oncogene, a SQSTM1-ALK fusion oncogene, a NPM1-ALK fusion oncogene, a BCL11A-ALK fusion oncogene and a BIRC6-ALK fusion oncogene.

The non-small cell lung cancer may be characterized by expression of a wild-type human ALK tyrosine kinase; e.g. a human ALK tyrosine kinase that comprises the sequence provided under UniProt Q9HC35 or a mature polypeptide thereof.

The non-small cell lung cancer may be characterized by not having a mutation in the gene coding for the ALK tyrosine kinase (ALK), leading to rearrangement of ALK with fusion partner to form a fusion oncogene and/or the subject does not have such a mutation.

The non-small cell lung cancer may be characterized by not having a mutation in the gene coding for the ALK tyrosine kinase (ALK), leading to rearrangement of the gene (EML4) coding for Echinoderm

microtubule-associated protein-like 4 (EMAPL4) (UniProt Q9HC35) with ALK (UniProt Q9HC35) and formation of an EML4-ALK fusion oncogene and/or the subject may be a subject that does not have such a mutation.

The non-small cell lung cancer may be characterized by not having a mutation in any gene selected from the group consisting of the gene coding for the ALK tyrosine kinase (ALK), the gene (EML4) coding for Echinoderm microtubule-associated protein-like 4 (EMAPL4) (UniProt Q9HC35).

The non-small cell lung cancer may be a cancer that is not characterized by a mutation selected from the group consisting of

- a sensitizing epidermal growth factor receptor (EGFR) mutation,
- a mutation in the gene coding for the ALK tyrosine kinase (ALK), leading to rearrangement of EML4 with ALK and formation of an EML4-ALK fusion oncogene,
- a mutation in the EGFR amino acid sequence, which induces or confers resistance of said subject to one or more EGFR tysrosine kinase inhibitors (EGFR-TKIs); and

the subject may have been treated with a programmed cell death-1 (PD-1)/ programmed cell death-1 (PD-1) inhibitor (e.g. nivolumab, genolimzumab, atezolizumab, durvalumab or avelumab) or with chemotherapy (e.g. chemotherapy comprising platinum, a taxane, pemetrexed and/or gemcitabine) and may have failed with such previous treatment.

The non-small cell lung cancer may be characterized by a mutation selected from the group consisting of

- a sensitizing epidermal growth factor receptor (EGFR) mutation,
- a mutation in the EGFR amino acid sequence, which induces or confers resistance of said subject to one or more EGFR tysrosine kinase inhibitors (EGFR-TKIs),
- a mutation in the gene coding for the ALK tyrosine kinase (ALK), leading to rearrangement of EML4 with ALK and formation of an EML4-ALK fusion oncogene; and

the subject may have been treated with an EGFR inhibitor (e.g. erlotinib, osimertinib, gefintinib, olmutinib, nazartinib and avitinib) or with a PD-1/PD-L1 inhibitor (e.g. nivolumab, genolimzumab, atezolizumab, durvalumab or avelumab) and has failed with such previous treatment.

The subject has received up to four prior systemic treatment regimens for advanced/metastatic disease to treat the lung cancer and has experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

Before receiving treatment according to the present invention, the subject has received platinum-based chemotherapy to treat the lung cancer. Alternatively, the subject may not be eligible for

platinum-based therapy and have received alternative chemotherapy, e.g., a treatment with gemcitabine-containing regimen.

The subject may have received prior treatment with checkpoint inhibitor(s) to treat the lung cancer, such as agent(s) targeting programmed cell death-1 (PD-1)/ programmed death-ligand 1 (PD-L1), such as a PD-1/PD-L1 inhibitor. Preferably, subjects must have received only one prior treatment with PD-1/PD-L1 inhibitor alone or in combination.

In particular, the subject may have experienced disease progression on or after treatment with checkpoint inhibitor(s), such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor. Further, the subject has experienced disease progression on or after last prior treatment with checkpoint inhibitor(s), such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor.

The inhibitor of PD-1 and/or PD-L1 may in particular comprise an antibody, or antigen-binding fragment thereof, capable of binding to PD-L1.

Known inhibitors of PD-1 and/or PD-L1 include pembrolizumab (Merck & Co), CBT-501 (genolimzumab; Genor Bio/CBT Pharma), nivolumab (BMS), REGN2810 (Cemiplimab; Regeneron), BGB-A317 (Tislelizumab; BeiGene/Celgene), Amp-514 (MEDI0680) (Amplimmune), TSR-042 (Dostarlimab; Tesaro/AnaptysBio), JNJ-63723283/JNJ-3283 (Johnson & Johnson), PF-06801591 (Pfizer), JS-001 (Tripolibamab/Toripalimab; Shanghai Junshi Bio), SHR-1210/INCSHR-1210 (Camrelizumab; Incyte corp), PDR001 (Spartalizumab; Novartis), BCD-100 (BioCad), AGEN2034 (Agenus), IBI-308 (Sintilimab; Innovent Biologics), RG7446/MPDL-3280A (atezolizumab; Roche), MSB-0010718C (avelumab; Merck Serono/Pfizer) and MEDI-4736 (durvalumab; AstraZeneca), KN-035 (envafolimab; 3DMed/Alphamab Co.).

In particular, the subject may have experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

Alternatively, the subject receiving treatment according to the invention may be one that has not received prior treatment with checkpoint inhibitor(s) to treat said lung cancer, such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. any of the PD-1/PD-L1 inhibitors recited above.

In other embodiments the tumor or cancer is an endometrial cancer. In the US as well as other developed countries, uterine endometrial cancer (EC) was the most common gynecological malignancy, with increasing incidence globally. In the United States, there were estimated 60,000 new cases reported and over 10,000 deaths in 2016. Worldwide in 2012, 527,600 women were diagnosed with uterine EC. A majority of EC cases are identified at an early stage and are treated

with surgery with or without radiotherapy or chemotherapy. However, patients with advanced disease have a poorer prognosis with a 5-year survival rate of less than 50% for patients with lymph node metastases and less than 20% for patients with peritoneal or distant metastases.

Multiagent chemotherapy is the preferred treatment for metastatic, recurrent, or high-risk disease; however, there is no consensus on a standard regimen. Carboplatin and paclitaxel are increasingly used in the first-line setting for advanced/metastatic or recurrent EC. Response rates with carboplatin and paclitaxel range from 40% to 62% with an OS of approximately 13 to 29 months. Patients who progress on combination therapy or who are unable to tolerate multi-agent chemotherapy may receive single-agent therapy, however, chemotherapeutic options in this setting have produced only modest activity, especially in the second-line setting and beyond. Single agent response rates range from 21% to 36% in the first-line setting and 4% to 27% in the second-line setting (NCCN, 2018d).

Most recently, pembrolizumab has demonstrated anti-tumor activity in patients with locally advanced or metastatic PD-L1 positive EC who experienced progression on or after standard therapy

In particular, the subject or the endometrial cancer treated according to the invention may have epithelial endometrial histology including: endometrioid, serous, squamous, clear-cell carcinoma, or carcinosarcoma.

The subject may have received up to four prior systemic treatment regimens for advanced/metastatic disease to treat said endometrial cancer and may have experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

The subject may be one that has not received prior treatment with checkpoint inhibitor(s) to treat said endometrial cancer, such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. a PD-1/PD-L1 inhibitor selected from the list of PD-1/PD-L1 inhibitors above.

According to other embodiments, the tumor or cancer is a urothelial cancer, including cancer of the bladder, ureter, urethra, or renal pelvis.

The subject may have received up to four prior systemic treatment regimens for advanced/metastatic disease to treat said urothelial cancer and may have experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

The subject may have received prior treatment with checkpoint inhibitor(s) to treat said urothelial cancer, such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. any one of the PD-1/PD-L1 inhibitors listed above.

Further, the subject may be one that has received platinum-based chemotherapy to treat said urothelial cancer; i.e. chemotherapy with an agent which is a are coordination complex of platinum. Examples of platinum-based chemotherapy include treatment with cisplatin, oxaliplatin, and carboplatin.

The subject may be one that is not eligible for platinum-based therapy and has received alternative chemotherapy, e.g., a treatment with gemcitabine-containing regimen.

In other embodiments according to the invention, the tumor or cancer is a breast cancer, such as a triple negative breast cancer (TNBC). TNBC generally refers to breast cancers that lack expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). The TNBC may in particular be HER2 negative, such as determined by Fluorescence in situ hybridization (FISH) or determination of protein expression by immunohistochemistry.

The subject may have received at least one prior systemic treatment regimen for locally advanced/metastatic disease to treat said breast cancer, such as at least one prior systemic treatment regimen including anthracycline-, taxane-, antimetabolite- or microtubule inhibitor-containing regimens.

In further embodiments, the subject may have received at the most 4 prior systemic treatment regimens for locally advanced/metastatic disease to treat said breast cancer, such including as at least one prior systemic treatment regimen including anthracycline-, taxane-, antimetabolite- or microtubule inhibitor-containing regimens.

The subject may have received prior treatment with checkpoint inhibitor(s) to treat the breast cancer, such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. any one of the PD-/PD-L1 inhibitors listed above.

The subject may have experienced disease progression on or after said prior treatment with checkpoint inhibitor(s) to treat the breast cancer, such as disease progression determined by radiography.

In other embodiments, the subject may be one that has not received prior treatment with checkpoint inhibitor(s) to treat the breast cancer, such a subject that has not received treatment

with as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. the PD-/PD-L1 inhibitors listed above.

The tumor or cancer may be a head and neck cancer, such as a squamous cell carcinoma of the head and neck (SCCHN). Squamous-cell carcinoma of the head and neck (SCCHN) is a major cause of death with over 600,000 cases diagnosed annually worldwide. In 2018, approximately 64,690 people will develop oral cavity, pharyngeal, or laryngeal cancers in the US and an estimated 13,740 deaths will occur over the same period. Head and neck cancers can arise in the oral cavity, pharynx, larynx, nasal cavity, paranasal sinuses, thyroid, and salivary glands. Tobacco use and alcohol greatly increase the risk of developing head and neck cancer. In addition, human papillomavirus (HPV) infection has a causal association with squamous cancers of the oropharynx (particularly tonsils and base of tongue) and recent evidence suggests that HPV may also be associated with increased risk of squamous cell carcinoma of the larynx. Patients with locally HPV-positive head and neck cancers have improved outcomes for response to treatment, PFS, and OS as compared with HPV-negative tumors.

Treatment of head and neck cancers is complex and requires a multidisciplinary approach. The prognosis of patients with recurrent or metastatic SCCHN is generally poor with a median survival of approximately 6 to 12 months depending on patient's performance status and disease-related factors. First-line therapy for fit patients includes cetuximab with cisplatin or carboplatin plus 5-fluorouracil (5-FU). The addition of cetuximab resulted in prolonged survival as compared with platinum and 5-FU alone (10.1 months vs. 7.4 months) as well as prolonged mPFS (3.3 months vs. 5.6 months). Single agent chemotherapy is recommended for patients with poorer performance status. In the past, the most widely used single agents included platinum compounds, taxanes, nab-paclitaxel, methotrexate, fluorouracil, and cetuximab.

