BINDING CONSTRUCTS

Field of the invention

The invention relates to constructs comprising (i) an LTBR binding agent and (ii) a PD-1 binding agent or a PD-L1 binding agent, particularly for the treatment of diseases such as cancer, and related aspects.

Background of the invention

Cancer can affect multiple cell types and tissues but the underlying cause is a breakdown in the control of cell division. This process is highly complex, requiring careful coordination of multiple pathways, many of which remain to be fully characterised.

Cancer immunotherapy involves the use of a subject's own immune system to treat or prevent cancer. These therapies are designed to provoke the body's immune system to eliminate cancer cells and exploit the fact that cancers accumulate genetic mutations resulting in the expression of tumor antigens. These tumor antigens consist of tumor-associated antigens such as proteins and tumor-specific antigens or neoantigens, which are peptides that are presented by major histocompatibility molecules (Xie et al 2023). However, malignant tumors can evade detection by the immune system via tumor cell intrinsic mechanisms and mechanisms linked to the tumor microenvironment leading to therapy resistance (Sharma et al 2017).

Checkpoint inhibitors such as, PD-1 and PD-L1 blocking antibodies, have had a significant impact on the treatment of cancer. PD-1 and PD-L1 antibodies work by blocking the interaction of PD-1 with its ligands, PD-L1 and/or PD-L2, which has been shown to enhance the antitumor activity of T-cells (Jiang et al. 2020).

Durable responses to immunotherapy however, have only been observed in a minority of cancer patients and thus there is a need for the development of new therapies. Key challenges in mounting durable anti-tumor responses include limited infiltration of immune effector cells in the tumor, immune cell dysfunction and the lack of priming of anti-tumor immune cells. Recently, it has been shown that the presence of high endothelial venules (HEV) and tertiary lymphoid structures (TLS) in cancer patients is associated with better survival and with better response to therapy (Cabrita et al 2020; Vanhersecke et al 2021; Asrir et al 2022). HEVs are specialized structures in the vasculature that serve as portals for immune cell entry into the tumor and TLS are ectopic immune cell aggregates that provide a favourable local immune environment and facilitate local priming of immune cells (Sautès-Fridman et al 2019; Schumacher et al 2022). Therapeutic strategies aimed at enhancing both HEV and TLS in patients would therefore be advantageous to improve antitumour immune responses.

Lymphotoxin beta receptor (LTBR), also known as tumour necrosis factor receptor superfamily member 3 (TNFRSF3), is a cell surface receptor for lymphotoxin involved in cytokine release and apoptosis. It is a member of the tumour necrosis factor receptor superfamily. LTBR plays a central role in the development and homeostasis of lymph nodes and secondary lymphoid organs (SLO), TLS and HEV by regulating the expression of several homeostatic lymphoid cytokines (e.g. CCL-19, CCL-21, CXCL-13) and adhesion molecules (ICAM-1, VCAM-1, MAdCAM1) via the NF-κB pathway (Dejardin et al. 2002, Schneider et al. 2004). LTBR is activated by two different trimeric ligands, LIGHT (TNFSF14) and lymphotoxin α1β2 (LTα1β2). Whereas LTα1β2 is specific for LTBR, LIGHT also binds to and activates HVEM (TNFRSF14), a receptor expressed on and implicated in the regulation of immune cells (Pasero et al. 2012).

It has been found that activation of LTBR by its ligands leads to ectopic formation of TLS and HEV (Schrama et al. 2001; Tang et al. 2017; Allen et al. 2017; Asrir et al. 2022). Presence of TLS and HEV in the tumour microenvironment typically correlates with immune infiltration and is also associated with better prognosis, suggesting that TLS and HEV are involved in anti-tumour immune responses (Dieu-Nosjean et al. 2008; Weinstein and Storkus 2015; Vanhersecke et al 2021; Asrir et al 2022). Therefore, activation of LTBR has the potential to promote formation of HEV and TLS in the tumour microenvironment and induce anti-tumour immune responses and improve current cancer immunotherapies.

Due to the broad expression of LTBR in organisms, an agonistic LTBR binding construct capable of inducing TLS and creating an activating immune environment bears a substantial risk of causing systemic immune-related adverse events. Johansson-Percival et al. reported weight loss in mice after systemic administration of an LTBR activating compound, VTP-LIGHT (Johansson-Percival et al. 2017). Overexpression of lymphotoxin α and lymphotoxin β induced hepatotoxicity in mice (Haybaeck et al 2009). Therefore, as postulated in the prior art, a therapeutic modality which activates LTBR specifically in the tumour but not in other tissues is needed to reduce the risk of toxicity and to generate a well-tolerated drug that can be employed for combination therapies (Tang et al. 2017).

US 2021/0188990 discloses multispecific binding molecules, such as bispecific antibodies, with a first specificity for LTBR and a second specificity for extra-domain B of fibronectin (EDB) (US 2021/0188990, paragraph 17) in particular for the purpose of killing cancer cells ((US 2021/0188990, paragraph 2). It is stated in paragraph 315 of US 2021/0188990 “Taken together, the data presented in Examples 2-4 suggested, that EDB-containing Fibronectin (a tumor associated antigen that is deposited in the extracellular matrix of a tumor…can lead to efficient clustering of LTBR leading to its efficient activation unlike antigens co-expressed with LTBR on the surface of tumor cells (e.g. Mesothelin)…In the absence of EDB-

containing Fibronectin…the bispecific antibodies of this invention were not able to activate LTBR…". Paragraph 316 of US 2021/0188990 states "In short, it is shown herein that targeting of LTBR and a tumor-associated antigen (TAA) that is co-expressed with LTBR on tumor cells with a bispecific antibody that binds to both LTBR and such TAA, was not capable of activating LTBR in a tumor-specific manner…Strikingly and surprisingly however, bispecific antibodies that bind with one binding domain to LTBR and with another binding domain to EDB of Fibronectin (a TAA that is present in the extracellular matrix), were capable of activating LTBR in a tumor-specific manner...". Therefore, this disclosure indicates that tumor antigen-dependent activation of LTBR using a bispecific antibody with one arm binding to LTBR and another arm (the 'targeting' arm) binding to a TAA is more effective when using a targeting arm that binds to an extracellular matrix antigen than to a cell surface antigen co-expressed on the same cell as LTBR. In contrast, the present inventors show that a bispecific antibody binding to LTBR expressed on the surface of a first cell and to PD1 or PDL1 expressed on the surface of a second cell is capable of effectively activating LTBR on the first cell. This is surprising as PD1 and PDL1 can both be considered as cell-surface antigens. Cell surface antigens, like PD1 and PDL1, may be preferred over extracellular matrix antigens for the clustering and activation of LTBR using a bispecific antibody as cell surface antigens are less rigid compared to antigens fixed in the extracellular matrix. Additionally, the use of certain cell surface antigens, like PD1 or PDL1, may have favourable properties such as localization and distance to LTBR which may lead to more optimal activation and signalling of LTBR in the context of activating LTBR in at tumour antigen dependent manner using a bispecific antibody.

There remains a need for new approaches to cancer therapies. Such approaches may demonstrate high response rates and durable response, reduction in the dose required for effect, an improved safety profile/reduced side effects, or the like.

Summary of the invention

The invention provides a construct comprising an LTBR binding agent and a PD-1 binding agent. The invention also provides a construct comprising an LTBR binding agent and a PD-L1 binding agent.

In a further aspect the invention provides a construct according to the invention for use in the treatment of cancer.

In a further aspect the invention provides a polynucleotide encoding the construct of the invention.

Certain embodiments of the invention may be expected to benefit from one or more of the following advantages over the prior art:

(a) increased avidity,

(b) agonism of LTBR (e.g. activation of NFKB pathways, such as the classical or alternative NFKB pathways),

(c) clustering of LTBR,

(d) reduced toxicity (e.g. liver toxicity),

(e) reduced doses,

(f) improved biodistribution,

(g) improved therapeutic efficacy,

(h) localisation to, and conditional activation (i.e. in a PD-1/PD-L1-dependent manner) at the target site,

(i) upregulation of intercellular adhesion molecules (e.g. ICAM-1),

(j) binding LTBR and PD-1/PD-L1 on the same cell simultaneously and activating LTBR on this cell,

(k) increased secretion of CCL-2, CCL-5 and/or CXCL-10,

(l) improved convenience of production,

(m) improved half-life and/or reduced rate of clearance.

Without wishing to be bound by theory, the inventors believe that certain embodiments of the invention may be expected to benefit from one or more of the following advantages over the combined administration of separate anti-LTBR and anti- PD-1/PD-L1 agents, for the following reasons:

(a) Macrophages are present in large numbers in many tumors and frequently express high levels of PD-L1 -often higher than that seen on tumor cells. Macrophages (and tumor cells) are often found in close proximity to fibroblasts. Therefore, trans activation of fibroblasts by macrophage- or tumor cell- anchored LTBRxPD-L1 may result in high levels of chemokine induction to promote lymphocyte infiltration / TLS formation.

(b) Cis activation of dendritic cells expressing PD-L1 and LTBR could enhance antigen presentation of DCs and increase immune responses of T cells.

(c) Similarly, proximity of PD-L1 expression to LTBR expressing vascular endothelial cells could lead to strong induction of HEV formation.

Further aspects of the invention will become clear from the below.

Summary of the sequences

SEQ ID NO: 1	Polypeptide sequence of BHA10 heavy chain variable region
SEQ ID NO: 2	Polypeptide sequence of BHA10 light chain variable region
SEQ ID NO: 3	Polypeptide sequence of Pembrolizumab heavy chain variable region
SEQ ID NO: 4	Polypeptide sequence of Pembrolizumab light chain variable region

SEQ ID NO: 5	Polypeptide sequence of Nivolumab heavy chain variable region
SEQ ID NO: 6	Polypeptide sequence of Nivolumab light chain variable region
SEQ ID NO: 7	Polypeptide sequence of Atezolizumab heavy chain variable region
SEQ ID NO: 8	Polypeptide sequence of Atezolizumab light chain variable region
SEQ ID NO: 9	Polypeptide sequence of Avelumab heavy chain variable region
SEQ ID NO: 10	Polypeptide sequence of Avelumab light chain variable region
SEQ ID NO: 11	Polypeptide sequence of MF7702 heavy chain variable region
SEQ ID NO: 12	Polypeptide sequence of MF7702 light chain variable region
SEQ ID NO: 13	Polypeptide sequence of E12V2 heavy chain variable region
SEQ ID NO: 14	Polypeptide sequence of E12V2 light chain variable region
SEQ ID NO: 15	Polypeptide sequence of full length human LTBR
SEQ ID NO: 16	Polypeptide sequence of human LTBR extracellular domain
SEQ ID NO: 17	Polypeptide sequence of full length *Macaca fascicularis* LTBR
SEQ ID NO: 18	Polypeptide sequence of *Macaca fascicularis* LTBR extracellular domain
SEQ ID NO: 19	Polypeptide sequence of full length mouse LTBR
SEQ ID NO: 20	Polypeptide sequence of mouse LTBR extracellular domain
SEQ ID NO: 21	Polypeptide sequence of full length human PD-1
SEQ ID NO: 22	Polypeptide sequence of human PD-1 extracellular domain
SEQ ID NO: 23	Polypeptide sequence of full length *Macaca fascicularis* PD-1
SEQ ID NO: 24	Polypeptide sequence of *Macaca fascicularis* PD-1 extracellular domain
SEQ ID NO: 25	Polypeptide sequence of full length mouse PD-1
SEQ ID NO: 26	Polypeptide sequence of mouse PD-1 extracellular domain
SEQ ID NO: 27	Polypeptide sequence of full length human PD-L1
SEQ ID NO: 28	Polypeptide sequence of human PD-L1 extracellular domain
SEQ ID NO: 29	Polypeptide sequence of full length *Macaca fascicularis* PD-L1
SEQ ID NO: 30	Polypeptide sequence of *Macaca fascicularis* PD-L1 extracellular domain
SEQ ID NO: 31	Polypeptide sequence of full length mouse PD-L1
SEQ ID NO: 32	Polypeptide sequence of mouse PD-L1 extracellular domain
SEQ ID NO: 33	Polypeptide sequence of human LIGHT (including ECD)
SEQ ID NO: 34	Polypeptide sequence of human LIGHT ECD
SEQ ID NO: 35	Polypeptide sequence of C5H9v2 heavy chain variable region
SEQ ID NO: 36	Polypeptide sequence of C5H9v2 light chain variable region
SEQ ID NO: 37	Polypeptide sequence of 5G11 heavy chain variable region
SEQ ID NO: 38	Polypeptide sequence of 5G11 light chain variable region