In the US and several other countries, pembrolizumab and nivolumab are approved for patients with progressive disease (PD) after platinum-containing chemotherapy. While data from trials exploring the single agent activity of PD-1 targeted appear encouraging, response rates remain low.

In particular, the tumor or cancer may be recurrent of metastatic SCCHN.

In particular embodiments relating to SCCHN, the tumor or cancer is cancer of the oral cavity, pharynx or larynx.

The subject may have received up to four prior systemic treatment regimens for recurrent/metastatic disease to treat the SCCHN and may have experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

The subject may have received platinum-based chemotherapy to treat the SCCHN, such as treatment with treatment with cisplatin, oxaliplatin, and carboplatin.

Alternatively, the subject may not be eligible for platinum-based therapy and may have received alternative chemotherapy to treat the SCCHN.

The subject may be one that has received prior treatment with checkpoint inhibitor(s) to treat the SCCHN, such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. the PD-/PD-L1 inhibitors listed above.

The subject my have experienced disease progression on or after said prior treatment with checkpoint inhibitor(s), such as disease progression determined by radiography.

In other embodiments the subject may be one that has not received prior treatment with checkpoint inhibitor(s), such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. a subject that has not received treatment with any of the PD-/PD-L1 inhibitors listed above.

In further embodiments, the tumor or cancer is a cervical cancer. Cervical cancer poses a significant medical problem worldwide with an estimated incidence of more than 500,000 new cases. In the US, approximately 12,800 new cases and 4,210 deaths are estimated to occur in 2017. Cervical cancer has a median age of diagnosis of 49 years in the US and even lower in developing countries. While the 5-year survival rate for patients in the US diagnosed with localized disease is 91%, the prognosis for patients with advanced disease remains poor. Five-year survival rates for advanced/metastatic disease are less than 35%.

First-line treatment for recurrent or metastatic cervical cancer is comprised of bevacizumab combined with paclitaxel and platinum (cisplatin or carboplatin) or paclitaxel and topotecan. Despite a 48% ORR and a median OS of approximately 18 months, almost all patients relapse after this first-line treatment. For second line therapy, pembrolizumab is approved in the US for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy and whose tumors express PD-L1 as determined by an FDA-approved test. No additional approved therapies are available however, patients are often treated with single agent modalities including, but not limited to: pemetrexed, topotecan, docetaxel, nab-paclitaxel, vinorelbine, and in some cases bevacizumab. Response rates with single agent treatment are very low (range: 0-15%) and for this reason, cervical cancer remains a population of very high unmet medical need.

The cervical cancer may in particular be of squamous cell, adenocarcinoma or adenosquamous histology.

The subject treated according to the invention may be a subject that has received at least one prior systemic treatment regimen for recurrent/metastatic disease to treat said cervical cancer, such as chemotherapy in combination with treatment targeting vascular endothelial growth factor A, such as treatment with bevacizumab, and has experienced disease progression on or after last prior systemic treatment, such as disease progression determined by radiography.

The subject treated according to the invention may be a subject that has received at the most 4 prior systemic treatment regimens for recurrent/metastatic disease, including chemotherapy in combination with treatment targeting vascular endothelial growth factor A, such as treatment with bevacizumab.

In some embodiments the subject treated according to the invention is a subject that has not received prior treatment with checkpoint inhibitor(s), such as agent(s) targeting PD-1/PD-L, such as a PD-1/PD-L1 inhibitor; e.g. a subject that has not received treatment with any of the PD-/PD-L1 inhibitors listed above.

Preferably, the subject is a female.

The binding agent used according to the present invention may in particular be administered by systemic administration.

Preferably, the binding agent is administered to said subject by intravenous injection or infusion.

Each treatment cycle treatment cycle may be two weeks (14 days), three weeks (21 days) or four weeks (28 days).

In particular embodiments of the invention, each dose is administered or infused every second week (1Q2W), every third week (1Q3W) or every fourth week (1Q4W).

In some embodiments one dose or each dose is administered or infused on day 1 of each treatment cycle

Each dose may be administered or infused over a minimum of 30 minutes, such as over a minimum of 60 minutes, a minimum of 90 minutes, a minimum of 120 minutes or a minimum of 240 minutes.

A further aspect of the invention provides composition, such as a pharmaceutical composition comprising a binding agent comprising a first binding region binding to human CD137 and a second binding region binding to human PD-L1, wherein the amount of binding agent in the composition is about 25-400 mg or about $1.7 \times 10^{-7} – 2.7 \times 10^{-6}$ mol, such as 25-400 mg or $1.7 \times 10^{-7} – 2.7 \times 10^{-6}$ mol.

The amount of binding agent administered in said composition may in particular be about 25-320 mg or about $1.7 \times 10^{-7} – 2.2 \times 10^{-6}$ mol, such as 25-320 mg or $1.7 \times 10^{-7} – 2.2 \times 10^{-6}$ mol;

about 30-320 mg or about $2.4 \times 10^{-7} – 2.2 \times 10^{-6}$ mol; such as 30-320 mg or $2.4 \times 10^{-7} – 2.2 \times 10^{-6}$ mol about 40-260 mg or about $2.7 \times 10^{-7} – 1.8 \times 10^{-6}$ mol, such as 40-260 mg or $2.7 \times 10^{-7} – 1.8 \times 10^{-6}$ mol; about 50-200 mg or about $3.4 \times 10^{-7} – 1.4 \times 10^{-6}$ mol, such as 50-200 mg or $3.4 \times 10^{-7} – 1.4 \times 10^{-6}$ mol; about 60-140 mg or about $4.1 \times 10^{-7} – 9.5 \times 10^{-7}$ mol, such as 60-140 mg or $4.1 \times 10^{-7} – 9.5 \times 10^{-7}$ mol; about 70-140 mg or about $4.8 \times 10^{-7} – 9.5 \times 10^{-7}$ mol, such as 70-140 mg or $4.8 \times 10^{-7} – 9.5 \times 10^{-7}$ mol; about 80-120 mg or about $5.5 \times 10^{-7} – 8.2 \times 10^{-7}$ mol, such as 80-120 mg or $5.5 \times 10^{-7} – 8.2 \times 10^{-7}$ mol; about 90-110 mg or about $6.1 \times 10^{-7} – 7.5 \times 10^{-7}$ mol, such as 90-110 mg or $6.1 \times 10^{-7} – 7.5 \times 10^{-7}$ mol; about 95-105 mg or about $6.5 \times 10^{-7} – 7.2 \times 10^{-7}$ mol, such as 95-105 mg or $6.5 \times 10^{-7} – 7.2 \times 10^{-7}$ mol; about 65-120 mg or about $4.4 \times 10^{-7} – 8.2 \times 10^{-7}$ mol, such as 65-120 mg or $4.4 \times 10^{-7} – 8.2 \times 10^{-7}$ mol; about 70-100 mg or about $4.8 \times 10^{-7} – 6.8 \times 10^{-7}$ mol, such as 70-100 mg or $4.8 \times 10^{-7} – 6.8 \times 10^{-7}$ mol; or about 75-90 mg or about $5.1 \times 10^{-7} – 6.1 \times 10^{-7}$ mol, such as 75-90 mg or $5.1 \times 10^{-7} – 6.1 \times 10^{-7}$ mol.

The composition or pharmaceutical composition may be formulated with a carrier, excipient and/or diluent as well as any other components suitable for pharmaceutical compositions, including known adjuvants, in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 1995. The pharmaceutically acceptable carriers or diluents as well as any known adjuvants and excipients should be suitable for the antibody or antibody conjugate of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen compound or pharmaceutical composition of the present invention (e.g., less than a substantial impact [10% or less relative inhibition, 5% or less relative inhibition, etc.] upon antigen binding).

A pharmaceutical composition of the present invention may include diluents, fillers, salts, buffers, detergents (e. g., a nonionic detergent, such as Tween-20 or Tween-80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition.

Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption-delaying agents, and the like that are physiologically compatible with a compound of the present invention.

Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the

like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well known in the pharmaceutical arts.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated.

Pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

Pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the composition. The combination of compounds of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and micro-encapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, poly-ortho esters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art, see e.g. Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In one embodiment, the binding agent used according to the present invention may be formulated to ensure proper distribution *in vivo*. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the

extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except in so far as any conventional media or agent is incompatible with the active compound, use thereof in the compositions of the present invention is contemplated. Other active or therapeutic compounds may also be incorporated into the compositions.

Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be an aqueous or a non-aqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Sterile injectable solutions may be prepared by incorporating the active compounds in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum-drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In one embodiment, the composition according to the invention comprises about 5.5 x 10^{-7} mol or about 80 mg of said binding agent, such as 5.5 x 10^{-7} mol or 80 mg.

In a currently preferred embodiment, the composition according to the invention comprises about 6.8 x 10^{-7} mol or about 100 mg of said binding agent, such as 6.8 x 10^{-7} mol or 100 mg of said binding agent.

In the composition according to the invention the binding agent may be as defined above; e.g. the binding agent may comprise any of the variable regions and constant regions defined above.

The present invention further comprises a dosage unit form of a binding agent or composition as disclosed above

Preferably, the dosage unit form is for systemic administration. In particular embodiments, the dosage unit form is for injection or infusion, such as intravenous injection or infusion into a subject.

In the composition or dosage unit form the binding agent is preferably in aqueous solution, such in 0.9% NaCl (saline). The dosage unit form may have a volume of 50-500 mL, such as 50-250 mL, 50-500 mL, 100-500 mL or 100-250 mL.

In yet a further aspect, the present application provides a binding agent for use in treatment of cancer, comprising a first binding region binding to human CD137, and a second binding region binding to human PD-L1.

The binding agent may be administered in suitable amounts. In particular, the amount of binding agent administered in each dose and/or in each treatment cycle may be

a) about 0.3-5 mg/kg body weight or about 25-400 mg in total; and/or
b) about 2.1 x 10^{-9} – 3.4 x 10^{-8} mol/kg body weight or about 1.7 x 10^{-7} – 2.7 x 10^{-6} mol in total.

Preferably, the amount of binding agent administered in each dose and/or in each treatment cycle is

a) about 1.25 mg/kg body weight or about 100 mg in total, such as 1.25 mg/kg body weight or 100 mg in total; and/or
b) about 8.5 x 10^{-9} mol/kg body weight or about 6.8 x 10^{-7} mol in total, such as 8.5 x 10^{-9} mol/kg body weight or 6.8 x 10^{-7} mol in total.

Additional items of the present disclosure include:

1. A method for reducing or preventing progression of a tumor or treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a binding agent,

wherein the binding agent comprises a first antigen binding region which binds to human CD137, and a second antigen binding region which binds to human PD-L1, wherein the binding agent is administered in at least one treatment cycle, and wherein the therapeutically effective amount of the binding agent is

(i) about 25 mg to about 400 mg in total; or

(ii) about 1.7 x 10^{-7} mol to about 2.7 x 10^{-6} mol in total.

2. The method of item 1, wherein the therapeutically effective amount of the binding agent is

(i) about 80 mg to about 240 mg in total; or

(ii) about 5.5 x 10^{-7} mol to about 1.6 x 10^{-6} mol in total.

3. The method of item 1, wherein the therapeutically effective amount of the binding agent is

(i) about 80 mg in total; or

(ii) about 5.5 x 10^{-7} mol in total.