Summary of the figures

Figure 1 Example 4, NFKB activation of HepG2 reporter cells expressing human LTBR as monoculture or cocultured with PD-1-overexpressing CHO cell line

Figure 2 Example 5, *in vitro*, PD-1/LTBR bispecific activity in chemokine induction assay using tumour cell line HCC1187 cultured in monoculture or with PD-1 positive CHO cell line

Figure 3 Example 6, *in vitro*, PD-L1/LTBR bispecific activity in chemokine induction assay using tumour cell line HCC1187 cultured in monoculture or with PD-L1 positive CHO cell line

Figure 4 Example 6, *in vitro*, PD-L1/LTBR bispecific activity in chemokine induction assay using tumour cell line HCC1187 cultured with PD-L1 positive CHO cell line

Figure 5 Example 7, *in vitro* PD-1/PD-L1 interaction blockade bioassay (human)

Figure 6 Example 8, *in vitro*, PD-L1/LTBR bispecific antibody activity in chemokine (CXCL-10) induction assay using immortalised mouse lung fibroblasts cultured in monoculture or with PD-L1 positive CHO cell line

Figure 7 Example 8, *in vitro*, PD-L1/LTBR bispecific antibody activity in chemokine (CCL-5) induction assay using immortalised mouse lung fibroblasts cultured in monoculture or with PD-L1 positive CHO cell line

Figure 8 Example 9, *in vitro* activity of PD-L1/LTBR bispecific antibody in an ICAM-1 upregulation assay using immortalised mouse lung fibroblasts cultured in in monoculture or with with a PD-L1 overexpressing cell line

Figure 9 Example 10, *in vitro* PD-1/PD-L1 interaction blockade bioassay (mouse)

Detailed description of the invention

Binding agents

Binding agents are capable of binding to a target with an affinity (suitably expressed as a KD value, a Ka value, a kon-rate and/or a koff-rate, as further described herein). Suitably the binding agent agonises, inversely agonises, antagonises or neutralises a specified target. In one embodiment the binding agent is a small molecule. In one embodiment the binding agent is a binding polypeptide. Polypeptides are said to be binding polypeptides when they comprise a domain wherein the domain comprises one or more stretches of amino acid residues which form an antigen-binding site, capable of binding to an epitope on a target with an affinity. 'Binding polypeptide' and 'antigen-binding polypeptide' are used synonymously herein. A polypeptide which binds to LTBR is synonymous with a polypeptide which is anti-LTBR, a polypeptide which binds to PD-1 is synonymous with a polypeptide which is anti-PD-1 and a polypeptide which binds to PD-L1 is synonymous with a polypeptide which is anti-PD-L1.

Binding polypeptides may include antibodies and fragments thereof (which are further described below), antibodies modified to comprise additional binding regions and antibody mimetics. Further binding polypeptides may include, for example, DARPins (Binz et al. 2003), Affimers™, Fynomers™, Centyrins, Nanofitins® and cyclic peptides.

Antibodies and fragments thereof

The binding agents are preferably antibodies or fragments thereof. If the binding agents are antibody fragments, these fragments may be comprised in an antibody such that the construct comprising the binding agents is itself an antibody. Such fragments may be comprised in a non-related antibody (or antigen-binding fragment thereof) such that the construct comprising the binding agents is a chimeric antibody (or chimeric fragment thereof). A conventional antibody or immunoglobulin (Ig) is a protein comprising four polypeptide chains: two heavy (H, or HC) chains and two light (L, or LC) chains. Each chain is divided into a constant region and a variable region. The variable region comprises a heavy chain variable region and a light chain variable region. The heavy (H) chain variable region is abbreviated herein as VH region, and the light (L) chain variable region is abbreviated herein as VL region. These domains, domains related thereto and domains derived therefrom, are referred to herein as variable domains. The VH and VL regions (or 'domains') can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDRs"), interspersed with regions that are more conserved, termed "framework regions" ("FRs"). The framework and complementarity determining regions have been precisely defined (Kabat et al 1991). In a conventional antibody, each VH and VL region is 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. The VH region CDRs and FRs are denoted HCDR1, HCDR2, HCDR3, HFR1, HFR2, HFR3 and HFR4. The VL region CDRs and FRs are denoted LCDR1, LCDR2, LCDR3, LFR1, LFR2, LFR3 and LFR4. The conventional antibody tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains is formed with the heavy and the light immunoglobulin chains inter-connected by e.g. disulfide bonds, and the heavy chains similarly connected. The heavy chain constant region includes three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable domain of the heavy chains and the variable domain of the light chains are binding domains that interact with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g. effector cells) and the first component (C1q) of the classical complement system.

An exception to conventional antibody structure is found in sera of Camelidae. In addition to conventional antibodies, these sera possess special IgG antibodies. These IgG

antibodies, known as heavy-chain antibodies (HCAbs), are devoid of the L chain polypeptide and lack the first constant domain (CH1). At its N-terminal region, the H chain of the homodimeric protein contains a dedicated immunoglobulin chain variable domain, referred to as the VHH, which serves to associate with its cognate antigen (Muyldermans 2013; Hamers-Casterman et al 1993).

The term “antibody” includes any antibody protein construct comprising at least one antibody variable domain comprising at least one antigen binding site (ABS). Antibodies include, but are not limited to, immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). The overall structure of Immunoglobulin G (IgG) antibodies assembled from two identical heavy (H)-chain and two identical light (L)-chain polypeptides is well established and highly conserved in mammals (Padlan 1994).

A fragment of an antibody (which may also referred to as “antibody fragment”, “immunoglobulin fragment”, “antigen-binding fragment” or “antigen-binding polypeptide”) as used herein refers to a portion of an antibody that specifically binds to the target (e.g. a molecule in which one or more immunoglobulin chains is not full length, but which specifically binds to the target). Examples of binding fragments encompassed within the term antibody fragment include:

(i) a Fab fragment (a monovalent fragment consisting of the VL, VH, CL and CH1 domains);

(ii) a F(ab')2 fragment (a bivalent fragment consisting of two Fab fragments linked by a disulphide bridge at the hinge region);

(iii) a Fd fragment (consisting of the VH and CH1 domains);

(iv) a Fv fragment (a variable domain or variable region, consisting of the VL and VH domains of a single arm of an antibody wherein the VL and VH domains together form an antigen binding site);

(v) a single chain variable fragment, scFv (consisting of VL and VH domains 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);

(vi) a VH (a variable domain consisting of a VH domain);

(vii) a VL (a variable domain consisting of a VL domain);

(viii) a domain antibody (dAb, consisting of either the VH or VL domain);

(ix) a minibody (consisting of a pair of scFv fragments which are linked via CH3 domains); and

(x) a diabody (consisting of a noncovalent dimer of scFv fragments that consist of a VH domain from one antibody connected by a small peptide linker a VL domain from another antibody);

(xi) a VHH (a heavy chain variable domain as described in detail above).

The antibody or fragment thereof may be a human antibody or derived from a human antibody. “Human antibody" refers to antibodies having variable and constant regions derived

from human germline immunoglobulin sequences. Human subjects administered with said human antibodies do not generate cross-species antibody responses (for example termed HAMA responses - human-anti-mouse antibody) to the primary amino acids contained within said antibodies. Said human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g. mutations introduced by random or site-specific mutagenesis or by somatic mutation), for example in the CDRs and in particular CDR3. However, the term is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences, may also be referred to as “recombinant human antibodies”. Substituting at least one amino acid residue in the framework region of a non human immunoglobulin variable domain with the corresponding residue from a human variable domain is referred to as “humanisation”. Humanisation of a variable domain may reduce immunogenicity in humans.

Suitably the construct comprises an antibody, or an antibody fragment. In one embodiment, the antibody fragment comprises an scFv, Fv, Fab, Fab’, F(ab')2, variable domain (e.g. VH, VL, VNAR or VHH), diabody or minibody. If the construct is an antibody or antibody fragment, then the binding regions of the antibody or antibody fragment (e.g. the Fab regions of the antibody or antibody fragment, or synthetically introduced further binding domains such as VHHs) provide the PD-1/PD-L1 binding agent(s) and the LTBR binding agent(s) of the construct of the invention.

In one embodiment, the antibody fragment is an scFv, Fab, Fab’, F(ab')2, variable domain (e.g. VH or VL), diabody, minibody or antibody.

In a particular embodiment, the construct is an antibody or scFv,

In one embodiment, an scFv may be fused to the C-terminus of an Fc. Suitably, a linker such as a linker comprising Glycine and Serine residues, such as a G_4S linker may be used. Suitably a disulfide bond may be situated in the VH44:VL100 position (Weatherill et al. 2012).

Most suitably the construct is an antibody (i.e. a full length antibody). Antibodies of the invention can be of any class, e.g. IgG, IgA, IgM, IgE, IgD, or subclass thereof, and can comprise a kappa or lambda light chain. In one embodiment, the antibody is an IgG antibody,

for example, at least one of subclasses, IgG1, IgG2, IgG3 or IgG4. In one embodiment, the antibody is an IgG1, most suitably human IgG1. In a further embodiment, the antibody may be in a format, such as an IgG format, that has been modified to confer desired properties, such as having the Fc mutated to reduce effector function, extend half-life, alter ADCC, or improve hinge stability. Such modifications are well known in the art and exemplary embodiments are described herein. Suitably, the antibody comprises PGLALA substitutions (substitutions Pro329Gly, Leu234Ala, and Leu235Ala, Schlothauer et al. 2016) in the Fc region. Suitably the antibody Fc region comprises 329Gly, 234Ala and 235Ala. Alternatively, the antibody comprises LALA substitutions (substitutions Leu234Ala, and Leu235Ala, Schlothauer et al. 2016) in the Fc region. Suitably the antibody Fc region comprises 234Ala and 235Ala. In a further embodiment the antibody comprises an STR-silenced Fc region (i.e. G236R in combination with the LALA substitutions described above, see Wilkinson et al. 2021). Therefore, in one embodiment, the Fc region comprises 234Ala, 235Ala and 236Arg. In a yet further embodiment, the Fc region is aglycosylated.

In one embodiment the antibody is multispecific, such as bispecific and multivalent, such as bivalent. Suitable constructs of these and other formats are detailed further below.

In a particular embodiment, the construct comprises, essentially consists of or consists of an antibody or a pair of scFvs. Most suitably the construct comprises an antibody.

Specificity, affinity, avidity and potency

"Specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antibody or fragment thereof can bind. The specificity of an antibody is the ability of the antibody to recognise a particular antigen as a unique molecular entity and distinguish it from another. An antibody that "specifically binds" to an antigen or an epitope is a term well understood in the art. A molecule is said to exhibit "specific binding" if it reacts more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen or epitope, than it does with alternative targets. An antibody "specifically binds" to a target antigen or epitope if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. An antibody (or fragment thereof) may be considered to specifically bind to a target if the binding is statistically significant compared to a non-relevant binder. Specific binding of an antibody, or fragment thereof, to an antigen or antigenic determinant can be determined in any suitable known manner, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, equilibrium dialysis, equilibrium binding, gel filtration, ELISA, or spectroscopy (e.g. using a fluorescence assay) and the different variants thereof known in the art.

In one embodiment by 'binding specifically' it is meant that the target is bound to with a KD of 10^{-8} M or less, such as 10^{-9} M or less whereas any other target is bound to with a KD of 10^{-7} M or more such as 10^{-6} M or more. More suitably, binding to any other target cannot be measured.

"Affinity", represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding polypeptide (KD), is a measure of the binding strength between an antigenic determinant and an antigen-binding site on the antibody (or fragment thereof): the lesser the value of the KD, the stronger the binding strength between an antigenic determinant and the antigen-binding polypeptide. Alternatively, the affinity can also be expressed as the affinity constant (KA), which is 1/KD. Affinity can be determined by known methods, depending on the specific antigen of interest. Any KD value less than 10^{-6} M is considered to indicate binding. Suitably, constructs of the invention will bind targets with a dissociation constant of 10^{-6} M or less, more suitably 10^{-7} M or less, more suitably 10^{-8} M or less and more suitably 10^{-9} M or less.

Suitably, the K_D of a polypeptide of the invention is determined using surface plasmon resonance (SPR) or biolayer interferometry (such as by the method described in Example 3).

"Avidity" is the measure of the strength of binding between a polypeptide, an antibody or fragment thereof, and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antibody and the number of pertinent binding sites present on the antibody.