4. The method of item 1, wherein the therapeutically effective amount of the binding agent is

(i) about 100 mg in total; or

(ii) about 6.8 x 10^{-7} mol in total.

5. The method of any one of items 1 to 4, wherein

a) the first antigen binding region comprises a heavy chain variable region (VH) comprising a CDR1 (HCDR1), a CDR2 (HCDR2), and a CDR3 (HCDR3) and a light chain variable region (VL) comprising a CDR1 (LCDR1), a CDR2 (LCDR2), and a CDR3 (LCDR3);

wherein HCDR1 comprises the amino acid sequence according to SEQ ID NO: 2,

wherein HCDR2 comprises the amino acid sequence according to SEQ ID NO: 3,

wherein HCDR3 comprises the amino acid sequence according to SEQ ID NO: 4,

wherein LCDR1 comprises the amino acid sequence according to SEQ ID NO: 6,

wherein LCDR2 comprises the amino acid sequence GAS, and

wherein LCDR3 comprises the amino acid sequence according to SEQ ID NO: 7;

b) the second antigen-binding region comprises a heavy chain variable region (VH) comprising a CDR1 (HCDR1), a CDR2 (HCDR2), and a CDR3 (HCDR3),and a light chain variable region (VL) comprising a CDR1 (LCDR1), a CDR2 (LCDR2), and a CDR3 (LCDR3);

wherein HCDR1 comprises the amino acid sequence according to SEQ ID NO: 9,

wherein HCDR2 comprises the amino acid sequence according to SEQ ID NO: 10,

wherein HCDR3 comprises the amino acid sequence according to SEQ ID NO: 11,

wherein LCDR1 comprises the amino acid sequence according to SEQ ID NO: 13,

wherein LCDR2 comprises the amino acid sequence DDN, and

wherein LCDR3 comprises the amino acid sequence according to SEQ ID NO: 14.

6. The method of any one of items 1 to 4, wherein the first binding region comprises:

(i) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to the amino acid sequence according to SEQ ID NO: 1, and

(ii) a light chain variable region (VL) region comprising an amino acid at least 95% identical to the amino acid sequence according to SEQ ID NO: 5;

wherein the second binding region comprises:

(iii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to the amino acid sequence according to SEQ ID NO: 8, and

(iv) a light chain variable region (VL) region comprising an amino acid sequence at least 95% to SEQ ID NO: 12.

7. The method of any one of items 1 to 6, wherein the first binding region comprises

(i) a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO: 1, and

(ii) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO: 5;

wherein the second binding region comprises:

(iii) a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO: 8, and

(iv) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO: 12.

8. The method of any one of items 1 to 7, wherein the binding agent comprises:

(1) a first polypeptide comprising:

(a) a first heavy chain comprising a first heavy chain variable region (VH1), and a first heavy chain constant region (CH1), and

(b) a first light chain comprising a first light chain variable region (VL1) and a first light chain constant region (CL1); and

(2) a second polypeptide comprising:

(c) a second heavy chain comprising a second heavy chain variable region (VH2) and a second heavy chain constant region (CH2), and

(d) a second light chain comprising a second light chain variable region (VL2) and a second light chain constant region (CL2);

wherein VH1 comprises the amino acid sequence according to SEQ ID NO: 1,

wherein VL1 comprises the amino acid sequence according to SEQ ID NO: 5,

wherein VH2 comprises the amino acid sequence according to SEQ ID NO: 8, and

wherein VL2 comprises the amino acid sequence according to SEQ ID NO: 12.

9. The method of item 8, wherein CH1 comprises an amino acid sequence at least 95% identical to the amino acid sequence according to the amino acid sequence of SEQ ID NO: 19 or 34, wherein between 1 and 10 consecutive amino acids have been deleted, and

wherein the CH2 comprises an amino acid sequence at least 95% identical to the amino acid sequence according to the amino acid sequence of SEQ ID NO: 20 or 35, wherein between 1 and 10 consecutive amino acids have been deleted.

10. The method of any one of items 1 to 9, wherein the binding agent is an antibody or fragment thereof.

11. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is non-small cell lung cancer (NSCLC).

12. The method of item 11, wherein the NSCLC subject:

(i) has received up to four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment;

(ii) has histological or cytological diagnosis of non-squamous NSCLC that does not have an epidermal growth factor (EGFR)-sensitizing mutation and/or anaplastic lymphoma (ALK) translocation / ROS1 rearrangement;

(iii) has received platinum-based therapy or alternative chemotherapy due to platinum ineligibility; and

(iv) has shown disease progression under prior treatment with a PD-1/PD-L1 inhibitor.

13. The method of item 11, wherein the NSCLC subject:

(i) has received up to four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment;

(ii) has histological or cytological diagnosis of non-squamous NSCLC that does not have an epidermal growth factor (EGFR)-sensitizing mutation and/or anaplastic lymphoma (ALK) translocation / ROS1 rearrangement;

(iii) has received platinum-based therapy or alternative chemotherapy due to platinum ineligibility; and

(iv) has not received prior treatment with a PD-1/PD-L1 inhibitor.

14. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is urothelial cancer (UC).

15. The method of item 14, wherein the UC subject:

(i) has received up to four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment;

(ii) has shown disease progression under prior treatment with a PD-1/PD-L1 inhibitor; and

(iii) has either received platinum-based chemotherapy or is not eligible for any platinum-based or any cisplatin-containing chemotherapy.

16. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is endometrial cancer (EC).

17. The method of item 16, wherein the EC subject:

(i) has received up to four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment;

(ii) has epithelial endometrial histology comprising endometrioid, serous, squamous, clear-cell carcinoma, or carcinosarcoma; and

(iii) has not received prior treatment with a PD-1/PD-L1 inhibitor.

18. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is triple-negative breast cancer (TNBC).

19. The method of item 18, wherein the TNBC subject:

(i) has TNBC defined as HER2-negative;

(ii) has received between one and four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment.

20. The method of item 18, wherein the TNBC subject has shown disease progression under prior treatment with a PD-1/PD-L1 inhibitor.

21. The method of item 18, wherein the TNBC subject has not received prior treatment with a PD-1/PD-L1 inhibitor.

22. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is squamous cell carcinoma of the head and neck (SCCHN).

23. The method of item 22, wherein the SCCHN subject:

(i) has received up to 4 prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment; and

(ii) has shown disease progression under prior treatment with platinum-based chemotherapy or alternative combination if not eligible for platinum-based chemotherapy.

24. The method of any one of items 1 to 10, wherein the tumor or cancer in the subject is cervical cancer.

25. The method of item 24, wherein the cervical cancer subject:

(i) has received between one and four prior systemic treatment regimens for advanced/metastatic disease and has experienced disease progression on or after last prior systemic treatment;

(ii) has cervical cancer of squamous cell, adenocarcinoma, or adenosquamous histology; and

(iii) has not received prior treatment with a PD-1/PD-L1 inhibitor.

26. The method of any one of the preceding items, wherein the therapeutically effective amount of the binding agent is administered at a fixed dose irrespective of body weight.

27. The method of to any one of the preceding items, wherein the binding agent is administered by systemic administration.

28. The method of any one of the preceding items, wherein the binding agent is administered by intravenous injection or infusion.

29. The method of any one of the preceding items, wherein each treatment cycle is three weeks (21 days).

30. The method of any one of the preceding items, wherein one dose is administered every third week (1Q3W).

31. The method of any one of the preceding items, wherein one dose is administered on day 1 of each treatment cycle.

SEQUENCES

Table 7

SEQ ID	NAME	SEQUENCE
1	VH_CD137-009-H7	EVQLVESGGGLVQPGRSLRLSCTASGFSLNDYWMSWVRQAPGK GLEWVGYIDVGGSLYYAASVKGRFTISRDDSKSIAYLQMNSLKTED TAVYYCARGGLTYGFDLWGQGTLVTVSS
2	VH_CD137-009-H7_CDR1	GFSLNDYW
3	VH_CD137-009-H7_CDR2	IDVGGSL
4	VH_CD137-009-H7_CDR3	ARGGLTYGFDL
5	VL_CD137-009-L2	DIVMTQSPSSLSASVGDRVTITCQASEDISSYLAWYQQKPGKAPK RLIYGASDLASGVPSRFSASGSGTDYTFTISSLQPEDIATYYCHYYAT ISGLGVAFGGGTKVEIK
6	VL_CD137-009-L2_CDR1	EDISSY
	VL_CD137-009-L2_CDR2	GAS
7	VL_CD137-009-L2_CDR3	HYYATISGLGVA
8	VH-PD-L1-547	EVQLLEPGGGLVQPGGSLRLSCEASGSTFSTYAMSWVRQAPGKG LEWVSGFSGSGGFTFYADSVRGRFTISRDSSKNTLFLQMSSLRAED TAVYYCAIPARGYNYGSFQHWGQGTLVTVSS
9	VH- PD-L1-547-CDR1	GSTFSTYA
10	VH- PD-L1-547-CDR2	FSGSGGFT
11	VH- PD-L1-547-CDR3	AIPARGYNYGSFQH
12	VL- PD-L1-547	SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPV LVVYDDNDRPSGLPERFSGSNSGNTATLTISRVEAGDEADYYCQV WDSSSDHVVFGGGTKLTVL
13	VL- PD-L1-547-CDR1	NIGSKS
	VL- PD-L1-547-CDR2	DDN
14	VL- PD-L1-547-CDR3	QVWDSSSDHVV
15	IgG1-Fc	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR

		TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
16	IgG1-Fc_F405L	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFLLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
17	IgG1-Fc_K409R	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
18	IgG1-Fc_FEA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
19	IgG1-FEAR-Fc	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPE**FE**GGPSVFLFPPKPKDTLMISR TPEVTCVVV**A**VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR

		EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYS**R**LTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
20	IgG1-FEAL-Fc	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPE**FE**GGPSVFLFPPKPKDTLMISR TPEVTCVVV**A**VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSF**L**LYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
21	Kappa-C	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
22	Lambda-C	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD SSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVT HEGSTVEKTVAPTECS
23	Human CD137 (UniProtKB - Q07011; incl. signal peptide sequence: aa 1-23)	MGNSCYNIVALLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICS PCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPG FHCLGAGCSMCEQDCKQGQELTKKGCKDCCFGTFNDQKRGICRP WTNCSLDGKSVLVNGTKERDVVCGPSPADLSPGASSVTPPAPAR EPGHSPQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCEL
24	Human CD137 (UniProtKB - Q07011; mature sequence)	LQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCDICRQ CKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQE LTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERD VVCGPSPADLSPGASSVTPPAPAREPGHSPQIISFFLALTSTALLFLL FFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE GGCEL
25	Human PD-L1 (UniProtKB - Q9NZQ7; incl. signal peptide sequence: aa 1-18)	MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECFPVEK QLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQ LSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNK INQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTT NSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPL

		AHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDT NSKKQSDTHLEET
26	Human PD-L1 (UniProtKB - Q9NZQ7; mature sequence)	FTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKN IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDA GVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTC QAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINT TTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLC LGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET
27	*Homo Sapiens* EGFR	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFE DHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLI ALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPM RNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDFQ NHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRG KSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLY NPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGA DSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHF KNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLI QAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSL KEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCK ATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEG EPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGP HCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVRKR TLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGT VYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDN PHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLNW CVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLAKLL GAEEKEYHAEGGKVPIKWMALESILHRIYTQSDVWSYGVTVWEL MTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMI DADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYR ALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSATSNN STVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPE YINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVG NPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFP

		KEAKPNGIFKGSTAENAEYLRVAPQSSEFIGA
28	VH_CD137-009	QSLEESGGRLVTPGTPLTLTCTVSGFSLNDYWMSWVRQAPGKGL EWIGYIDVGGSLYYASWAKGRFTISRTSTTVDLKMTSLTTEDTATY FCARGGLTYGFDLWGPGTLVTVSS
29	VL_CD137-009	DIVMTQTPASVSEPVGGTVTINCQASEDISSYLAWYQQKPGQRP KRLIYGASDLASGVPSRFSASGSGTEYALTISDLESADAATYYCHYY ATISGLGVAFGGGTEVVVK
30	IgG1-Fc without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
31	IgG1-Fc_F405L without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFLLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
32	IgG1-Fc_K409R without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
33	IgG1-Fc_FEA without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISR

		TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
34	IgG1-FEAR-Fc without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
35	IgG1-FEAL-Fc without C-terminal Lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFLLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG

The present invention is further illustrated by the following examples, which should not be construed as limiting the scope of the invention.