"Potency" is a measure of the activity of a therapeutic agent expressed in terms of the amount required to produce an effect of given intensity. A highly potent agent evokes a greater response at low concentrations compared to an agent of lower potency that evokes a smaller response at low concentrations. Potency is a function of affinity and efficacy. Efficacy refers to the ability of therapeutic agent to produce a biological response upon binding to a target and the quantitative magnitude of this response. The term half maximal effective concentration (EC50) refers to the concentration of a therapeutic agent which causes a response halfway between the baseline and maximum after a specified exposure time. The therapeutic agent may cause inhibition or stimulation. It is commonly used, and is used herein, as a measure of potency.

LTBR

Lymphotoxin beta receptor (LTBR), also known as tumour necrosis factor receptor superfamily member 3 (TNFRSF3), is a cell surface receptor for lymphotoxin involved in apoptosis and cytokine release. It is a member of the tumour necrosis factor receptor superfamily.

The construct of the invention comprises an LTBR binding agent. LTBR binding agents are disclosed in the prior art, for example anti-LTBR binding polypeptide BHA10 (WO2004002431, comprising heavy and light chain variable regions of SEQ ID NO: 1 and 2, respectively).

The LTBR is suitably situated on the surface of stromal and/or cancer cells. Suitably the LTBR is situated on the surface of a cell wherein the cell also comprises PD-L1 on its surface.

The LTBR binding agent suitably binds to the extracellular domain of LTBR.

In one embodiment, LTBR is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 15 (full length human LTBR). Suitably, LTBR comprises such as consists of SEQ ID NO: 15 (full length human LTBR).

Suitably, the LTBR is the extracellular domain of human LTBR, i.e. the LTBR binding agent binds to the extracellular domain of human LTBR. In one embodiment, LTBR is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 16 (human LTBR extracellular domain). Suitably, LTBR comprises such as consists of SEQ ID NO: 16 (human LTBR extracellular domain).

In one embodiment, LTBR is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 17 (full length *Macaca fascicularis* LTBR). Suitably, LTBR comprises such as consists of SEQ ID NO: 17 (full length *Macaca fascicularis* LTBR).

Suitably, the LTBR is the extracellular domain of human LTBR, i.e. the LTBR binding agent binds to the extracellular domain of *Macaca fascicularis* LTBR. In one embodiment, LTBR is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 18 (*Macaca fascicularis* LTBR extracellular domain). Suitably, LTBR comprises such as consists of SEQ ID NO: 18 (*Macaca fascicularis* LTBR extracellular domain).

In one embodiment, LTBR is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 19 (full length mouse LTBR). Suitably, LTBR comprises such as consists of SEQ ID NO: 19 (full length mouse LTBR).

Suitably, the LTBR is the extracellular domain of mouse LTBR, i.e. the LTBR binding agent binds to the extracellular domain of mouse LTBR. In one embodiment, LTBR is a

polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 20 (mouse LTBR extracellular domain). In one embodiment, the LTBR comprises, such as consists of SEQ ID NO: 20 (mouse LTBR extracellular domain).

The construct of the invention may bind to LTBR with an EC50 of 1.0E-06 M or less, such as 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 5.0E-09 M or less, such as 3.0E-09 M or less, such as 2.8E-09 M or less, such as 1.0E-09 M or less. Suitably the EC50 is established using a chemokine induction assay, for example assayed using an ELISA. More suitably the EC50 is established using the assay set out in Example 2.

Suitably the LTBR binding agent binds to LTBR specifically, i.e. the binding agent does not bind significantly to targets other than human LTBR, such as does not bind significantly to targets other than human, *Macaca fascicularis* or mouse LTBR, such as does not bind significantly to targets other than LTBR.

In one embodiment, the LTBR binding agent is the LTBR ligand, LIGHT, more suitably human LIGHT.

In one embodiment, the LTBR binding agent is not the LTBR ligand, human LIGHT, or more suitably the LTBR binding agent is not LIGHT.

Suitably LIGHT is a polypeptide comprising, consisting essentially of, or consisting of a sequence sharing at least 70%, such as at least 80%, such as at least 90%, such as at least 99%, such as 100% identity, with SEQ ID NO: 33 or SEQ ID NO: 34.

The construct of the invention may bind to LTBR with a binding affinity (KD) of 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 7.0E-09 M or less, such as 6.7E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less, such as 1.0E-11 M or less.

In one embodiment the construct is an LTBR agonist. In an alternative embodiment, the construct is an LTBR antagonist.

Suitably the LTBR binding agent agonises (i.e. activates), inversely agonises, antagonises or neutralises LTBR. In one embodiment the LTBR binding agent is an LTBR agonist. Suitably the agonist activates NFKB signalling pathways. If the LTBR binding agent is an LTBR agonist, then suitably the LTBR binding agent (a) induces clustering of LTBR and more suitably (b) activates the classical NFKB pathway (suitably leading to expression of adhesion molecules such as ICAMs, such as ICAM-1, VCAM-1 and/or MAdCAM-1) and/or

activates the alternative NFKB pathway (suitably leading to expression of chemokines, such as CCL-2, CCL-5, CCL-19, CCL-21, CXCL-10 and/or CXCL-13).

In one embodiment, the construct binds to mouse LTBR. In an alternative embodiment, the construct binds to *Macaca fascicularis* LTBR. In an alternative embodiment, the construct binds to human LTBR.

In one embodiment, the LTBR binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1. More suitably, the LTBR binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 1. In one embodiment, the LTBR binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 2. More suitably, the LTBR binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 2.

In one embodiment, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively. More suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

In one embodiment, the LTBR binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 37. More suitably, the LTBR binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 37. In one embodiment, the LTBR binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 38. More suitably, the LTBR binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 38.

In one embodiment, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 37 and SEQ ID NO: 38, respectively. More suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 37 and SEQ ID NO: 38, respectively.

PD-1

Programmed Cell Death Protein 1 (PD-1) also known as CD279 is a protein localised to the surface of T and B cells which plays a role in regulating the immune system. PD-1 is an immune checkpoint. In humans, PD-1 protein is encoded by the *PDCD1* gene. PD-1 is a 55 kDa transmembrane protein.

A construct of the invention may comprise a PD-1 binding agent. PD-1 binding agents are disclosed in the prior art, for example anti-PD-1 binding polypeptide Nivolumab (WO2006121168A1, comprising heavy and light chain variable regions of SEQ ID NO: 5 and 6, respectively).

The PD-1 is suitably situated on the surface of exhausted T and/or B-cells including in the tumor microenvironment.

The PD-1 binding agent suitably binds to the extracellular domain of PD-1.

In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 21 (full length human PD-1). Suitably, PD-1 comprises such as consists of SEQ ID NO: 21 (full length human PD-1).

Suitably, the PD-1 is the extracellular domain of human PD-1, i.e. the PD-1 binding agent binds to the extracellular domain of human PD-1. In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 22 (human PD-1 extracellular domain). Suitably, PD-1 is a polypeptide comprising or consisting of SEQ ID NO: 22.

In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 23 (full length *Macaca fascicularis* PD-1). Suitably, PD-1 comprises such as consists of SEQ ID NO: 23 (full length *Macaca fascicularis* PD-1).

Suitably, the PD-1 is the extracellular domain of *Macaca fascicularis* PD-1, i.e. the PD-1 binding agent binds to the extracellular domain of *Macaca fascicularis* PD-1. In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 24 (*Macaca fascicularis* PD-1 extracellular domain). Suitably, PD-1 is a polypeptide comprising or consisting of SEQ ID NO: 24.

In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least

90% identity, such as at least 95% identity with SEQ ID NO: 25 (full length mouse PD-1). Suitably, PD-1 comprises such as consists of SEQ ID NO: 25 (full length mouse PD-1).

Suitably, the PD-1 is the extracellular domain of mouse PD-1, i.e. the PD-1 binding agent binds to the extracellular domain of mouse PD-1. In one embodiment, PD-1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 26 (mouse PD-1 extracellular domain). Suitably, PD-1 comprises such as consists of SEQ ID NO: 26 (mouse PD-1 extracellular domain).

The construct of the invention may bind to PD-1 with an EC50 of 1.0E-06 M or less, such as 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 5.0E-09 M or less, such as 3.0E-09 M or less, such as 1.1E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 5.4E-10 M or less, such as 3.5E-10 M or less, such as 3.0E-10 M or less, such as 1.7E-10 M or less, such as 1.5E-10 M or less, such as 1.0E-10 M or less. Suitably the EC50 is established using the assay set out in Example 2.

Suitably the PD-1 binding agent binds to PD-1 specifically, i.e. the binding agent does not bind significantly to targets other than human PD-1, such as does not bind significantly to targets other than human, *Macaca fascicularis* or mouse PD-1, such as does not bind significantly to targets other than PD-1.

The construct of the invention may bind to PD-1 with a binding affinity (KD) of 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 8.0E-10 M, such as 7.2E-10 M, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less, such as 1.0E-11 M or less.

In one embodiment the PD-1 binding agent is a PD-1 antagonist. Suitably, the PD-1 binding agent reduces transmission of inhibitory signals from PD-1 receptor to T cells by for example by reducing recruitment of by the receptor of tyrosine phosphatase SHP2 (thereby dephosphorylating proximal signalling elements).

In one embodiment, the construct binds to mouse PD-1. In an alternative embodiment, the construct binds to *Macaca fascicularis* PD-1. In an alternative embodiment, the construct binds to human PD-1.

In one embodiment, the PD-1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 3. More suitably, the PD-1 binding agent comprises a heavy chain variable region comprising, such as consisting of,

SEQ ID NO: 3. In one embodiment, the PD-1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 4. More suitably, the PD-1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 4.

In one embodiment, the PD-1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 5. More suitably, the PD-1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 5. In one embodiment, the PD-1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 6. More suitably, the PD-1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 6.

In one embodiment, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 3 and SEQ ID NO: 4, respectively. More suitably, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

In one embodiment, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 5 and SEQ ID NO: 6, respectively. More suitably, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

PD-L1

Programmed death-ligand 1 (PD-L1) also known as CD274 or B7-H1 is a transmembrane protein is widely expressed on tumor cells and tumor infiltrated leukocytes, especially tumor associated macrophages which plays a role in regulating the immune system by acting as a ligand for PD-1 (Saito et al. 2022). In humans, PD-L1 protein is encoded by the *CD274* gene. PD-L1 is a 40 kDa transmembrane protein.

A construct of the invention may comprise a PD-L1 binding agent. PD-L1 binding agents are disclosed in the prior art, for example anti-PD-L1 binding polypeptide MF7702

(WO2018056821A1, comprising heavy and light chain variable regions of SEQ ID NO: 11 and 12, respectively).

The PD-L1 is suitably situated on tumor cells and tumor infiltrated leukocytes, especially tumor associated macrophages.

The PD-L1 binding agent suitably binds to the extracellular domain of PD-L1.

In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 27 (full length human PD-L1). Suitably, PD-L1 comprises such as consists of SEQ ID NO: 27 (full length human PD-L1).

Suitably, the PD-L1 is the extracellular domain of human PD-L1, i.e. the PD-L1 binding agent binds to the extracellular domain of human PD-L1. In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 28 (human PD-L1 extracellular domain). Suitably, PD-L1 is a polypeptide comprising or consisting of SEQ ID NO: 28.

In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 29 (full length *Macaca fascicularis* PD-L1). Suitably, PD-L1 comprises such as consists of SEQ ID NO: 29 (full length *Macaca fascicularis* PD-L1).

Suitably, the PD-L1 is the extracellular domain of *Macaca fascicularis* PD-L1, i.e. the PD-L1 binding agent binds to the extracellular domain of *Macaca fascicularis* PD-L1. In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 30 (*Macaca fascicularis* PD-L1 extracellular domain). Suitably, PD-L1 is a polypeptide comprising or consisting of SEQ ID NO: 30.

In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95% identity with SEQ ID NO: 31 (full length mouse PD-L1). Suitably, PD-L1 comprises such as consists of SEQ ID NO: 31 (full length mouse PD-L1).

Suitably, the PD-L1 is the extracellular domain of mouse PD-L1, i.e. the PD-L1 binding agent binds to the extracellular domain of mouse PD-L1. In one embodiment, PD-L1 is a polypeptide sharing at least 50% identity, such as at least 60% identity, such as at least 70% identity, such as at least 80% identity, such as at least 90% identity, such as at least 95%

identity with SEQ ID NO: 32 (mouse PD-L1 extracellular domain). Suitably, PD-L1 comprises such as consists of SEQ ID NO: 32 (mouse PD-L1 extracellular domain).

The construct of the invention may bind to PD-L1 with an EC50 of 1.0E-06 M or less, such as 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 5.0E-09 M or less, such as 3.0E-09 M or less, such as 1.1E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 5.4E-10 M or less, such as 3.5E-10 M or less, such as 3.0E-10 M or less, such as 1.7E-10 M or less, such as 1.5E-10 M or less, such as 1.0E-10 M or less. Suitably the EC50 is established using the assay set out in Example 3.