EXAMPLES

Example 1: Generation of CD137 antibody

The antibodies CD137-005 and CD137-009 were generated as described in example 1 of WO2016/110584. In short, rabbits were immunized with a mixture of proteins containing a human CD137-Fc fusion protein. Single B cells from blood were sorted and screened for production of CD137 specific antibody by ELISA and flow cytometry. From screening-positive B cells, RNA was extracted and sequencing was performed. The variable regions of heavy and light chain were gene synthesized and cloned into a human IgG1 kappa expression vector or human IgG1 lambda

expression vector including a human IgG1 heavy chain containing the following amino acid mutations: L234F, L235E, D265A and F405L (FEAL) or F405L (FEAL) wherein the amino acid position number is according to EU numbering (correspond to SEQ ID NO: 20). The variable region sequences of the chimeric CD137 antibody (CD137-009) are shown in the Sequence Listing SEQ ID NO: 28 and SEQ ID NO: 29 herein.

Example 2: Humanization of the rabbit (chimeric) CD137 antibody

Humanized antibody sequences from the rabbit anti-CD137-009 were generated at Antitope (Cambridge, UK). Humanized antibody sequences were generated using germline humanization (CDR-grafting) technology. Humanized V region genes were designed based on human germline sequences with closest homology to the VH and Vκ amino acid sequences of the rabbit antibody. A series of seven VH and three Vκ (VL) germline humanized V-region genes were designed. Structural models of the non-human parental antibody V regions were produced using Swiss PDB and analyzed in order to identify amino acids in the V region frameworks that may be important for the binding properties of the antibody. These amino acids were noted for incorporation into one or more variant CDR-grafted antibodies. The germline sequences used as the basis for the humanized designs are shown in Table 8.

Table 8: Closest matching human germline V segment and J segment sequences.

Antibody	**Heavy chain**		**Light chain (κ)**	
	Human V region germline segment	**Human J region germline segment**	**Human V region germline segment**	**Human J region germline segment**
Rabbit anti-CD137-009	hIGHV3-49*04	hIGHJ4	hIGKV1-33*01	IGKJ4

Variant sequences with the lowest incidence of potential T cell epitopes were then selected using Antitope's proprietary in silico technologies, iTope™ and TCED™ (T Cell Epitope Database) (Perry, L.C.A, Jones, T.D. and Baker, M.P. New Approaches to Prediction of Immune Responses to Therapeutic Proteins during Preclinical Development (2008). Drugs in R&D 9 (6): 385-396; 20 Bryson, C.J., Jones, T.D. and Baker, M.P. Prediction of Immunogenicity of Therapeutic Proteins (2010). Biodrugs 24 (1):1-8). Finally, the nucleotide sequences of the designed variants have been codon-optimized.

The variable region sequences of the humanized CD137 antibody (CD137-009-HC7LC2) are shown in the Sequence Listing SEQ ID NO: 1 and SEQ ID NO: 5 herein.

Example 3: Generation of PD-L1 antibody

Immunization and hybridoma generation were performed at Aldevron GmbH (Freiburg, Germany). A cDNA encoding amino acid 19-238 of human PD-L1 was cloned into Aldevron proprietary expression plasmids. Antibody PD-L1-547 was generated by immunization of OmniRat animals (transgenic rats expressing a diversified repertoire of antibodies with fully human idiotypes; Ligand Pharmaceuticals Inc., San Diego, USA) using intradermal application of human PD-L1 cDNA-coated gold-particles using a hand-held device for particle-bombardment ("gene gun"). Serum samples were collected after a series of immunizations and tested in flow cytometry on HEK cells transiently transfected with the aforementioned expression plasmids to express human PD-L1. Antibody-producing cells were isolated and fused with mouse myeloma cells (Ag8) according to standard procedures. RNA from hybridomas producing PD-L1 specific antibody was extracted and sequencing was performed. The variable regions of heavy and light chain (SEQ ID NOs: 8 and 12) were gene synthesized and cloned into a human IgG1 lambda expression vector including a human IgG1 heavy chain containing the following amino acid mutations: L234F, L235E, D265A and K409R (FEAR) wherein the amino acid position number is according to EU numbering (correspond to SEQ ID NO: 19).

Example 4: Generation of bispecific antibodies by 2-MEA-induced Fab-arm exchange

Bispecific IgG1 antibodies were generated by Fab-arm-exchange under controlled reducing conditions. The basis for this method is the use of complementary CH3 domains, which promote the formation of heterodimers under specific assay conditions as described in WO2011/131746. The F405L and K409R (EU numbering) mutations were introduced into the relevant antibodies to create antibody pairs with complementary CH3 domains.

To generate bispecific antibodies, the two parental complementary antibodies, each antibody at a final concentration of 0.5 mg/mL, were incubated with 75 mM 2-mercaptoethylamine-HCl (2-MEA) in a total volume of 100 µL PBS at 31°C for 5 hours. The reduction reaction was stopped by removing the reducing agent 2-MEA using spin columns (Microcon centrifugal filters, 30k, Millipore) according to the manufacturer's protocol.

Bispecific antibodies were generated by combining the following antibodies from Example 1 and 4:

- CD137-009-FEAL antibody combined with the PD-L1-547-FEAR antibody

- PD-L1-547-FEAL antibody combined with the CD137-009-FEAR antibody
- GEN1046 (PD-L1-547-FEAL antibody combined with CD137-009-HC7LC2-FEAR antibody),
- b12-FEAL antibody combined with the PD-L1-547-FEAR antibody, with CD137-009-FEAR or with CD137-009-HC7LC2-FEAR antibody, using as the first arm the antibody b12 which is a gp120 specific antibody (Barbas, CF. J Mol Biol. 1993 Apr 5;230(3):812-23)
- PD-L1-547-FEAL or CD137-009-FEAL with b12-FEAR antibody.

Example 5: Simultaneous binding of GEN1046 to PD-L1 and CD137-expressing cells

To measure the dose-response of simultaneous binding of GEN1046 to human PD-L1- and CD137-expressing cells, transgenic K562 cells were differently labelled with fluorescent dyes and the formation of doublets analyzed by flow cytometry.

K562 cells transgenic for human PD-L1 (K562_hPD-L1; 6 x 10^6 cells) were fluorescently labelled with the CellTrace™ Violet Cell Proliferation Kit (Cat. no. C34557, Thermo Fisher Scientific GmbH, Dreieich, Germany) in 2 mL of a 2.5 µM staining solution for 10 minutes at 37°C. In parallel, K562 cells transgenic for human CD137 (K562_h4-1BB; 6 x 10^6 cells) were fluorescently labelled with the CellTrace™ Far Red Cell Proliferation Kit (Cat. no. C34564, Thermo Fisher Scientific GmbH, Dreieich, Germany) in 2 mL of a 0.5 µM staining solution for 10 minutes at 37°C. Staining was stopped by adding 4 mL fetale bovine serum (FBS; Cat. no. S0115, Biochrom GmbH, Berlin, Germany). After washing once in RPMI1640 (Cat. no. 11875093, Thermo Fisher Scientific GmbH, Dreieich, Germany) supplemented with 10% FBS, stained K562_hPD-L1 and K562_h4-1BB cells were combined at a ratio of 1:1 and adjusted to 1.25 x 10^6 cells/mL in RPMI1640, 10% FBS. Combined K562_hPD-L1 and K562_h4-1BB cells were transferred into polystyrene 5 mL round-bottom tubes (Cat No. 10579511, Fisher Scientific, Schwerte, Germany) (1 x 10^6 cells/tube). Cells were incubated with serial dilutions of antibodies (range 0.001 to 100 µg/mL in 10-fold dilution steps) in RPMI1640, 10% FBS at 37°C for 15 minutes. Samples were immediately analyzed on a FACS Canto™ II flow cytometer (Becton Dickinson GmbH, Heidelberg, Germany) without prior mixing in order to preserve formed doublets. K562_hPD-L1/K562_h4-1BB doublets were identified as CellTrace™ Violet / CellTrace™ Far Red double-positive population by FlowJo 10.4 software. The percent double-positive cells was plotted as a function of antibody concentration using GraphPad Prism version 8.01 (GraphPad Software, Inc).

Figure 1A shows that the addition of GEN1046 induced the formation of CellTrace™ Violet / CellTrace™ Far Red double-positive doublets. K562_hPD-L1/K562_h4-1BB co-cultures incubated with an intermediate GEN1046 concentration of 0.1 µg/mL displayed the most prominent doublet formation, whereas only moderate doublet formation was observed at a low GEN1046 concentration of 0.001 µg/mL and a minimal to absent doublet formation was detectable at a high

GEN1046 concentration of 100 µg/mL. This observation is in-line with the bell-shaped dose response curve displayed in Figure 1B covering the tested antibody concentration range of 0.001 µg/mL to 100 µg/mL. In contrast to GEN1046, the combination of monovalent PD-L1 and CD137 control antibodies, PD-L1-547-FEALxb12-FEAR and b12-FEALxCD137-009-HC7LC2-FEAR, resulted in no doublet formation at all antibody concentrations tested.

Example 6: Effect of GEN1046 in CD137 reporter assay

A schematic representation of the anticipated mode of action of PD-L1xCD137 bispecific antibodies is shown in Figure 2.

To determine the dose-response of GEN1046 to mediate PD-L1-binding dependent CD137 agonist activity, a luciferase based CD137 activation reporter assay was performed with adherent growing human tumor cell lines as PD-L1 source.