Suitably the PD-L1 binding agent binds to PD-L1 specifically, i.e. the binding agent does not bind significantly to targets other than human PD-L1, such as does not bind significantly to targets other than human, *Macaca fascicularis* or mouse PD-L1, such as does not bind significantly to targets other than PD-L1.

The construct of the invention may bind to PD-L1 with a binding affinity (KD) of 1.0E-07 M or less, such as 9.0E-08 M or less, such as 5.0E-08 M or less, such as 1.0E-08 M or less, such as 9.0E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 8.0E-10 M, such as 7.2E-10 M, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less, such as 1.0E-11 M or less.

In one embodiment the PD-L1 binding agent is a PD-L1 antagonist. Suitably, the PDL-1 binding agent reduces transmission of inhibitory signals from PD-1 receptor to T cells by for example reducing recruitment by the receptor of tyrosine phosphatase SHP2 (thereby dephosphorylating proximal signalling elements).

In one embodiment, the construct binds to mouse PD-L1. In an alternative embodiment, the construct binds to *Macaca fascicularis* PD-L1. In an alternative embodiment, the construct binds to human PD-L1.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 7. More suitably, the PD-L1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 7. In one embodiment, the PD-L1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 8. More suitably, the PD-L1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 8.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 9. More suitably, the PD-L1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 9. In one embodiment, the PD-L1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 10. More suitably, the PD-L1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 10.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 11. More suitably, the PD-L1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 11. In one embodiment, the PD-L1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 12. More suitably, the PD-L1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 12.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 13. More suitably, the PD-L1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 13. In one embodiment, the PD-L1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 14. More suitably, the PD-L1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 14.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 7 and SEQ ID NO: 8, respectively. More suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with

SEQ ID NO: 9 and SEQ ID NO: 10, respectively. More suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 9 and SEQ ID NO: 10, respectively.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 11 and SEQ ID NO: 12, respectively. More suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 11 and SEQ ID NO: 12, respectively.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 13 and SEQ ID NO: 14, respectively. More suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 13 and SEQ ID NO: 14, respectively.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 35. More suitably, the PD-L1 binding agent comprises a heavy chain variable region comprising, such as consisting of, SEQ ID NO: 35. In one embodiment, the PD-L1 binding agent comprises a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 36. More suitably, the PD-L1 binding agent comprises a light chain variable region comprising, such as consisting of, SEQ ID NO: 36.

In one embodiment, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 35 and SEQ ID NO: 36, respectively. More suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 35 and SEQ ID NO: 36, respectively.

Polypeptide and polynucleotide sequences

For the purposes of comparing two closely-related polypeptide sequences, the “% sequence identity” between a first polypeptide sequence and a second polypeptide sequence may be calculated using NCBI BLAST v2.0, using standard settings for polypeptide sequences (BLASTP). For the purposes of comparing two closely-related polynucleotide sequences, the

"% sequence identity" between a first nucleotide sequence and a second nucleotide sequence may be calculated using NCBI BLAST v2.0, using standard settings for nucleotide sequences (BLASTN).

Polypeptide or polynucleotide sequences are said to be the same as or "identical" to other polypeptide or polynucleotide sequences if they share 100% sequence identity over their entire length. Residues in sequences are numbered from left to right, i.e. from N- to C-terminus for polypeptides; from 5' to 3' terminus for polynucleotides.

A "difference" between polypeptide sequences refers to an insertion, deletion or substitution of a single amino acid residue in a position of the second sequence, compared to the first sequence. Two polypeptide sequences can contain one, two or more such amino acid differences. Insertions, deletions or substitutions in a second sequence which is otherwise identical (100% sequence identity) to a first sequence result in reduced % sequence identity. For example, if the identical sequences are 9 amino acid residues long, one substitution in the second sequence results in a sequence identity of 88.9%. If first and second polypeptide sequences are 9 amino acid residues long and share 6 identical residues, the first and second polypeptide sequences share greater than 66% identity (the first and second polypeptide sequences share 66.7% identity).

As used herein, numbering of polypeptide sequences and definitions of CDRs and FRs (i.e. HCDR1, HCDR2, HCDR3, HFR1, HFR2, HFR3, HFR4, LCDR1, LCDR2, LCDR3, LFR1, LFR2, LFR3 and LFR4) are as defined according to the Kabat system (Kabat et al., 1991, herein incorporated by reference in its entirety), unless mentioned otherwise. A "corresponding" amino acid residue between a first and second polypeptide sequence is an amino acid residue in a first sequence which shares the same position according to the Kabat system with an amino acid residue in a second sequence, whilst the amino acid residue in the second sequence may differ in identity from the first. Suitably corresponding residues will share the same number (and letter) if the framework and CDRs are the same length according to Kabat definition. Alignment can be achieved manually or by using, for example, a known computer algorithm for sequence alignment such as NCBI BLAST v2.0 (BLASTP or BLASTN) using standard settings.

Constructs

A construct according to the invention comprises multiple binding agents and is therefore multivalent (e.g. bivalent). The binding agents are physically connected within the construct. These multiple binding agents bind to different targets and therefore the construct is multispecific (e.g. bispecific). Such a construct may comprise (a) no more than one LTBR binding agent and no more than one PD-1 binding agent, (b) no more than one LTBR binding

agent and more than one PD-1 binding agent, (c) more than one LTBR binding agent and no more than one PD-1 binding agent or (d) more than one LTBR binding agent and more than one PD-1 binding agent. If the construct comprises more than one LTBR binding agent and/or PD-1 binding agent, then the LTBR binding agents may be the same or different and the PD-1 binding agents may be the same or different. Suitably the LTBR binding agents are the same. Suitably the PD-1 binding agents are the same. Most suitably, the LTBR binding agents are the same and the PD-1 binding agents are the same.

Such a construct may comprise (a) no more than one LTBR binding agent and no more than one PD-L1 binding agent, (b) no more than one LTBR binding agent and more than one PD-L1 binding agent (c) more than one LTBR binding agent and no more than one PD-L1 binding agent, or (d) more than one LTBR binding agent and more than one PD-L1 binding agent. If the construct comprises more than one LTBR binding agent and/or PD-1 binding agent or PD-L1 binding agent, then the LTBR binding agents may be the same or different and the PD-L1 binding agents may be the same or different. Suitably the LTBR binding agents are the same. Suitably the PD-L1 binding agents are the same. Most suitably, the LTBR binding agents are the same and the PD-L1 binding agents are the same.

The valency and specificity of a construct of the invention may be expressed as a ratio e.g. 1:1, 1:2, etc.. A bispecific construct which is monovalent for PD-1 and monovalent for LTBR may be described as 1:1. A bispecific construct which is bivalent for PD-1 and monovalent for LTBR may be described as 2:1. A bispecific construct which is bivalent for PD-1 and bivalent for LTBR may be described as 2:2, and so on.

A bispecific construct which is monovalent for PD-L1 and monovalent for LTBR may be described as 1:1. A bispecific construct which is bivalent for PD-L1 and monovalent for LTBR may be described as 2:1. A bispecific construct which is bivalent for PD-L1 and bivalent for LTBR may be described as 2:2, and so on.

A construct according to the invention may comprise at least one PD-1 binding agent and at least one PD-L1 binding agent, as well as at least one LTBR binding agent. Such a construct would be considered trispecific. A trispecific construct which is monovalent for PD-1, monovalent for PD-L1 and monovalent for LTBR may be described as 1:1:1. A trispecific construct which is bivalent for PD-1, monovalent for PD-L1 and monovalent for LTBR may be described as 2:1:1, and so on.

A construct according to the invention may comprise at least one further binding agent (such as an antibody or antigen-binding fragment thereof), which is neither an LTBR binding agent, a PD-1 binding agent nor a PD-L1 binding agent.

A particularly suitable construct may comprise or consist of an antibody, wherein the first variable region binds to LTBR and the second variable region binds to PD-1.

A particularly suitable construct may comprise or consist of an antibody, wherein the first variable region binds to LTBR and the second variable region binds to PD-L1.

A further suitable construct may comprise multiple antibodies.

Other suitable constructs may comprise a plurality of antibody fragments as described above, wherein each antibody fragment contributes a binding domain.

The binding agents can be linked to each other directly (i.e. without use of a linker) or via a linker. Suitably, the linker is a polypeptide. Suitably the linker is selected so as to allow binding of the binding agents to their targets. If intended for administration to a subject, the linker is suitably non-immunogenic in the subject to which the binding agents are administered.

In one embodiment the binding agents are linked to each other directly in the form of a bispecific, bivalent antibody. A bispecific antibody may be referred to as a "bsAb".

A particularly suitable bispecific antibody format is that generated via the knobs-into-holes approach (Merchant et al. 1998). Alternatively, or in addition, one or more (most suitably, all) Golay mutations in the CH1/CL regions may be introduced to obtain efficient light chain pairing (Golay et al. 2016).

In one embodiment, bispecific constructs having more than one valency for LTBR and/or PD-1 or PD-L1 may comprise one or more scFvs, wherein each scFv is fused to the C-terminal of an Fc by a linker, such as a linker comprising glycine and serine residues, such as a G_4S linker.

In some embodiments the LTBR binding agent and the PD-1 binding agent or PD-L1 binding agent are as follows:

(a) the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 3 and SEQ ID NO: 4, respectively and more suitably, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 3 and SEQ ID NO: 4, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(b) the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 5 and SEQ ID NO: 6, respectively and more suitably, the PD-1 binding agent comprises a heavy chain

variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 5 and SEQ ID NO: 6, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(c) the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 7 and SEQ ID NO: 8, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 7 and SEQ ID NO: 8, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(d) the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 11 and SEQ ID NO: 12, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 11 and SEQ ID NO: 12, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(e) the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 9 and SEQ ID NO: 10, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 9 and SEQ ID NO: 10, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least

70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(f) the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 13 and SEQ ID NO: 14, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 13 and SEQ ID NO: 14, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively; or

(g) the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 35 and SEQ ID NO: 36, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 35 and SEQ ID NO: 36, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 37 and SEQ ID NO: 38, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 37 and SEQ ID NO: 38, respectively.

Indications

Cancer

In one embodiment, the cancer may be a non-haematological cancer. In a further embodiment, the cancer may be a solid cancer. In this context, the LTBR binding agent is most suitably an LTBR agonist (as discussed in more detail above).

Administration

The invention is typically intended for use with mammalian subjects, in particular human subjects. The construct will typically be administered to a subject in need thereof, in particular a mammalian subject in need thereof, in particular a human subject in need thereof.

The construct may be administered by any suitable route, which may depend on the nature of the specific agents. Exemplary routes include oral, parenteral, buccal, sublingual, nasal or rectal administration. Suitably, the construct is administered parenterally, such as intravenously.

The construct may be provided in the form of a pharmaceutical composition comprising the construct and a pharmaceutically acceptable carrier or excipient.

If delivered orally, the construct may suitably be delivered in a solid pharmaceutical composition (such as a tablet, capsule or lozenge) or in a liquid pharmaceutical composition (such as a suspension, emulsion or solution).

A liquid formulation will generally consist of a suspension or solution of the construct in a suitable liquid carrier e.g. an aqueous solvent such as water, ethanol or glycerine, or a non-aqueous solvent, such as polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring and/or colouring agent.

A tablet formulation can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations, such as magnesium stearate, starch, lactose, sucrose and cellulose.

Suitably, the pharmaceutical composition is in unit dose form, such as a tablet, capsule, vial or prefilled syringe. Suitably the unit dose form is for intravenous administration.

The pharmaceutical composition may for example contain from 0.1% to 99.99% by weight, for example from 10 to 60% by weight, of the active material, depending on the method of administration. The pharmaceutical composition may contain from 0.01% to 99% by weight, for example 40% to 90% by weight, of the carrier, depending on the method of administration. The pharmaceutical composition may contain from 0.05 mg to 2000 mg of the active material, for example from 1.0 mg to 500 mg, depending on the method of administration. The pharmaceutical composition may contain from 50 mg to 1000 mg of the carrier, for example from 100 mg to 400 mg, depending on the method of administration.

The dose of the pharmaceutical composition used will vary in the usual way with the seriousness of the cancer, the weight of the sufferer, and other similar factors. However, as a general guide, suitable unit doses may be 0.05 mg to 1000 mg, more suitably 1.0 mg to 500 mg, and such unit doses may be administered more than once a day, for example two or three a day. Such therapy may extend for a number of weeks, months or longer.

The dose provided to a subject will typically be a safe and effective dose, i.e. an amount providing an acceptable balance of desired benefits and undesired side effects. A “safe and effective amount" is intended to include an amount of a compound that is effective to achieve a desirable effect in treatment of a disease-state. A desirable effect is typically clinically significant and/or measurable, for instance in the context of (a) inhibiting the disease-state, i.e., slowing or arresting its development; and/or (b) relieving the disease-state, i.e., causing regression of the disease state or a reduction in associated symptoms. The safe and effective amount is one that is sufficient to achieve the desirable effect.

For avoidance of doubt, a “safe and effective amount” as recited herein can be achieved by any suitable dosage regimen. Hence, for example, references herein to administering a safe and effective amount of a composition, such as by a particular administration route, include achieving the safe and effective amount via a single dose or by plural doses, such as administered by the specified administration route. For instance, intravenously administering a safe and effective amount includes both intravenously administering a single dose and intravenously administering any plural number of doses, provided that a safe and effective amount is thereby achieved by intravenous administration.

The invention is further exemplified by the following non-limiting examples.

EXAMPLES

Example 1: Generation of bispecific constructs targeting LTBR and Programmed cell death protein (PD-1) or Programmed death-ligand 1 (PD-L1).

Bispecific agonistic constructs with either bivalent or monovalent binding for LTBR and monovalent (1:1) or bivalent (2:2) binding for PD-1 or PD-L1 were prepared.

The variable regions of human-specific constructs were cloned into an expression vector containing the constant region of human IgG1. To ensure the correct assembly of light and heavy chains, the knob-into-holes strategy (Merchant et al. 1998) and Golay mutations in CH1/CL to obtain efficient light chain pairing (Golay et al. 2016) were employed. In addition, to prevent the binding to FcyRs, PGLALA (Pro329Gly, Leu234Ala, and Leu235Ala, Schlothauer et al. 2016) were introduced in the Fc region.

For the murine-specific constructs, the variable regions of mouse-specific constructs were cloned into the expression vector containing the constant region of mouse IgG2a or mouse IgG1. To ensure the correct assembly of light and heavy chains, published mutations in CH1/CL to promote sufficient light chain paring and electrostatic steering mutations into the CH3 domain were introduced to ensure specific heavy chain pairing (Wang et al., 2020). Like

for the human-specific constructs, to prevent the binding to FcyRs, PGLALA (Pro329Gly, Leu234Ala, and Leu235Ala, Schlothauer et al. 2016) were introduced in the Fc region. To generate 2:1 scFvs containing sequences of variable regions, 5G11 (Clone 1.9, VH/VL sequences SEQ ID NOs: 37 and 38 respectively) was fused to the C-terminal of Fc by a G4S linker. An additional disulfide bond was also introduced in the VH44:VL100 position (Weatherill et al., 2012).

In parallel, monospecific control antibody, anti-human LTBR, clone BHA10 (VH and VL sequences disclosed in WO2004002431, provided herein as SEQ ID NO: 1 and 2, respectively) and anti-human PD-L1 construct MF7702 (VH and VL sequences disclosed in WO2018056821A1, provided herein as SEQ ID NO: 11 and 12, respectively) and monospecific control anti-mouse antibody 5G11 (Clone 1.9, VH/VL sequences SEQ ID NOs: 37 and 38 respectively) were cloned into an expression vector containing either the constant region of human IgG1 or rat IgG2a. PGLALA (Pro329Gly, Leu234Ala, and Leu235Ala, ref. Schlothauer et al. 2016) mutations were introduced in the constant region of the human IgG1 heavy chains to abrogate binding to Fc gamma receptors.

The bispecific and monospecific controls were expressed by transient transfection of CHO suspension cells. Antibodies were purified from cell culture supernatants by affinity chromatography using protein A resin and followed by one or two chromatography steps: preparative size exclusion (SEC) and/or ion exchange to enrich the heterodimer. The purity and heterogeneity of the samples were assessed by SEC-HPLC and mass spectrometry. All bispecifics were confirmed to have a monomer content of ≥95% and contain <10 % impurities prior to functional readout.

The resulting bispecific constructs and monospecific controls are listed in Tables 1A and 1B, and the sequences of listed clones' variable regions can be found in Table 2.

Table 1A List of monospecific and bispecific constructs, all of human IgG1 isotype

Construct ID	Format	LTBR clone	PD-1 clone	PD-L1 clone	Species Specificity
1.1	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-1 mAb	BHA10	Pembrolizumab KEGG DRUG ID: D10574	N/A	Human LTBR Human PD-1
1.2	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-1 mAb	BHA10	Nivolumab KEGG DRUG ID: D10316	N/A	Human LTBR Human PD-1

Construct ID	Format	LTBR clone	PD-1 clone	PD-L1 clone	Species Specificity
1.3	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-L1 mAb	BHA10	N/A	Atezolizumab KEGG DRUG ID: D10773	Human LTBR Human & Mouse PD-L1
1.4	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-L1 mAb	BHA10	N/A	MF7702 (WO2018/ 056821A1)	Human LTBR Human PD-L1
1.5	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-L1 mAb	BHA10	N/A	Avelumab KEGG DRUG ID: D10817	Human LTBR Human & Mouse PD-L1
1.6	1:1 heterodimer consisting of LTBR mAb (BHA10 clone) paired with PD-L1 mAb	BHA10	N/A	E12V2 (WO2020/ 011964A1)	Human LTBR Human PD-L1
1.7	Anti-PDL-1 antibody	N/A	N/A	MF7702 (WO2018/ 056821A1)	Human PD-L1
1.8	Anti-huLTBR antibody	BHA10	N/A	N/A	Human LTBR

Table 1B Continued list of monospecific and bispecific constructs

Construct ID	Format	LTBR clone	PD-1 clone	PD-L1 clone	Species Specificity
1.9	Anti-moLTBR antibody (Rat IgG2a)	5G11	N/A	N/A	Mouse LTBR
2.0	2:1 Heterodimeric IgG-scFv, consisting of anti-PDL-L1 mAb with C terminal	5G11	N/A	C5H9v2 (US11174316B2)	Mouse LTBR, human or mouse PD-L1

	fusion anti-LTBR scFv, 5G11 clone (Mouse IgG2a)				

Table 2 Variable region amino acid sequences of LTBR, PD-1 and PD-L1 antibodies

Clone name	Heavy chain variable region	Light chain variable region
BHA10	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTTYYLHWVRQAPGQGLEWMGWIYPGNVHAQYNEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARSWEGFPYWGQGTTVTVSS; SEQ ID NO: 1	DIQMTQSPSSLSASVGDRVTITCKASQNVGINVAWYQQKPGKAPKSLISSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQYDTYPFTFGQGTKVEIK; SEQ ID NO: 2
Pembrolizumab	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSS; SEQ ID NO: 3	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIK; SEQ ID NO: 4
Nivolumab	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSS; SEQ ID NO: 5	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIK; SEQ ID NO: 6
Atezolizumab	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS; SEQ ID NO: 7	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIK; SEQ ID NO: 8

Avelumab	EVQLLESGGGLVQPGGSLR LSCAASGFTFSSYIMMWVR QAPGKGLEWVSSIYPSGGIT FYADTVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAR IKLGTVTTVDYWGQGTLVTV SS; SEQ ID NO: 9	QSALTQPASVSGSPGQSITISCTGTSS DVGGYNYVSWYQQHPGKAPKLMIYD VSNRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYTSSSTRVFGTG TKVTVL; SEQ ID NO: 10
MF7702	QVQLVQSGSELKKPGASVK VSCKASGYTFTNYAINWVRQ APGQGLEWMGWINPNTGN PTYAQGFTGRFVFSLDTSVS TAYLQISSLKAEDTAVYYCA RDRKYVTNWVFAEDFQHW GRGTLVTVSS; SEQ ID NO: 11	DIQMTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSL QSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQSYSTPPTFGQGTKVEIK; SEQ ID NO: 12
E12V2	EVQLLESGGGLVQPGGSLR LSCAASGFTFSSYIMMWVR QAPGKGLEWVSSIYPSGGIT FYADTVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAR IKLGTVTTVDYWGQGTLVTV SS; SEQ ID NO: 13	QSALTQPASVSGSPGQSITISCTGTSS DVGGYNYVSWYQQHPGKAPKLMIYD VSNRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYTSSSTRVFGTG TKVTVL; SEQ ID NO: 14
C5H9v2	EVQLLESGGGLVQPGGSLR LSCAASGFTFSSYAMSWVR QAPGKGLEWVSSIWRNGIVT VYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAK WSAAFDYWGQGTLVTVSS SEQ ID NO: 35	DIQMTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSL QSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQDNGYPSTFGGGTKVEIK SEQ ID NO: 36
5G11	QVQLKESGPGLVQPSQTLSL TCTVSGFSLTTYSVHWVRQ HSGKSLEWMGRMWTDGDT SYNSAFTSRLSISRDTSKSQ VFLKMNSLQTEDTGTYYCAR GYWYFDFWGPGTMVTVSS; SEQ ID NO: 37	DVQMTQSPSYLAASPGESVSIRCKAS KSISNNLAWYQEKPGKANKLLIHSGST LQSGTPSRFSGSGSGTDFTLTIRSLEF EDFAVYYCQQYNEYPYTFGAGTKLEL K; SEQ ID NO: 38

Example 2: Binding to human PD-1 expressing cells

To assay binding on PD-1-expressing cells, PD-1-CHO-K1 stable cell line (Genescript, cat no. M00529), and Jurkat cells (ATCC, cat no TIB-152) stimulated with PHA-L (Sigma, cat no 11249738001) for 24 h were used.

$1x10^6$ cells were resuspended in FACS buffer (1% BSA, 0.1% Sodium Azide in PBS), and 50 µl was added to each well ($1x10^5$ cells/well) of a 96-U bottom plate. The cells were washed once and resuspended in 50 µl/well of FACS buffer containing a 1:3, 11-point series dilutions of constructs 1.1, 1.2 and human IgG1 isotype control (Southern Biotech, cat no 0151K-01), followed by 1h incubation at 4°C. After extensive washing, cells were further stained with a secondary detection antibody, R-Phycoerythrin-conjugated AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (Stratech, cat.no 109-115-098-JIR), for 1h incubation at 4°C. Next, cells were washed and resuspended in FACS buffer and read on Beckman Coulter CytoFLEX. The data was analysed using FloJo V10 and GraphPad Prism v9. The EC50 values can be found in Table **3**.

Table 3 EC50 values of binding curves to PD-1-positive cell lines

Construct ID	CHO-PD-1 [M]	Jurkat [M]
1.1	1.7E-09	1.9E-09
1.2	5.0E-10	8.8E-10
Human IgG1 isotype control	No binding	No binding

The result confirmed that PD-1/LTBR bispecific constructs recognised human PD-1 in native conformation in a concentration-dependent manner.

Example 3 Binding to human PD-L1 expressing cells

To assay binding on PD-L1 expressing cells, PD-L1-CHO-K1 ((BPS Bioscience, cat no 60543) stable cell line was used.

$1x10^6$ cells were resuspended in FACS buffer (1% BSA, 0.1% Sodium Azide in PBS), and 50 ul was added to each well ($1x10^5$ cells/well) of a 96-U bottom plate. The cells were washed once and resuspended in 50ul/well of FACS buffer containing a 1:3, 11-point series dilutions of constructs 1.3 to 1.7, Atezolizumab (Evidentic, batch no B0006B01), which was employed as a positive control, and human IgG1 isotype control followed by 1h incubation at 4°C. After extensive washing, cells were further stained with a secondary detection antibody, R-Phycoerythrin-conjugated AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (Stratech, cat.no 109-115-098-JIR), for 1h incubation at 4°C. Next, cells were washed and

resuspended in FACS buffer and read on Beckman Coulter CytoFLEX. The data was analysed using FloJo V10 and Graphpad Prism v9. The EC50 values can be found in Table 4.

Table 4 EC50 values of binding curves to PD-L1 positive cell lines

Construct ID	CHO-PD-L1
1.3	3.5E-10
Atezolizumab	6.0E-10
1.4	2.1E-10
1.5	1.6E-09
1.6	1.4E-10
1.7	9.8E-11

The result confirmed that PD-L1/LTBR bispecific constructs recognised human PD-L1 in native conformation in a concentration-dependent manner.

Example 4: NFKB activation of HepG2 reporter cells expressing human LTBR cocultured with PD-1-overexpressing CHO cell line

For PD-1-mediated crosslinking, the commercially available PD-1-CHO-K1 recombinant cell line (Genescript, cat no. M00529) was used.