Endogenously PD-L1-expressing human ES-2 (ovarian clear cell carcinoma; ATCC® CRL-1978™) and MDA-MB-231 (breast adenocarcinoma; ATCC® HTB-26™) cells were seeded in white flat-bottom 96-well plates (Cat. No. 136101, Thermo Fisher Scientific GmbH, Dreieich, Germany) at a density of 3 x 10^4 cells/well in DMEM (Cat. No. 10566016, Thermo Fisher Scientific GmbH, Dreieich, Germany) and incubated overnight at 37°C. Cryo-conserved Thaw-and-use GloResponse™ NFkB-Luc2P/4-1BB Jurkat reporter cells (Cat. No. CS196003, Promega GmbH, Walldorf, Germany) were thawed the next day and the contents of a single vial transferred to a 15 mL tube containing 9.5 mL prewarmed RPMI-1640 supplemented with 1% FBS. Culture medium of the adherent ES-2 and MDA-MB-231 cells was discarded and the co-culture initiated by seeding 50 µL NFkB-Luc2P/4-1BB Jurkat cell suspension on top of the ES-2 or MDA-MB-231 cell monolayer. Cells were incubated with serial dilutions of antibodies (in-assay concentration range 0.00128 to 100 µg/mL in 5-fold dilution steps) in RPMI 1640, 10% FBS at 37°C for 6 hours. Next, the assay plate was removed from the incubator and equilibrated to room-temperature (RT) for 10 minutes. Bio-Glo™ luciferase reagent (Cat. No. G7941, Promega GmbH, Walldorf, Germany) was reconstituted and prewarmed to RT. 75 µL of the luciferase reagent was added per well and incubated for 10 minutes at RT in the dark. The induced luminescence was measured using an Infinite F200 Pro plate reader (Tecan Deutschland GmbH, Crailsheim, Germany).

Upon addition of GEN1046 to ES-2:Jurkat (Figure 3A) and MDA-MB-231:Jurkat reporter cell co-cultures (Figure 3B), luciferase expression as a read-out for CD137 agonist activation was effectively induced in a concentration-dependent manner following a bell-shaped dose response curve. Whereas an intermediate dose level of around 0.1 µg/mL GEN1046 resulted in the most prominent

luminescence signals, lower dose levels as well as higher dose levels were less effective in induction of luciferase expression. Importantly, at very low (0.00128 µg/mL GEN1046) and very high GEN1046 concentrations (100 µg/mL GEN1046), no luciferase expression was detectable. For both co-cultures analyzed, incubation with the b12-FEAL control antibody led to no luciferase expression.

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Example 7: Polyclonal T-cell proliferation assay to measure effects of bispecific antibodies binding to PD-L1 and CD137

To measure induction of T-cell proliferation in polyclonally activated T cells, PBMCs were incubated with a sub-optimal concentration of anti-CD3 antibody (clone UCHT1), to activate T cells, combined
10 with bispecific antibody GEN1046 or control antibodies. Within the PBMC population, cells expressing PD-L1 can be bound by the PD-L1-specific arm of the bispecific antibody, whereas activated T cells in the population can be bound by the CD137-specific arm. In this assay, trans-activation of the T cells via the CD137-specific arm, induced by cross-linking with the PD-L1-expressing cells via the bispecific antibody and by blockade of PD-L1:PD-1 interaction, is measured as
15 T-cell proliferation.

PBMCs were obtained from the buffy coat of a healthy donor (Sanquin, Amsterdam, The Netherlands) using a Ficoll gradient (Lonza, lymphocyte separation medium, cat. no. 17-829E). PBMCs were labeled using 0.5 µM carboxyfluorescein succinimidyl ester (CFSE) (Life technologies, cat. no. C34554) in PBS, according to the manufacturer's instructions. 75,000 CFSE-labeled PBMCs
20 were seeded per well in a 96-well round-bottom plate (Greiner bio-one, cat. no. 650180) and incubated with a sub-optimal concentration of anti-CD3 antibody (Stemcell, clone UCHT1, cat. no. 60011; 0.03 µg/mL final concentration) that was pre-determined to induce sub-optimal T cell proliferation, and bispecific or control antibodies (0.0032 – 10 µg/mL), in 200 µL IMDM GlutaMAX supplemented with 5% human AB serum and 1% penecilin/streptomycin, at 37°C, 5% CO_2, for four
25 days.

Proliferation of different T-cell subsets was analyzed by flow cytometry. Cells were washed in PBS and stained to exclude dead cells with Fixable Viability Stain 510 (50 µL/well; BD Biosciences, cat. no. 564406) at 4°C for 20 min. After another wash in FACS buffer, cells were stained to distinguish various cellular subsets with a PE-CF594-conjugated CD56-specific antibody (BD BioSciences, cat. no.
30 564849), a Pacific Blue-conjugated CD4-specific antibody (BioLegend, cat. no. 300521), a AF700-conjugated CD8-specific antibody (BioLegend, cat. no. 301028), a BV711-conjugated CD197-specific antibody (CCR7; BioLegend, cat. no. 353228), a PE-Cy7-conjugated CD45RO-specific antibody (BioLegend, cat. no. 304230), a APC-conjugated CD274-specific antibody (PD-L1; BioLegend cat. no.

329708) and a BV605-conjugated CD137-specific antibody (BioLegend, cat. no. 309822) in FACS buffer at 4°C for 30 min. Cells were washed three times in FACS buffer and subsequently measured on a FACS Fortessa (BD Biosciences) in 80 µL FACS buffer. CFSE dilution was measured in total T cells and in different T cell subsets (e.g. $CCR7^+CD45RO^+$ central memory T cells and $CCR7^-CD45RO^+$ effector memory T cells). Detailed analyses of T-cell proliferation based on CFSE-peaks indicating cell divisions were made by FlowJo 10.4 software and exported expansion index values were used to plot dose-response curves in GraphPad Prism version 6.04 (GraphPad Software, Inc). The expansion index determines the fold-expansion of the overall culture; an expansion index of 2.0 represents a doubling of the cell count, whereas an expansion index of 1.0 represents no change of the overall cell count.

Figure 4A shows that the bispecific antibody GEN1046 induced expansion of T cells, which was increased compared to CD3 pre-stimulation alone, isotype control antibody b12-FEAL and a monovalent PD-L1-control antibody, PD-L1-547-FEALxb12-FEAR, having one irrelevant arm and one corresponding to the parental bivalent antibody PD-L1-547-FEAR. GEN1046-induced T-cell proliferation was most optimal at 0.4 µg/mL, while at lower and higher concentrations the GEN1046-induced T-cell expansion was less pronounced. When $CCR7^+CD45RO^+$ central memory T cells and $CCR7^-CD45RO^+$ effector memory T cells were analyzed separately (Figure 4B), a similar pattern emerged, where GEN1046 enhanced T-cell proliferation, which was optimal at 0.4 µg/mL.

Example 8: Antigen-specific $CD8^+$ T cell proliferation assay to measure effects by bispecific antibodies binding to PD-L1 and CD137

To measure induction of T cell proliferation by the bispecific antibody targeting PD-L1 and CD137 in an antigen-specific assay, dendritic cells (DCs) were transfected with claudin-6 in vitro-transcribed RNA (IVT-RNA) to express the claudin-6 antigen. T cells were transfected with PD-1 IVT-RNA and with the claudin-6-specific, HLA-A2-restricted T cell receptor (TCR). This TCR can recognize the claudin-6-derived epitope presented in HLA-A2 on the DC. The PD-L1xCD137 bispecific antibody GEN1046 can cross-link PD-L1 endogenously expressed on monocyte-derived dendritic cells or on tumor cells and CD137 on the T cells, leading to inhibition of the inhibitory PD-1/PD-L1 interaction and at the same time clustering of CD137, resulting in T cell proliferation. Clustering of the CD137 receptor expressed on T cells leads to activation of the CD137 receptor which thereby delivers a co-stimulatory signal to the T cell.

$HLA\text{-}A2^+$ peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors (Transfusionszentrale, University Hospital, Mainz, Germany). Monocytes were isolated from PBMCs

by magnetic-activated cell sorting (MACS) technology using anti-CD14 MicroBeads (Miltenyi; cat. no. 130-050-201), according to the manufacturer's instructions. The peripheral blood lymphocytes (PBLs, CD14-negative fraction) were frozen for future T-cell isolation. For differentiation into immature DCs (iDCs), $1x10^6$ monocytes/ml were cultured for five days in RPMI GlutaMAX (Life technologies GmbH, cat. no. 61870-044) containing 5% human AB serum (Sigma-Aldrich Chemie GmbH, cat. no. H4522-100ML), sodium pyruvate (Life technologies GmbH, cat. no. 11360-039), non-essential amino acids (Life technologies GmbH, cat. no. 11140-035), 100 IU/mL penicillin-streptomycin (Life technologies GmbH, cat. no.15140-122), 1000 IU/mL granulocyte-macrophage colony-stimulating factor (GM-CSF; Miltenyi, cat. no. 130-093-868) and 1,000 IU/mL interleukin-4 (IL-4; Miltenyi, cat. no. 130-093-924). Once during these five days, half of the medium was replaced with fresh medium. iDCs were harvested by collecting non-adherent cells and adherent cells were detached by incubation with PBS containing 2mM EDTA for 10 min at 37°. After washing, iDCs were frozen in RPMI GlutaMAX containing 10 % v/v DMSO (AppliChem GmbH, cat. no A3672,0050) + 50% v/v human AB serum for future antigen-specific T cell assays.

One day prior to the start of an antigen-specific $CD8^+$ T cell proliferation assay, frozen PBLs and iDCs from the same donor were thawed. $CD8^+$ T cells were isolated from PBLs by MACS technology using anti-CD8 MicroBeads (Miltenyi, cat. no. 130-045-201), according to the manufacturer's instructions. About 10-15 x 10^6 $CD8^+$ T cells were electroporated with 10 µg of in vitro translated (IVT)-RNA encoding the alpha-chain plus 10 µg of IVT-RNA encoding the beta-chain of a claudin-6-specific murine TCR (HLA-A2-restricted; described in WO 2015150327 A1) plus 0.4 – 10 µg IVT-RNA encoding PD-1 in 250 µL X-Vivo15 (Biozym Scientific GmbH, cat. no.881026) in a 4-mm electroporation cuvette (VWR International GmbH, cat. no. 732-0023) using the BTX ECM® 830 Electroporation System device (BTX; 500 V, 1 x 3 ms pulse). Immediately after electroporation, cells were transferred into fresh IMDM medium (Life Technologies GmbH, cat. no. 12440-061) supplemented with 5% human AB serum and rested at 37°C, 5% CO_2 for at least 1 hour. T cells were labeled using 1.6 µM carboxyfluorescein succinimidyl ester (CFSE; Invitrogen, cat. no. C34564) in PBS according to the manufacturer's instructions, and incubated in IMDM medium supplemented with 5% human AB serum, O/N.

Up to 5 x 10^6 thawed iDCs were electroporated with 0.3 - 1 µg IVT-RNA encoding full length claudin-6, in 250 µL X-Vivo15 medium, using the electroporation system as described above (300 V, 1x12 ms pulse) and incubated in IMDM medium supplemented with 5% human AB serum, O/N.

The next day, cells were harvested. Cell surface expression of claudin-6 and PD-L1 on DCs and TCR and PD-1 on T cells was checked by flow cytometry. DCs were stained with an Alexa647-conjugated CLDN6-specific antibody (non-commercially available; in-house production) and with anti-human

CD274 antibody (PD-L1, eBioscienes, cat. no.12-5983) and T cells were stained with an anti-Mouse TCR ß Chain antibody (Becton Dickinson GmbH, cat. no. 553174) and with anti-human CD279 antibody (PD-1, eBioscienes, cat. no. 17-2799). 5,000 electroporated DCs were incubated with 50,000 electroporated, CFSE-labeled T cells in the presence of bispecific or control antibodies in IMDM GlutaMAX supplemented with 5% human AB serum in a 96-well round-bottom plate. T cell proliferation was measured after 5 days by flow cytometry. Detailed analyses of T-cell proliferation based on CFSE-peaks indicating cell divisions were made by FlowJo 10.4 software and exported expansion index values were used to plot dose-response curves in GraphPad Prism version 6.04 (GraphPad Software, Inc). The expansion index determines the fold-expansion of the overall culture; an expansion index of 2.0 represents a doubling of the cell count, whereas an expansion index of 1.0 represents no change of the overall cell count.

Figure 5 shows that GEN1046 dose-dependently enhanced T-cell proliferation compared to isotype control antibody b12-FEAL, reflected by an increase in expansion index at concentrations of ≥0.004 µg/mL. GEN1046-induced T-cell proliferation was most optimal at 0.03-0.11 µg/mL, and slightly decreased at the highest concentrations tested, indicative of a bell-shaped dose response curve.

Example 9: Antigen-specific $CD8^+$ T-cell proliferation assay to measure cytokine release induced by bispecific antibodies binding to PD-L1 and CD137

The induction of cytokine release by bispecific antibody GEN1046 targeting PD-L1 and CD137 was measured in an antigen-specific assay, performed essentially as described in Example 8.

T cells were electroporated with 10 µg TCR α chain- and 10 µg β chain-encoding RNA, with or without 2 µg PD-1-encoding IVT RNA. Electroporated T cells were not CFSE-labeled (as described supra), but transferred into fresh IMDM medium (Life Technologies GmbH, cat. no. 12440-061) supplemented with 5% human AB serum, immediately after electroporation. iDCs were electroporated with 5 µg claudin-6 (CLDN6)-encoding RNA, as described supra. After O/N incubation, DCs were stained with Alexa647-conjugated CLDN6-specific antibody and T cells with anti-mouse TCR β chain antibody and with anti-human CD279 antibody, as described supra.

5,000 electroporated DCs were incubated with 50,000 electroporated T cells in the presence of different concentrations of bispecific antibody GEN1046 or control antibody b12-FEAL in IMDM GlutaMAX supplemented with 5% human AB serum in a 96-well round-bottom plate. Following a 48-hour incubation period, the plates were centrifuged at 500 x g for 5 min and the supernatant was carefully transferred from each well to a fresh 96-well round bottom plate and stored at -80°C until cytokine analysis on the MSD® platform. The collected supernatants from the antigen-specific

proliferation assay were analyzed for cytokine levels of 10 different cytokines by an MSD V-Plex Human Proinflammatory panel 1 (10-Plex) kit (Meso Scale Diagnostics, LLC., cat. no. K15049D-2) on a MESO QuickPlex SQ 120 instrument (Meso Scale Diagnostics, LLC., cat. no. R31QQ-3), according to the manufacturer's instructions.

The addition of GEN1046 led to a dose-dependent increase in secretion of primarily IFN-γ, TNF-α, IL-13 and IL-8 (Figure 6), which was most optimal at concentrations of 0.04-0.33 µg/mL. Lower dose levels as well as a higher dose level of 1 µg/mL were less effective in inducing these cytokines, indicative of a bell-shaped dose response curve. When comparing T cell:DC co-cultures where T cells were not electroporated with PD-1 RNA to those where T cells were electroporated with 2 µg PD-1 RNA, slightly higher cytokine levels were detectable for co-cultures without PD-1 RNA electroporation. This was observed for both the GEN1046 dose response curve as well as for the b12-FEAL control antibody values.

Example 10: *Ex vivo* TIL expansion assay to evaluate the effects of the CD137xPD-L1 bispecific antibody on tumor infiltrating lymphocytes.

To evaluate the effects of CD137-009-FEALxPD-L1-547-FEAR on tumor infiltrating lymphocytes (TIL), an *ex vivo* culture of human tumor tissue was performed as follows. Fresh human tumor tissue resection specimens were washed three times by transferring the isolated tumor chunks from one well in a 6-well plate (Fisher Scientific cat. no. 10110151) containing wash medium to the next using a spatula or serological pipette. Wash medium was composed of X-VIVO 15 (Biozym, cat. no. 881024) supplemented with 1% Pen/Strep (Thermo Fisher, cat. no. 15140-122) and 1% Fungizone (Thermo Fisher, cat. no. 15290-026). Next, the tumor was dissected with a surgical knife (Braun/Roth, cat. no. 5518091 BA223) and cut into pieces with a diameter of about 1-2 mm. Two pieces each were put into one well of a 24-well plate (VWR international, cat. no. 701605) containing 1 mL TIL medium (X-VIVO 15, 10% Human Serum Albumin (HSA, CSL Behring, cat. no. PZN-6446518) 1% Pen/Strep, 1% Fungizone and supplemented with 10 U/mL IL-2 (Proleukin®S, Novartis Pharma, cat. no. 02238131)). CD137-009-FEALxPD-L1-547-FEAR was added at the indicated final concentrations. Culture plates were incubated at 37°C and 5% CO_2. After 72 hours, 1 mL of fresh TIL medium containing the indicated concentration of the bispecific antibody was added to each well. Wells were monitored via a microscope for the occurrence of TIL clusters every other day. Wells were transferred individually when more than 25 TIL microclusters were detected in the respective well. To split TIL cultures, the cells in the wells of a 24-well plate were re-suspended in the 2 mL medium and transferred into a well of a 6-well plate. Each well was in addition supplemented with another 2 mL of TIL medium.

After a total culture period of 10-14 days, TILs were harvested and analyzed by flow cytometry. Cells were stained with the following reagents, all diluted 1:50 in staining-buffer, (D-PBS containing 5% FCS and 5 mM EDTA), anti-human CD4-FITC (Miltenyi Biotec, cat. no. 130-080-501), anti-human CD3-PE-Cy7 (BD Pharmingen, cat. no. 563423), 7-aminoactinomycin D (7-AAD, Beckman Coulter, cat. no. A07704), anti-human CD56-APC (eBioscience, cat. no. 17-0567-42), and anti-human CD8-PE (TONBO, cat. 50-0088). To allow for quantitative comparison of the acquired cells between different treatment groups, cell pellets were re-suspended after the last washing step in FACS-buffer supplemented with BD™ CompBeads (BD biosciences, cat. no. 51-90-9001291). Flow cytometric analysis was performed on a BD FACSCanto™ II flow cytometer (Becton Dickinson) and acquired data was analyzed using FlowJo 7.6.5 software. The relative viable TIL count, $CD3^+CD8^+$ T cell count, $CD3^+CD4^+$ T cell count and $CD3^-CD56^+$ NK cell count per 1,000 beads correlating to the corresponding well in a 6-well plate was calculated by normalization of the acquired 7AAD-negative cell fraction to the acquired bead counts.

Figure 7 shows the analysis of a TIL expansion from a human non-small-cell lung carcinoma tissue specimen. Here, the following concentrations of CD137-009-FEALxPD-L1-547-FEAR were added: 0.01, 0.1 and 1 µg/mL; a tissue specimen from the same patient without antibody addition served as negative control. After 10 days of culture, the TILs were harvested and analyzed by flow cytometry. Five samples (from 5 original wells) for each antibody concentration derived from different wells of the 24-well plate were measured. In all samples cultured with the bispecific antibody the viable count of TILs was increased in comparison to the without antibody control samples. Overall, a significant (up to 10-fold) expansion of viable TILs was observed, when 0.1 µg/mL CD137-009-FEALxPD-L1-547-FEAR was added to cultures (Figure 7A). When analyzed separately, a strong effect on $CD3^+CD8^+$ T cell expansion was observed, which was significant at 0.1 µg/mL CD137-009-FEALxPD-L1-547-FEAR (Figure 7B; 7.4-fold expansion over control). $CD3^+CD4^+$ T cells were only slightly expanded, and their expansion was not significant compared to cultures without antibody (Figure 7C). The most prominent TIL expansion was seen for $CD3^-CD56^+$ NK cells (Figure 7D; up to 64-fold expansion over control), which was significant at 0.1 µg/mL CD137-009-FEALxPD-L1-547-FEAR.

Example 11: Pharmacodynamic evaluation of GEN1046 in peripheral blood in patients with advanced solid tumors.

To investigate the biological activity of GEN1046 at various dose levels in patients with advanced tumors, blood and serum samples were collected at baseline and at multiple timepoints on treatment. Based on the mechanism of action of GEN1046, it was anticipated that dose levels with

biological activity will modulate circulating levels of interferon-γ (IFN-γ) and interferon-gamma–inducible protein 10 (IP-10) and induce proliferation of peripheral CD8 T cells .

To determine serum levels of (IFN-γ) and IP-10, serum samples were collected from patients at baseline and at multiple timepoints post administration of GEN1046 in cycle 1 and cycle 2 (days 1 [2h and between 4-6h post-administration], 2, 3, 8, and 15). Serum levels of IFN-γ and IP-10 were measured by a Meso Scale Discovery (MSD) multiplex immune-assay (cat. no. K15209G) following the manufacturer's instructions.

To measure peripheral modulation of immune cells subsets, immunophenotyping of peripheral blood was conducted in whole blood collected in EDTA tubes at baseline and at multiple timepoints post GEN1046 administration in cycle 1 and cycle 2 (days 2, 3, 8 and 15). 100 µL of whole blood was added to fluorochrome-conjugated monoclonal antibodies that bind specifically to cell surface antigens: CD45RA-FITC (clone LEU-18, BD Biosciences cat. no. 335039), CCR7-BV510 (clone 3D12, BD Biosciences, cat. no. 563449), CD8-PerCP-Cy5.5 (clone RPA- T8, BD Biosciences, cat. no. 560662). After incubation on ice, the stained samples were treated with FACS Lysing Solution (BD Biosciences, Catalog No 349202) to lyse erythrocytes. Excess antibody and cell debris were removed by washing with Stain Buffer (BD Biosciences, cat. no. 554656). Following lyse/wash, cells were fixed and permeabilized by incubation with Permeabilizing Solution 2 buffer (BD Biosciences, cat. no. 340973). Next, cells were washed and resuspended in Stain Buffer and incubated on ice with antibody to Ki67 (BV421 B56, BD Biosciences, cat. no. 562899) to detect proliferating cells. After incubation, excess antibody was removed by washing with Stain Buffer. Cells were resuspended in Stain Buffer and acquired on a BD FACSCanto™ II flow cytometer (Becton Dickinson) within 1 hour of staining.