To assess the ability of the PD-1/LTBR bispecific (constructs 1.1 and 1.2) to activate LTBR conditionally, the molecules were tested in NFKB luciferase reporter HEPG2 (Signosis, cat.no SL0017-FP) in the presence or absence of a CHO- overexpressing PD-1 cell line. Clustering endogenous LTBR on HepG2 cells leads to activation of endogenous NfKb and translocation from cytoplasm and nucleus, where it binds to the promoter region and induces luciferase expression. After that, a luciferase substrate/lysis reagent mix (ONE-GLO EX Promega) is added to cells, which allows quantification in the bioluminescence reaction, where enzymatic activity is proportional to luciferase expression.

Monospecific anti-LTBR agonist antibody (construct 1.8) and recombinant human LIGHT (LTBR ligand, Acro Biosystem, cat. no LIT-H5242-100ug) were used as positive controls. In addition, human IgG1 isotype antibody was included as a negative control.

HepG2 NF-Kb Lc (30000 cells/well) were either co-seeded with PD-1- overexpressing CHO cells (20000 cells/well) or seeded alone in their growth media. A 1:10 series dilution of bispecific antibodies and controls was added to the cells and incubated for 6h at 37°C, 5% CO_2, prior to the addition of detection reagents. After 6 h ONE-Glo Ex Solution (Promega, cat. no E8120) was added to the well and incubated for 10 min before being read on a BMG Pherastar FSX plate reader. Background-corrected RLU values were plotted against stimulant

concentration for all conditions tested in GraphPad Prism and shown in Figure 1a and Figure 1b, respectively. The EC50 values can be found in Table 5.

Table 5 EC50 values of HepG2 NF-KB activation-induced luciferase activity

Construct ID	EC50 (monoculture) [M]	EC50 (coculture) [M]
1.1	No activity	1.0e-010
1.2	No activity	2.7e-010
1.8	1.0e-010	3.05e-010
LIGHT	6.9e-011	2.2e-010
Human IgG1 isotype antibody	No activity	No activity

The results in Figures 1a-b confirmed that the PD-1/LTBR bispecific constructs (constructs 1.1 and 1.2) activate LTBR in a PD-1-dependent way, with minimal or no activity in the absence of PD-1. This was in contrast to the controls of monospecific antibody construct 1.8 and LIGHT, which activate HepG2 cells at a similar extent in the presence or absence of PD-1-positive cells. The PD-1/LTBR bispecific constructs in coculture demonstrated comparable activity to the control LIGHT. Due to the complexity of the bispecific constructs, residual impurities in the sample may have reduced activity.

Example 5: *In vitro* PD-1/LTBR bispecific activity in chemokine induction assay using tumour cell line HCC1187 cultured with PD-1 overexpressing CHO cell

An activation assay measuring chemokine secretion was used to evaluate the ability of the LTBR/PD-1 bispecific constructs to conditionally cluster and activate LTBR endogenously expressed on tumour cells (HCC1187).

HCC1187 cells (20000/well) were co-seeded either with PD-1 overexpressing CHO cell line (20000/well) or alone in their growth medium. A 1:10, 7-point serial dilution series in duplicates of bispecific antibodies and controls were prepared in assay medium as 2-fold concentration stocks, added to the cells, and incubated for 24h at 37°C, 5% CO_2.

After incubation, the supernatants were collected and cleared by centrifugation, and the chemokine level was determined by HTRF (Homogeneous Time Resolved Fluorescence) Human CCL-2 kit (62HCCL2PEG) following the manufacturer's instructions. HTRF emissions were measured at two different wavelengths, 620nm (donor) and 665nm (acceptor), on the BMG Pherastar FSX plate reader. A standard curve was analysed in Prism, according to the

manufacturer's recommendation, and used to calculate cytokine concentrations for the test wells. Dose-response curves were plotted using GraphPad Prism Version 9, applying non-linear fits (log(agonist) vs response (variable slope- four parameters). EC50 values can be found in Table 6.

Table 6 EC50 values of upregulation of CCL-2 secreted by HCC1187 in the presence and absence of CHO-PD-1

Construct ID	EC50 (monoculture) [M]	EC50 (co-culture) [M]
1.1	No activity	1.037e-010
1.2	No activity	1.165e-010
1.8	5.699e-010	7.506e-010
LIGHT	3.247e-011	1.013e-010
huIgG1 isotype	No activity	No activity

Stimulation with a LTBR/PD-1 agonist construct results in the concentration-dependent upregulation of chemokine when in co-culture, as represented in Figures 2a and 2b. This was in contrast to the control monospecific antibody 1.8 and LIGHT, which activates HCC1187 cells at a similar extent in the presence or absence of PD-1-positive cells and could therefore potentially lead to toxicity due to the broad expression of LTBR in normal tissue. The PD-1/LTBR bispecific constructs have the ability to conditionally cluster LTBR with comparable potency to the ligand LIGHT.

Example 6: *In vitro* PD-L1/LTBR bispecific activity in chemokine induction assay using tumour cell line HCC1187 cultured with PD-L1 positive CHO line

An activation assay measuring chemokine secretions was used to evaluate the ability of LTBR/PD-L1 bispecific antibodies to activate and conditionally cluster LTBR endogenously expressed on tumour cells (HCC1187). The experiment was carried out as described in Example 5; however, instead of CHO-PD-1, the CHO-PD-L1 (BPS Bioscience, cat no 60543) cell line was employed.

Monospecific anti-LTBR agonist antibody (1.8) and recombinant human LIGHT (LTBR ligand, Acro Biosystem, cat. no LIT-H5242-100 ug) were used as positive controls. In addition, some cases included the monospecific anti-PD-L1 antibody control (construct 1.7) as a negative control. EC50 values can be found in Table 7.

Table 7 EC50 values of secretion of CCL-2 on HCC1187 in the presence and absence of CHO-PD-L1

Construct ID	EC50 (monoculture) [M]	EC50 (coculture) [M]
1.3	No activity	8.5e-011
1.4	No activity	3.7e-011
1.7	No activity	No activity
1.5	No activity	4.2e-011
1.6	No activity	1.5e-011
1.8	6.1e-010	9.6e-010
LIGHT	4.2e-011	5.4e-011

Figures 3 and 4 demonstrated the ability of PD-L1/LTBR bispecific constructs to augment conditionally activate LTBR by promoting receptor clustering in a PD-L1 dependent manner. These findings, together with Example 3, might suggest that decreased PD-L1 affinity led to reduced potency.

Example 7: *In vitro* human PD-1/PD-L1 interaction blockade bioassay

The ability of human PD-L1/LTBR bispecific antibodies to block the PD-1/PD-L1 interaction was measured in a PD-1/PD-L1 Blockade Bioassay (Promega, cat. no J1250), following the manufacturer's instructions. In short, PD-L1 APC CHO-K1 cells were thawed out, washed once in PBS, resuspended in recovery medium (90% Ham's F-12/10% FBS), and plated in a 96 well TC plate. Plates were incubated at 37°C overnight in a CO_2 incubator. The next day, a series dilution of PD-L1/LTBR bispecific and control anti-PD-L1 antibody (Atezolizumab analog, Evidentic, cat. no N7611H31) were added. Human IgG1 isotype control was employed as a negative control (SouthernBiotech, 0151K-01). Jurkat PD-1 reporter cells were thawed in a 37°C water bath, washed once with medium, then diluted into assay medium and added to the plate. The plate was incubated for 6 hours at 37°C overnight in a CO_2 incubator, then removed from the incubator and equilibrated to room temperature for 20 minutes. NanoGlo substrate (Promega cat no. N1 120) was added, and the plates were incubated for 20 minutes at room temperature prior to measurement of luminescence signal (RLU). Data was expressed as fold/background signal.

As shown in Figure 5, all tested PD-L1/LTBR bispecific antibodies have the ability to block PD-1/PD-L1 interaction in a dose-dependent manner. The IC50 values can be found in Table 8.

Table 8: IC50 values for blocking PD-1/PD-L1 interaction in PD-1/PD-L1 Blockade Bioassay

Construct ID	IC50
1.4	4.597e-009
1.7	3.088e-009
1.5	3.624e-010
1.6	5.830e-009
Atezolizumab analog	6.228e-010
huIgG1 isotype control	Inactive

Example 8: *In vitro* activity of a PD-L1/LTBR bispecific antibody in a chemokine induction assay using immortalised mouse lung fibroblasts cultured with a PD-L1 overexpressing cell line

An activation assay measuring chemokine secretion was used to evaluate the ability of a LTBR/PD-L1 bispecific to conditionally activate LTBR endogenously expressed on immortalised mouse lung fibroblasts (Creative Bioarray).

Immortalised mouse lung fibroblasts (10000/well) were co-seeded with a CHO-overexpressing PD-L1 cell line (20000/well) or alone in their growth medium. A 1:10, 7-point serial dilution series in duplicates of bispecific antibodies and controls were prepared in assay medium as 10-fold concentration stocks, added to the cells, and incubated for 48h at 37°C, 5% CO_2. After incubation, the supernatants were collected and cleared by centrifugation, and the chemokine level was determined by mouse CCL-5 Due Set ELISA, (R&D, cat no Y478)and mouse CXCL-10 Due Set ELISA, (R&D, CAT NO DY466) following the manufacturer's instructions. HTRF emissions were measured at two different wavelengths, 620nm (donor) and 665nm (acceptor), on the BMG Pherastar FSX plate reader. A standard curve was analysed in Prism, according to the manufacturer's recommendation, to interpolate cytokine concentrations for the test wells. Dose-response curves were plotted using GraphPad Prism Version 9, applying non-linear fits (log(agonist) vs response (variable slope- four parameters). EC50 values can be found in Table 9.

Table 9: EC50 values for induction of CCL-5 and CXCL-10 on immortalised mouse lung fibroblasts (iMLF) in the presence or absence of a CHO-PD-L1 cell line

Construct ID	EC50 (CCL-5)	EC50 (CXCL-10)
2.0	6.8E-011	1.6E-010
1.9	4.4E-012	1.5E-011

The results shown in Figures 6b and 7b confirm that the mouse PD-L1/LTBR bispecific antibody activates LTBR in a PD-L1-dependent manner, with no activity in the absence of PD-L1 (Figures 6a and 7b). This contrasts to the monospecific control, Clone 2.0.

Example 9: *In vitro* activity of a PD-L1/LTBR bispecific antibody in an ICAM-1 upregulation assay using immortalised mouse lung fibroblasts cultured with a PD-L1 overexpressing cell line

The ability of an LTBR/PD-L1 bispecific antibody to upregulate adhesion molecule ICAM-1 on immortalised lung fibroblasts (iMLF) was measured in a co-culture assay. In short, prior to culture, iMLF were stained with Cell Tracker Deep Red (Thermo Fisher, cat. no. C34565) following the manufacturer's instructions. To confirm conditional LTBR activation, the bispecific and control (clone 2.0) antibodies were tested in monoculture or co-culture, as described below. Stained immortalised mouse lung fibroblasts (10000/well) were co-seeded with CHO- overexpressing PD-L1 cell line (20000/well) or alone in their growth medium. A 1:10, 7-point serial dilution series in duplicates of bispecific antibodies and controls were prepared in assay medium as 10-fold concentration stocks, added to the cells, and incubated for 48h at 37°C, 5% CO_2. After removing the supernatant, the cells were fixed with 5% formaldehyde, following the washing steps. The primary antibody, anti-ICAM-1 (Biolegend, cat. no. 116101), was added in 1:100 dilution following the incubation step. Next, after extensive washing, the secondary antibody, anti-rat IgG AF488 (Invitrogen, cat. no A-21208) and nuclear stain Hoechst 33342 (Invitrogen, cat no. H3570) were added in. After 1 hour of incubation in the dark, the cells were washed extensively. The cells were imaged with the CellInsight CX7 HCS platform (ThermoFisher) using Cellomics Scan V6.6.2 software on Thermo Scientific HCS studio. The “Spot Detector BioApplication V4” was chosen to obtain average fluorescent intensity readings per cell. iMLF cells were segmented upon using CTDR staining so fluorescent readings were obtained specifically from these cells. Data was baseline corrected by subtracting the fluorescence intensity values obtained from the untreated conditions. Data was then plotted as dose-response curves using GraphPad Prism 10, where non-linear regression: log(agonist) vs response – variable slope (four parameters) was applied. The EC50 values can be found in Table 10.

Table 10: EC50 values for upregulation of ICAM-1 on immortalised mouse lung fibroblast (iMLF) in the presence or absence of CHO-PD-L1 cell line

Construct ID	EC50
2.0	7.9E-011
1.9	4.8E-012

As demonstrated in Figures 8a and 8b, the PD-L1/LTBR bispecific antibody activates LTBR in a PD-L1-dependent manner, leading to the upregulation of adhesion molecules (ICAM-1) on fibroblasts with a higher potency than control monospecific antibody. This is in contrast to the non-conditional LTBR monospecific control, which activates iMLF with similar potency in the presence or absence of PD-L1. Activation in the absence of PD-L1, as occurred with the LTBR monospecific control, may lead to toxicity due to the broad expression of LTBR in normal tissue.