Administration of GEN1046 to cancer patients resulted in modulation of circulating levels of IFN-γ, and IP-10 and proliferating effector memory CD8 T cells (Table 9 and Figure 13). In the preliminary data set shown in Table 9, levels of IFN-γ increased more than 2-fold in the first treatment cycle across all dose levels tested. Maximal increases were detected at the 50 mg and 80 mg dose levels, and most of the patients in the 80 mg cohort (75%) had fold-increase >2 (Table 9). GEN1046 also elicited proliferation of effector memory CD8+ T cells as measured by an increase in the frequency of $Ki67^+$ $CD8^+$ $CD45RA^-CCR7^-$ T cells. Comparable to the changes observed with modulation of circulating levels of IFNγ, maximal and more consistent modulation of proliferating $CD8^+$ effector memory T cells was observed in patients in the 80 mg cohort. Particularly in the 400 mg cohort the magnitude of the changes in both the circulating levels of IFN-γ and proliferating effector memory CD8 T cells were lower compared to the 25-200 mg cohorts. These results showed that GEN1046 elicited an immune response characterized by modulation of immune effector cells and soluble

factors critical for the generation of antitumor immune responses, with responses of greater magnitude at the 80 mg dose level.

In the data set shown in Figure 13, an increase in IFN-γ and IP-10 was observed in the first treatment cycle at dose levels ≤ 200 mg (Figure 13A-B) Although also an increase in IFN-γ and IP-10 was observed at dose levels ≥400 mg, the maximal fold change from baseline during the first treatment cycle was significantly lower compared to the lower dose levels. GEN1046 also elicited proliferation of total $CD8^+$ T cells and effector memory CD8+ T cells as measured by an increase in the frequency of $Ki67^+$ $CD8^+$ T cells and $Ki67^+$ $CD8^+$ $CD45RA^-CCR7^-$ T cells (Figure 13C-D). Comparable to the changes observed with modulation of circulating levels of IFNγ and IP-10, maximal and more consistent modulation of proliferating $CD8^+$ effector memory T cells was observed in patients treated with dose levels ≤ 200 mg, In the ≥400 mg cohorts the magnitude of the changes in proliferating effector memory CD8 T cells and total CD8 T cells were significantly lower compared to the 25-200 mg cohorts. These results showed that GEN1046 elicited an immune response characterized by modulation of immune effector cells and soluble factors critical for the generation of antitumor immune responses, with responses of greater magnitude at the ≤200 mg dose levels.

Table 9. GEN1046 Modulation of Peripheral Pharmacodynamic Endpoints in cancer patients: Peak Fold-change from Baseline during Cycle 1 by Dose Level [a]

	GEN1046 25 mg	**GEN1046 50 mg**	**GEN1046 80 mg**	**GEN1046 200 mg**	**GEN1046 400 mg**
Interferon-γ[b]					
n	4	4	8	8	6
Min	1.17	1.06	1.45	1.47	1.18
Q1	2.05	1.89	2.82	2.35	1.32
Median	3.90	4.63	4.49	3.48	2.56
Q3	9.99	6.90	5.94	4.89	3.37
Max	15.11	7.27	12.17	5.20	102.08
Proliferating Effector Memory CD8 T cells[c]					
n	3	2	8	8	7
Min	2.00	2.00	1.00	0.67	1.00
Q1	2.00	2.00	2.00	1.40	1.06
Median	2.00	2.50	3.42	2.83	1.50
Q3	3.50	3.00	9.75	5.25	2.00
Max	5.00	3.00	31.40	6.67	7.00

Preliminary data as of 27-Jan-2020.

n: number of patients per dose cohort; Min: lowest measured value; Q1: 25th percentile; Q3: 75th percentile; Max: maximum measured value.

[a] Pharmacodynamic assessments, including changes in circulating levels of interferon-gamma and effector memory T cells, were conducted using blood samples from patients with advanced solid tumors enrolled in the dose escalation phase of an open-label, multi-center safety trial of GEN1046 (NCT03917381).

[b] Circulating levels of interferon-gamma were measured in serum samples at baseline, and at multiple timepoints post administration of GEN1046 in cycle 1 and cycle 2 (days 1 [2h and between 4-6h post-administration], 2, 3, 8, and 15). Interferon-gamma levels in serum samples were determined by Meso Scale Discovery (MSD) multiplex immune assay.

[c] Immunophenotyping of peripheral blood was conducted in whole blood collected at baseline and at multiple timepoints post administration of GEN1046 in cycle 1 and cycle 2 (days 2, 3, 8 and 15). The frequency of proliferating ($Ki67^+$) effector memory CD8 T cells ($CD8^+CD45RA^-CCR7^-$ T cells) were assessed in whole blood samples by flow cytometry.

Example 12: Preliminary data from clinical trial

Trial design:

Clinical trial on GCT1046-01 (ClinicalTrials.gov Identifier: NCT03917381) was designed as a two-part trial, including an ongoing dose escalation part and a planned expansion part.

The trial was designed as an open-label, multi-center, Phase I/IIa safety trial of GEN1046 (DuoBody®-PD-L1x4-1BB). The trial consists of 2 parts; a First-in-Human (FIH) dose escalation (Phase I) and an expansion (Phase IIa). Figure 8 shows a schematic representation of the clinical trial design.

Dose escalation

The dose escalation was designed to evaluate GEN1046 in subjects with solid malignant tumors to determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) and/or the recommended phase 2 dose (RP2D).

For dose escalation, subject was required to be a man or woman ≥ 18 years of age and was required to have measurable disease according to RECIST 1.1.

Subjects was required to have a histologically or cytologically confirmed non-CNS solid tumor that was metastatic or unresectable and for whom there was no available standard therapy likely to confer clinical benefit, or subjects who are not candidates for such available therapy, and for whom, in the opinion of the investigator, experimental therapy with GEN1046 could be beneficial.

In the dose escalation, subjects received one infusion of GEN1046 every third week (1Q3W) until protocol defined treatment discontinuation criteria are met; e.g. Radiographic disease progression

or clinical progression. GEN1046 was be administered using i.v. infusion over a minimum of 60 minutes on Day 1 of each 3-week treatment cycle (21 days). The concept of the design of the trial is shown in Figure 8.

The 1Q3W dose escalation was designed to potentially (dependent on data collected during the trial) evaluate GEN1046 at 7 main dose levels: 25, 80, 200, 400, 800, 1200 and 1600 mg fixed, and 6 optional intermediate dose levels 50, 140, 300, 600, 1000 and 1400 mg fixed.

The recommended phase 2 dose (RP2D) was based on a review of the available safety and dosing information and could be lower than the maximum tolerated dose (MTD).

Expansion

The aim of the expansion is to provide further data on the safety, tolerability, MoA, PK and anti-tumor activity of the selected dose/schedule.

Expansion was designed to initiate recruitment in up to 6 tumor types (7 parallel cohorts), *i.e.*, in NSCLC, EC, UC, TNBC, SCCHN, and cervical cancer. Further expansion cohorts in additional tumor types may be opened based on preliminary efficacy signals generated in the dose escalation. The sponsor will determine the priority of opening the disease-specific expansion cohorts based on the data obtained in the dose escalation.

NSCLC Expansion Cohorts

The NSCLC expansion cohorts should include subjects with squamous histology as well as subjects with non-squamous histology.

Since response rates and other disease related outcomes may differ in a PD-1/PD-L1 naïve population versus a PD-1/L1 pre-treated population, NSCLC patients were separated into different cohorts to ensure sufficient evidence of preliminary efficacy. Cohort 2 aims to explore preliminary efficacy in PD-1/L1 naive patients with NSCLC where SOC with PD-1/L1 inhibitors is restricted or unavailable. If preliminary clinical evidence suggests a substantial improvement over available therapies in a population with high unmet medical need (*e.g.*, PD-L1 low or negative) as determined by the DMC's review of the totality of the data, the Sponsor may request to open Cohort 2 in areas where access to PD-1/L1 inhibitors is not restricted.

UC Expansion Cohort

The UC cohort was designed to include both subjects who are eligible to receive platinum-based chemotherapy and subjects who are not eligible to receive platinum-based chemotherapy.

SCCHN and TNBC Expansion Cohorts

The SCCHN and TNBC cohorts may include both subjects who have received prior treatment with a PD-1/PD-L1 inhibitor and subjects who have not received treatment with a prior PD-1/L1 inhibitor.

Inclusion criteria

Subjects are eligible to be included in the trial only if all of the following criteria apply:

Subject must be a man or woman ≥ 18 years of age Subject and must have measurable disease according to RECIST 1.1.

Subjects must have histologically or cytological confirmed diagnosis of relapsed or refractory, advanced and/or metastatic NSCLC, EC, UC, TNBC, SCCHN, or cervical cancer who are no longer candidates for or refuse standard therapy (if subjects had access and were eligible for the respective treatments), and who have failed anticancer therapy as follows:

Expansion Cohort 1 (NSCLC): PD-1/L1 pre-treated

NSCLC subjects who have received up to 4 prior systemic treatment regimens (adjuvant and maintenance treatment is considered being part of one treatment line) for advanced/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects must have histological or cytological diagnosis of non-squamous NSCLC that does not have an epidermal growth factor (EGFR)-sensitizing mutation and/or anaplastic lymphoma (ALK) translocation / ROS1 rearrangement. EGFR sensitizing mutations are those mutations that are amenable to treatment with an approved tyrosine kinase inhibitor (TKI). Documentation of EGFR and ALK status should be available per local assessment. If documentation of EGFR and ALK status is unavailable, sponsor medical monitor approval is required prior to enrollment.

Subjects should have received platinum-based therapy (or alternative chemotherapy due to platinum ineligibility, *e.g.*, a gemcitabine-containing regimen).

Subjects must have received prior treatment with a PD-1/L1 inhibitor alone or in combination and must have radiographic disease progression on treatment. Sponsor approval is required for subjects with a BOR of SD or PD on a CPI containing regimen with a treatment duration of up to 16 weeks.

Expansion Cohort 2 (NSCLC) - PD-1/L1 naive

NSCLC subjects who have received up to 4 prior systemic treatment regimens (adjuvant and maintenance treatment is considered being part of one treatment line) for advanced/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects must have histological or cytological diagnosis of non-squamous NSCLC that does not have an epidermal growth factor (EGFR)-sensitizing mutation and/or anaplastic lymphoma kinase (ALK)

translocation/ROS1 rearrangement. EGFR sensitizing mutations are those mutations that are amendable to treatment with an approved tyrosine kinase inhibitor (TKI). Documentation of EGFR and ALK status should be available per local assessment. If documentation of EGFR and ALK status is unavailable, sponsor medical monitor approval is required prior to enrollment.

Subjects should have received platinum-based therapy (or alternative chemotherapy due to platinum ineligibility, *e.g.*, a gemcitabine-containing regimen).

Subjects must not have received prior treatment with a PD-1/L1 inhibitor.

Expansion Cohort 3 (UC):

UC (of the bladder, ureter, urethra, or renal pelvis) subjects who have received up to 4 prior systemic treatment regimens (adjuvant and maintenance treatment is considered being part of one treatment line) for locally advanced/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects must have received prior treatment with a PD-1/L1 inhibitor alone or in combination and must have radiographic disease progression on treatment. Sponsor approval is required for subjects with a BOR of SD or PD on a CPI containing regimen with a treatment duration of up to 16 weeks.

Local results from the most recent PD-L1 test should be provided prior to enrollment (if available).

Cohort 3a: For subjects who are eligible to receive platinum-based therapy:

Subjects must have received platinum-based chemotherapy.

Cohort 3b: For subjects ineligible to receive platinum-based therapy:

Subjects must not be eligible for any platinum-based or any cisplatin-containing chemotherapy.