The ability to upregulate adhesion molecules is an important feature of the bispecific antibody since it is well-known that ICAM-1 regulates leukocyte recruitment from the circulation to sites of inflammation.

Example 10: *In vitro* mouse PD-1/PD-L1 interaction blockade bioassay

The capability of PD-L1/LTBR bispecific to block the PD-1/PD-L1 pathway was assessed in a luciferase-based mouse PD-1/PD-L1 Blockade assay kit (Promega, cat. no CS303201), following the manufacturer's instructions. Briefly, a mouse PD-L1 expressing CHO-K1 cell line was co-cultured with a PD-1 effector cells line in the presence of series dilution of PD-L1/LTBR Bispecific and monospecific control (anti-mouse PD-L1, clone 10F9.G2, Bu et al. 2022) for 6h. The mouse LTBR monospecific agonist (Clone 2.0) was employed as a negative control. Luciferase reporter activity was determined by luminescence signal (RLU). Data was expressed as fold/background signal. The EC50 values can be found in Table 11.

Table 11: IC50 values for blocking PD-1/PD-L1 interaction in PD-1/PD-L1 Blockade Bioassay

Construct ID	IC50
2.0	Inactive
1.9	3.0E-009
10F9.G2	5.3E-010

As shown in the Figure 9, the PD-L1/LTBR bispecific blocked PD-1/PD-L1 interaction in a dose-deponent manner with a high potency.

The findings in the examples above confirmed that the mouse PD-L1/LTBR bispecific antibody is a bifunctional molecule, targeting PD-L1 (as an antagonist) and LTBR (as an agonist).

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be

understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

The term "or" as used in a phrase such as "A or B" herein is intended to include A alone, B alone, and A and B. Therefore, "a construct comprising (i) an LTBR binding agent and (ii) a PD-1 binding agent or a PD-L1 binding agent" may comprise (i) an LTBR binding agent and (ii) a PD-1 binding agent; (i) an LTBR binding agent and (ii) a PD-L1 binding agent; or (i) an LTBR binding agent and (ii) a PD-1 binding agent and (iii) a PD-L1 binding agent.

Concentration percentages stated herein are w/v unless specified otherwise. The unit prefixes μ and u are used interchangeably herein for 'micro'.

The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process, or use claims and may include, by way of example and without limitation, the claims which follow.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Clauses of the invention:

A series of clauses setting out embodiments of the invention are as follows.

Clause 1A. A construct comprising an LTBR binding agent and a PD-1 binding agent.

Clause 2A. The construct according to clause 1A, wherein the construct comprises an antibody.

Clause 3A. The construct according to clause 2A, wherein the construct comprises an IgG1 antibody.

Clause 4A. The construct according to clause 2A, wherein the construct consists of an antibody.

Clause 5A. The construct according to clause 4A, wherein the construct consists of an IgG1 antibody.

Clause 6A. The construct according to any one of clauses 1A to 3A, wherein the construct comprises one or more antibody fragments.

Clause 7A. The construct according to clause 6A, wherein the construct comprises an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 8A. The construct according to any one of clauses 1A to 7A wherein the construct comprises only one LTBR binding agent.

Clause 9A. The construct according to any one of clauses 1A to 8A wherein the construct comprises only one PD-1 binding agent.

Clause 10A. The construct according to any one of clauses 1A to 7A or 9A wherein the construct comprises more than one LTBR binding agent.

Clause 11A. The construct according to any one of clauses 1A to 8A or 10A wherein the construct comprises more than one PD-1 binding agent.

Clause 12A. The construct according to any one of clauses 1A to 11A wherein the construct comprises a further binding agent which is neither an LTBR binding agent nor a PD-1 binding agent.

Clause 13A. The construct according to clause 12A wherein the further binding agent is a PD-L1 binding agent.

Clause 14A. The construct according to clause 12A wherein the further binding agent is not a PD-L1 binding agent.

Clause 15A. The construct according to any one of clauses 1A to 11A wherein the construct is a bispecific antibody.

Clause 16A. The construct according to clause 15A wherein the construct is a bivalent antibody.

Clause 17A. The construct according to clause 1A wherein the construct is a bispecific pair of scFvs.

Clause 18A. The construct according to either clause 1A or 17A wherein the construct is a bivalent pair of scFvs.

Clause 19A. The construct according to either clause 17A or 18A wherein the scFvs comprise a disulfide bond in the VH44:VL100 position.

Clause 20A. The construct according to any one of clauses 1A to 19A wherein the construct has a 1:1, 2:1, 1:2 or 2:2 format.

Clause 21A. The construct according to clause 20A wherein the construct has a 1:1 or 2:1 format.

Clause 22A. The construct according to clause 1A wherein the construct consists essentially of a bispecific, bivalent antibody.

Clause 23A. The construct according to clause 22A wherein the construct consists of a bispecific, bivalent antibody.

Clause 24A. The construct according to any one of clauses 1A to 23A wherein the LTBR binding agent is a binding polypeptide comprising an LTBR binding domain.

Clause 25A. The construct according to clause 24A, wherein the binding polypeptide comprising an LTBR binding domain is an antibody.

Clause 26A. The construct according to clause 24A, wherein the binding polypeptide comprising an LTBR binding domain is an antibody fragment.

Clause 27A. The construct according to clause 26A, wherein the LTBR binding domain is selected from an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 28A. The construct according to clause 27A, wherein the LTBR binding domain is a variable domain.

Clause 29A. The construct according to clause 26A, wherein the LTBR binding domain is a VH or a VL.

Clause 30A. The construct according to clause 29A, wherein the LTBR binding domain is a VH.

Clause 31A. The construct according to clause 26A, wherein the LTBR binding domain is a VHH.

Clause 32A. The construct according to any one of clauses 1A to 31A wherein the LTBR binding agent specifically binds to LTBR.

Clause 33A. The construct according to clause 32A wherein the LTBR binding agent specifically binds to LTBR in that any other target is bound to with a KD of 10^{-7} M or more, such as 10^{-6} M or more.

Clause 34A. The construct according to any one of clauses 1A to 33A wherein the LTBR binding agent specifically binds to the extracellular domain of LTBR.

Clause 35A. The construct according to any one of clauses 1A to 34A, wherein the LTBR is on the surface of stromal cells.

Clause 36A. The construct according to any one of clauses 1A to 35A, wherein the LTBR is on the surface of cancer cells.

Clause 37A. The construct of any one of any one of clauses 1A to 36A wherein the LTBR binding agent is an LTBR agonist.

Clause 38A. The construct of clause 37A wherein the LTBR agonist is an agonist of NFKB pathways.

Clause 39A. The construct of clause 38A wherein the LTBR agonist (a) induces clustering of LTBR and/or (b) activates the classical NFKB pathway (such as leading to expression of adhesion molecules such as ICAMs, such as ICAM-1, VCAM-1 and/or MAdCAM-1) and/or activates the alternative NFKB pathway (such as leading to expression of chemokines, such as CCL-2, CCL-5, CCL-19, CCL-21, CXCL-13 and/or CXCL-13).

Clause 40A. The construct of any one of clauses 1A to 39A wherein the PD-1 binding agent blocks interaction of PD-1 and PD-L1.

Clause 41A. The construct of any one of clauses 1A to 39A wherein the PD-1 binding agent is a PD-1 antagonist.

Clause 42A. The construct of any one of clauses 1A to 41A wherein the LTBR is human, *Macaca fascicularis* or mouse LTBR.

Clause 43A. The construct of any one of clauses 1A to 42A wherein the construct comprises at least one further LTBR binding agent.

Clause 44A. The construct according to any one of clauses 1A to 43A wherein the binding agent is a binding polypeptide comprising a binding domain.

Clause 45A. The construct according to any one of clauses 1A to 44A wherein the PD-1 binding agent specifically binds to PD-1.

Clause 46A. The construct according to clause 45A wherein the PD-1 binding agent specifically binds to PD-1 in that any other target is bound to with a KD of 10^{-7} M or more, such as 10^{-6} M or more.

Clause 47A. The construct according to any one of clauses 1A to 46A wherein the PD-1 binding agent specifically binds to the extracellular domain of PD-1.

Clause 48A. The construct according to any one of clauses 44A to 47A, wherein the binding polypeptide comprising a PD-1 binding domain is an antibody.

Clause 49A. The construct according to any one of clauses 44A to 47A, wherein the binding polypeptide comprising a PD-1 binding domain is an antibody fragment.

Clause 50A. The construct according to clause 49A, wherein the PD-1 binding domain is selected from an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 51A. The construct according to clause 50A, wherein the PD-1 binding domain is a variable domain.

Clause 52A. The construct according to clause 50A, wherein the PD-1 binding domain is a VH or a VL.

Clause 53A. The construct according to clause 52A, wherein the PD-1 binding domain is a VH.

Clause 54A. The construct according to clause 50A, wherein the PD-1 binding domain is a VHH.

Clause 55A. The construct according to any one of clauses 1A to 54A, wherein the PD-1 is on the surface of stromal cells.

Clause 56A. The construct according to any one of clauses 1A to 55A, wherein the PD-1 is on the surface of cancer cells.

Clause 57A. The construct of any one of clauses 1A to 56A wherein the construct comprises at least one further PD-1 binding agent.

Clause 58A. The construct according to any one of clauses 1A to 57A, wherein the LTBR binding agent and the PD-1 binding agent are directly linked.

Clause 59A. The construct according to any one of clauses 1A to 57A, wherein the LTBR binding agent and the PD-1 binding agent are indirectly linked.

Clause 60A. The construct according to clause 59A, wherein the LTBR binding agent and the PD-1 binding agent are indirectly linked via a linker.

Clause 61A. The construct according to any one of clauses 1A to 60A, wherein the construct comprises an Fc.

Clause 62A. The construct according to clause 61A, wherein the Fc comprises 329Gly, 234Ala and 235Ala.

Clause 63A. The construct of any one of clauses 1A to 62A wherein the is human, *Macaca fascicularis* or mouse and/or the LTBR is human, *Macaca fascicularis* or mouse LTBR.

Clause 64A. The construct of any one of clauses 1A to 63A wherein the LTBR binding agent is capable of binding LTBR on a first cell and the PD-1 binding agent is capable of binding PD-1 on a second cell simultaneously.

Clause 65A. The construct of any one of clauses 1A to 64A wherein the LTBR binding agent is capable of binding LTBR on a cell and the PD-1 binding agent is capable of binding PD-L1 on the same cell simultaneously.

Clause 66A. The construct of any one of clauses 1A to 65A wherein the construct binds to LTBR with an avidity of 1.0E-08 M or less, such as 9.0E-09 M or less, such as 7.0E-09 M or less, such as 6.7E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less.

Clause 67A. The construct of any one of clauses 1A to 66A wherein the construct binds to PD-1 with an avidity of 9.0E-10 M or less, such as 8.0E-10, such as 7.2E-10, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less.

Clause 68A. The construct of any one of clauses 1A to 67A wherein the construct binds to LTBR with an affinity (K_D) of 1.0E-08 M or less, such as 9.0E-09 M or less, such as 7.0E-09 M or less, such as 6.7E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less.

Clause 69A. The construct of any one of clauses 1A to 68A wherein the construct binds to PD-1 with an affinity (K_D) of 9.0E-10 M or less, such as 8.0E-10, such as 7.2E-10, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less.

Clause 70A. The construct of any one of clauses 1A to 69A wherein the LTBR binding agent has an EC50 of 5.0E-09 M or less, such as 3.0E-09 M or less, such as 2.8E-09 M or less.

Clause 71A. The construct of any one of clauses 1A to 70A wherein the PD-1 binding agent has an EC50 of 3.0E-09 M or less, such as 1.1E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 5.4E-10 M or less, such as 3.5E-10 M or less, such as 3.0E-10 M or less, such as 1.7E-10 M or less, such as 1.5E-10 M or less.

Clause 72A. The construct of either clause 70A or 71A wherein the EC50 is established in a chemokine induction assay.

Clause 73A. The construct of clause 72A wherein the chemokine induction is measured with an ELISA.

Clause 74A. The construct of either clause 72A or 73A wherein the chemokine is selected from CCL-2, CCL-5 or CXCL-10.

Clause 75A. The construct of clause 72A wherein the EC50 is established using the assay set out in Example 2.

Clause 76A. The construct of any one of clauses 1A to 75A wherein the construct induces clustering of LTBR.