Expansion Cohort 4 (EC):

EC subjects who have received up to 4 prior systemic treatment regimens (adjuvant and maintenance treatment is considered being part of one treatment line) for advanced/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects must have epithelial endometrial histology including: endometrioid, serous, squamous, clear-cell carcinoma, or carcinosarcoma. Sarcomas and mesenchymal EC are excluded.

Subjects must not have received prior treatment with a PD-1/L1 inhibitor (established local label / access need to be respected).

Expansion Cohort 5 (TNBC):

TNBC defined as HER2-negative [HER2 is negative by FISH] assay (non-amplified ratio of HER2 to CEP17 < 2.0 single probe average HER2 gene copy number < 4 signals/cell) or alternatively HER2

protein expression by IHC result is 1+ negative or IHC 0 – negative and ER and PgR negative status (defined as < 1% of cells expressing hormonal receptors via IHC analysis) as per local assessment. Subjects who have received at least one but no more than 4 prior systemic treatment regimens including but not limited to anthracycline-, taxane-, antimetabolite- or microtubule inhibitor-containing regimens (adjuvant and maintenance treatment is considered being part of one treatment line) for locally advanced/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects with a prior history of a breast cancer with a different phenotype must have confirmation of TNBC from a biopsy obtained after the subject's last prior systemic therapy.

Cohort 5a – Subjects who have received prior treatment with a PD-1/L1 inhibitor:
Subjects must have received prior treatment with a PD-1/L1 inhibitor alone or in combination and must have radiographic disease progression on treatment.

Cohort 5b – Subjects who have not received prior treatment with a PD-1/L1 inhibitor:
Subjects must not have received prior treatment with a PD-1/L1 inhibitor.

*Expansion Cohort 6 (SCCHN**):***

Recurrent or metastatic SCCHN (oral cavity, pharynx, larynx) subjects who have received up to 4 prior systemic treatment regimens for recurrent/metastatic disease with radiographic PD on or after last prior treatment (adjuvant and maintenance treatment is considered being part of one treatment line).

Subjects must have disease progression on or after prior therapy with platinum-based chemotherapy (alternative combination chemotherapy is acceptable if the subject's platinum ineligibility status is documented).

Cohort 6a – Subjects who have received prior treatment with a PD-1/L1 inhibitor:
Subjects must have received prior treatment with a PD-1/L1 inhibitor alone or in combination and must have radiographic disease progression on treatment. Sponsor approval is required for subjects with a BOR of SD or PD on a CPI containing regimen with a treatment duration of up to 16 weeks.

Cohort 6b – Subjects who have not received prior treatment with a PD-1/L1 inhibitor:
Subjects must not have received prior treatment with a PD-1/L1 inhibitor.

Expansion Cohort 7 (Cervical Cancer):
Cervical cancer subjects who have received at least one but no more than 4 prior systemic treatment regimens including chemotherapy in combination with bevacizumab (according to the applicable

labeling) unless the subject is ineligible for bevacizumab according to local standards (chemotherapy administered in the adjuvant or neoadjuvant setting, or in combination with radiation therapy should not be counted as a prior line of therapy) for recurrent/metastatic disease with radiographic disease progression on or after last prior treatment.

Subjects must have cervical cancer of squamous cell, adenocarcinoma, or adenosquamous histology.

Subjects must not have received prior treatment with a PD-1/L1 inhibitor (established local label / access need to be respected).

Results

Dose escalation

The following preliminary results were obtained during dose escalation. Table 10 shows Best Overall Response (RECIST v1.1) by Dose Level upon enrolment and dosing of a total of 30 patients (Data Extraction Date: 03-Feb-2020).

Tables 11 and 12 show Objective Response Rate and Confirmed Objective Response Rate, respectively (RECIST v1.1) by Dose Level upon enrolment and dosing of a total of 61 patients (Data cut-off: October 12, 2020).

Best percent change from baseline in tumor size in all patients is shown in Figure 9. Disease control occurred in 40/61 (65.6%) patients in the dose escalation phase. Partial response (PR) was achieved in four patients with triple-negative breast cancer, ovarian cancer, or non-small cell lung cancer (NSCLC); 36 patients maintained stable disease.

Clinical activity observed in patients with NSCLC (best change from baseline in tumor size) is show in in Figure 10 (Data cut-off: October 12, 2020). Of six patients with NSCLC, all of whom had received prior checkpoint immunotherapy, two achieved unconfirmed PR, two maintained stable disease, and two experienced progressive disease.

Table 10: Best Overall Response (RECIST v1.1) by Dose Level.

	25 mg (n=2)	50 mg (n=5)	80 mg (n=8)	140 mg (n=1)	200 mg (n=8)	400 mg (n=6)	Total (n=30)
Complete Response	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Partial Response	0 (0)	0 (0)	2[1] (25)	0 (0)	1[1] (12.5)	0 (0)	3 (10)
Stable Disease	0 (0)	3 (60)	5 (62.5)	0 (0)	5 (62.5)	6 (100)	19 (63.3)
Progressive Disease	2 (100)	2 (40)	1 (12.5)	1 (100)	2 (25)	0 (0)	8 (26.6)
[1] uPR							

Table 11: Objective Response Rate - dose escalation

	Total	25 mg	50 mg	80 mg	100 mg	140 mg	200 mg	400 mg	800 mg	1200 mg
N	61	4	5	9	6	6	9	9	9	4
Best Overall Response										
CR (Complete Response)	0	0	0	0	0	0	0	0	0	0
PR (Partial Response)	4 (6.6%)	0	0	2 (22.2%)	1 (16.7%)	0	1 (11.1%)	0	0	0
SD (Stable Disease)	36 (59.0%)	1 (25.0%)	3 (60.0%)	6 (66.7%)	3 (50.0%)	3 (50.0%)	5 (55.6%)	7 (77.8%)	5 (55.6%)	3 (75.0%)
PD (Progressive Disease)	14 (23.0%)	2 (50.0%)	2 (40.0%)	1 (11.1%)	0	2 (33.3%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	1 (25.0%)
NE (Not Evaluable)	7 (11.5%)	1 (25.0%)	0	0	2 (33.3%)	1 (16.7%)	1 (11.1%)	0	2 (22.2%)	0
Objective Response (CR+PR) Rate	4 (6.6%)	0	0	2 (22.2%)	1 (16.7%)	0	1 (11.1%)	0	0	0
Disease Control (PR+PR+SD) Rate	40 (65.6%)	1 (25.0%)	3 (60.0%)	8 (88.9%)	4 (66.7%)	3 (50.0%)	6 (66.7%)	7 (77.8%)	5 (55.6%)	3 (75.0%)

Table 12: Confirmed Response Rate – dose escalation

	Total	25 mg	50 mg	80 mg	100 mg	140 mg	200 mg	400 mg	800 mg	1200 mg
N	61	4	5	9	6	6	9	9	9	4
Confirmed Best Overall Response										
CR (Complete Response)	0	0	0	0	0	0	0	0	0	0
PR (Partial Response)	2 (3.3%)	0	0	1 (11.1%)	1 (16.7%)	0	0	0	0	0
SD (Stable Disease)	38 (62.3%)	1 (25.0%)	3 (60.0%)	7 (77.8%)	3 (50.0%)	3 (50.0%)	6 (66.7%)	7 (77.8%)	5 (55.6%)	3 (75.0%)
PD (Progressive Disease)	14 (23.0%)	2 (50.0%)	2 (40.0%)	1 (11.1%)	0	2 (33.3%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	1 (25.0%)
NE (Not Evaluable)	7 (11.5%)	1 (25.0%)	0	0	2 (33.3%)	1 (16.7%)	1 (11.1%)	0	2 (22.2%)	0
Confirmed Objective Response (CR+PR) Rate	2 (3.3%)	0	0	1 (11.1%)	1 (16.7%)	0	0	0	0	0
Confirmed Disease Control (PR+PR+SD) Rate	40 (65.6%)	1 (25.0%)	3 (60.0%)	8 (88.9%)	4 (66.7%)	3 (50.0%)	6 (66.7%)	7 (77.8%)	5 (55.6%)	3 (75.0%)

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Expansion:

Expansion cohort 1: As of October 12, 2020, 24 patients were enrolled in expansion cohort 1, which includes patients with NSCLC (PD-1/L1 pre-treated). 12 patients could be assessed post-baseline, with confirmed progression on or after checkpoint inhibitor therapy (Figure 11).

Conclusions:

GEN1046 is a first-in-class, next-generation, PD-L1x4-1BB bispecific antibody with an acceptable safety profile and encouraging early clinical activity, unlike the existing 4-1BB agonists.

In the dose escalation phase of this phase I/IIa study, GEN1046 demonstrated a manageable safety profile and preliminary clinical activity in a heavily pretreated population with advanced solid tumors.

Most adverse events were mild to moderate; treatment-related Grade 3 transaminase elevations resolved with corticosteroids. No treatment-related bilirubin increases or Grade 4 transaminase elevations were observed. Six patients had dose limiting toxicities (DLTs); Maximum tolerated dose (MTD) was not reached.

Clinical benefit across different dose levels was observed in patients, including those resistant to prior immunotherapy and those with tumors typically less sensitive to immune checkpoint inhibitors (ICIs).

Disease control was achieved in 65.6% of patients, including partial responses in triple negative breast cancer (1), ovarian cancer (1), and ICI pre-treated NSCLC (2).

Modulation of pharmacodynamic endpoints was observed across a broad range of dose levels demonstrating biological activity.

Example 13: Pharmacokinetic/Pharmacodynamic model

An integrated semi-mechanistic PK/PD (Pharmacokinetic/Pharmacodynamic) model was developed that assumes distribution of GEN1046 into central and peripheral PK compartments, as well as partitioning into tumor and lymph compartments. The model leverages PK and pharmacodynamic data as well as physiological parameters from literature for parameterizations of expressions of PD-L1 and 4-1BB, and T-cell trafficking into these cells. Model compartments consists of well-mixed 2-

and 3-dimensional spaces and free drug transfer between all compartments. In addition, the model incorporates dynamic binding of GEN1046 to PD-L1 and 4-1BB to predict trimer (crosslinking to PD-L1 and 4-1BB) formation and receptor occupancy (RO) for PD-L1 and 4-1BB in tumor. Simulations showed that trimer formation is optimal at a dose of 80 mg, and model predicted RO in tumor for PD-L1 and 4-1BB was deemed sufficient at doses between 80 to 140 mg. Increasing doses ≥200 mg resulted in reduced trimer formation. In addition, based on available clinical pharmacodynamic data, higher magnitude and consistent modulation of peripheral pharmacodynamic endpoints (IFNγ and proliferating Ki67+ effector memory CD8+ T cells) were seen at dose levels ≤200 mg. In light of PK/pharmacodynamic modeling predictions and available clinical data, the optimal dose of GEN1046 was predicted to be in the range of 80 to 140 mg. At 100 mg dose 1Q3W, maximal trimer formation and average RO for PD-L1 (%) is maintained at reasonable levels during the entire dosing interval.

Model Predicted Maximal Trimer Formation and Receptor Occupancy for PDL1 at 100 mg 1Q3W is shown in Figure 12.