Clause 77A. The construct of any one of clauses 1A to 76A wherein the LTBR binding agent comprises (a) a heavy chain variable region sharing at least 50% identity with SEQ ID NO: 1 and (b) a light chain variable region sharing at least 50% identity SEQ ID NO: 2.

Clause 78A. The construct of clause 77A wherein the LTBR binding agent comprises (a) a heavy chain variable region sharing at least 70% identity with SEQ ID NO: 1 and (b) a light chain variable region sharing at least 70% identity SEQ ID NO: 2.

Clause 79A. The construct of any one of clauses 1A to 78A wherein the PD-1 binding agent comprises (a) a heavy chain variable region sharing at least 50% identity with SEQ ID NO: 3 or SEQ ID NO: 5 and (b) a light chain variable region sharing at least 50% identity with SEQ ID NO: 4 or SEQ ID NO: 6.

Clause 80A. The construct of clause 79A wherein the PD-1 binding agent comprises (a) a heavy chain variable region sharing at least 70% identity with SEQ ID NO: 3 or SEQ ID NO: 5 and (b) a light chain variable region sharing at least 70% identity with SEQ ID NO: 4 or SEQ ID NO: 6.

Clause 81A. The construct of any one of clauses 1A to 80A wherein the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 3 and SEQ ID NO: 4, respectively and more suitably, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 3 and SEQ ID NO: 4, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 82A. The construct of any one of clauses 1A to 80A wherein the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 5 and SEQ ID NO: 6, respectively and more suitably, the PD-1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 5 and SEQ ID NO: 6, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 83A. A method of activating LTBR in a tumour in a subject, the method comprising administering to the subject the construct according to any one of clauses 1A to 82A.

Clause 84A. The construct of any one of clauses 1A to 82A wherein the construct is for use as a medicament.

Clause 85A. A construct according to clause 84A for use in the treatment of cancer.

Clause 86A. A method of treating cancer comprising administering to a subject in need thereof the construct according to any one of clauses 1A to 82A.

Clause 87A. Use of a construct according to any one of clauses 1A to 82A in the manufacture of a medicament for the treatment of cancer.

Clause 88A. The construct for use, method or use according to any one of clauses 85A to 87A wherein the cancer is a solid cancer.

Clause 89A. The construct for use, method or use according to any one of clauses 85A to 87A wherein the cancer is a non-haematological cancer.

Clause 90A. A set of one or more polynucleotides encoding the construct of any one of clauses 1A to 82A.

Clause 91A. A set of one or more expression vectors collectively comprising the polynucleotides of clause 90A.

Clause 92A. A cell comprising the expression vectors or polynucleotides of either clause 90A or 91A.

Clause 93A. A method of producing a construct according to any one of clauses 1A to 82A, the method comprising culturing the cell of clause 92A under suitable conditions such that the polynucleotide is expressed, and the construct is produced.

A further series of clauses setting out embodiments of the invention are as follows.

Clause 1B. A construct comprising an LTBR binding agent and a PD-L1 binding agent.

Clause 2B. The construct according to clause 1B, wherein the construct comprises an antibody.

Clause 3B. The construct according to clause 2B, wherein the construct comprises an IgG1 antibody.

Clause 4B. The construct according to clause 2B, wherein the construct consists of an antibody.

Clause 5B. The construct according to clause 4B, wherein the construct consists of an IgG1 antibody.

Clause 6B. The construct according to any one of clauses 1B to 3B, wherein the construct comprises one or more antibody fragments.

Clause 7B. The construct according to clause 6B, wherein the construct comprises an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 8B. The construct according to any one of clauses 1B to 7B wherein the construct comprises only one LTBR binding agent.

Clause 9B. The construct according to any one of clauses 1B to 8B wherein the construct comprises only one PD-L1 binding agent.

Clause 10B. The construct according to any one of clauses 1B to 7B or 9B wherein the construct comprises more than one LTBR binding agent.

Clause 11B. The construct according to any one of clauses 1B to 8B or 10B wherein the construct comprises more than one PD-L1 binding agent.

Clause 12B. The construct according to any one of clauses 1B to 11B wherein the construct comprises a further binding agent which is neither an LTBR binding agent nor a PD-L1 binding agent.

Clause 13B. The construct according to clause 12B wherein the further binding agent is a PD-1 binding agent.

Clause 14B. The construct according to clause 12B wherein the further binding agent is not a PD-1 binding agent.

Clause 15B. The construct according to any one of clauses 1B to 11B wherein the construct is a bispecific antibody.

Clause 16B. The construct according to clause 15B wherein the construct is a bivalent antibody.

Clause 17B. The construct according to clause 1B wherein the construct is a bispecific pair of scFvs.

Clause 18B. The construct according to either clause 1B or 17B wherein the construct is a bivalent pair of scFvs.

Clause 19B. The construct according to either clause 17B or 18B wherein the scFvs comprise a disulfide bond in the VH44:VL100 position.

Clause 20B. The construct according to any one of clauses 1B to 19B wherein the construct has a 1:1, 2:1, 1:2 or 2:2 format.

Clause 21B. The construct according to clause 20B wherein the construct has a 1:1 or 2:1 format.

Clause 22B. The construct according to clause 1B wherein the construct consists essentially of a bispecific, bivalent antibody.

Clause 23B. The construct according to clause 22B wherein the construct consists of a bispecific, bivalent antibody.

Clause 24B. The construct according to any one of clauses 1B to 23B wherein the LTBR binding agent is a binding polypeptide comprising an LTBR binding domain.

Clause 25B. The construct according to clause 24B, wherein the binding polypeptide comprising an LTBR binding domain is an antibody.

Clause 26B. The construct according to clause 24B, wherein the binding polypeptide comprising an LTBR binding domain is an antibody fragment.

Clause 27B. The construct according to clause 26B, wherein the LTBR binding domain is selected from an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 28B. The construct according to clause 27B, wherein the LTBR binding domain is a variable domain.

Clause 29B. The construct according to clause 26B, wherein the LTBR binding domain is a VH or a VL.

Clause 30B. The construct according to clause 29B, wherein the LTBR binding domain is a VH.

Clause 31B. The construct according to clause 26B, wherein the LTBR binding domain is a VHH.

Clause 32B. The construct according to any one of clauses 1B to 31B wherein the LTBR binding agent specifically binds to LTBR.

Clause 33B. The construct according to clause 32B wherein the LTBR binding agent specifically binds to LTBR in that any other target is bound to with a KD of 10^{-7} M or more, such as 10^{-6} M or more.

Clause 34B. The construct according to any one of clauses 1B to 33B wherein the LTBR binding agent specifically binds to the extracellular domain of LTBR.

Clause 35B. The construct according to any one of clauses 1B to 34B, wherein the LTBR is on the surface of stromal cells.

Clause 36B. The construct according to any one of clauses 1B to 35B, wherein the LTBR is on the surface of cancer cells.

Clause 37B. The construct of any one of any one of clauses 1B to 36B wherein the LTBR binding agent is an LTBR agonist.

Clause 38B. The construct of clause 37B wherein the LTBR agonist is an agonist of NFKB pathways.

Clause 39B. The construct of clause 38B wherein the LTBR agonist (a) induces clustering of LTBR and/or (b) activates the classical NFKB pathway (such as leading to expression of adhesion molecules such as ICAMs, such as ICAM-1, VCAM-1 and/or MAdCAM-1) and/or activates the alternative NFKB pathway (such as leading to expression of chemokines, such as CCL-2, CCL-5, CCL-19, CCL-21, CXCL-10 and/or CXCL-13).

Clause 40B. The construct of any one of clauses 1B to 39B wherein the PD-L1 binding agent blocks interaction of PD-1 and PD-L1.

Clause 41B. The construct of any one of clauses 1B to 39B wherein the PD-L1 binding agent is a PD-L1 antagonist.

Clause 42B. The construct of any one of clauses 1B to 41B wherein the LTBR is human, *Macaca fascicularis* or mouse LTBR.

Clause 43B. The construct of any one of clauses 1B to 42B wherein the construct comprises at least one further LTBR binding agent.

Clause 44B. The construct according to any one of clauses 1B to 43B wherein the binding agent is a binding polypeptide comprising a binding domain.

Clause 45B. The construct according to any one of clauses 1B to 44B wherein the PD-L1 binding agent specifically binds to PD-L1.

Clause 46B. The construct according to clause 45B wherein the PD-L1 binding agent specifically binds to PD-L1 in that any other target is bound to with a KD of 10^{-7} M or more, such as 10^{-6} M or more.

Clause 47B. The construct according to any one of clauses 1B to 46B wherein the PD-L1 binding agent specifically binds to the extracellular domain of PD-L1.

Clause 48B. The construct according to any one of clauses 44B to 47B, wherein the binding polypeptide comprising a PD-L1 binding domain is an antibody.

Clause 49B. The construct according to any one of clauses 44B to 47B, wherein the binding polypeptide comprising a PD-L1 binding domain is an antibody fragment.

Clause 50B. The construct according to clause 49B, wherein the PD-L1 binding domain is selected from an scFv, Fab, Fab', F(ab')2, variable domain, VH, VL, VHH, diabody or minibody.

Clause 51B. The construct according to clause 50B, wherein the PD-L1 binding domain is a variable domain.

Clause 52B. The construct according to clause 50B, wherein the PD-L1 binding domain is a VH or a VL.

Clause 53B. The construct according to clause 52B, wherein the PD-L1 binding domain is a VH.

Clause 54B. The construct according to clause 50B, wherein the PD-L1 binding domain is a VHH.

Clause 55B. The construct according to any one of clauses 1B to 54B, wherein the PD-L1 is on the surface of stromal cells.

Clause 56B. The construct according to any one of clauses 1B to 55B, wherein the PD-L1 is on the surface of cancer cells.

Clause 57B. The construct of any one of clauses 1B to 56B wherein the construct comprises at least one further PD-L1 binding agent.

Clause 58B. The construct according to any one of clauses 1B to 57B, wherein the LTBR binding agent and the PD-L1 binding agent are directly linked.

Clause 59B. The construct according to any one of clauses 1B to 57B, wherein the LTBR binding agent and the PD-L1 binding agent are indirectly linked.

Clause 60B. The construct according to clause 59B, wherein the LTBR binding agent and the PD-L1 binding agent are indirectly linked via a linker.

Clause 61B. The construct according to any one of clauses 1B to 60B, wherein the construct comprises an Fc.

Clause 62B. The construct according to clause 61B, wherein the Fc comprises 329Gly, 234Ala and 235Ala.

Clause 63B. The construct of any one of clauses 1B to 62B wherein the is human, *Macaca fascicularis* or mouse and/or the LTBR is human, *Macaca fascicularis* or mouse LTBR.

Clause 64B. The construct of any one of clauses 1B to 63B wherein the LTBR binding agent is capable of binding LTBR on a first cell and the PD-L1 binding agent is capable of binding PD-L1 on a second cell simultaneously.

Clause 65B. The construct of any one of clauses 1B to 64B wherein the LTBR binding agent is capable of binding LTBR on a cell and the PD-L1 binding agent is capable of binding PD-L1 on the same cell simultaneously.

Clause 66B. The construct of any one of clauses 1B to 65B wherein the construct binds to LTBR with an avidity of 1.0E-08 M or less, such as 9.0E-09 M or less, such as 7.0E-09 M or less, such as 6.7E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less.

Clause 67B. The construct of any one of clauses 1B to 66B wherein the construct binds to PD-L1 with an avidity of 9.0E-10 M or less, such as 8.0E-10, such as 7.2E-10, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less.

Clause 68B. The construct of any one of clauses 1B to 67B wherein the construct binds to LTBR with an affinity (K_D) of 1.0E-08 M or less, such as 9.0E-09 M or less, such as 7.0E-09 M or less, such as 6.7E-09 M or less, such as 5.0E-09 M or less, such as 1.0E-09 M or less.

Clause 69B. The construct of any one of clauses 1B to 68B wherein the construct binds to PD-L1 with an affinity (K_D) of 9.0E-10 M or less, such as 8.0E-10, such as 7.2E-10, such as 5.0E-10 M or less, such as 1.0E-10 M or less, such as 9.0E-11 M or less, such as 5.0E-11 M or less.

Clause 70B. The construct of any one of clauses 1B to 69B wherein the LTBR binding agent has an EC50 of 5.0E-09 M or less, such as 3.0E-09 M or less, such as 2.8E-09 M or less.

Clause 71B. The construct of any one of clauses 1B to 70B wherein the PD-L1 binding agent has an EC50 of 3.0E-09 M or less, such as 1.1E-09 M or less, such as 1.0E-09 M or less, such as 9.0E-10 M or less, such as 5.4E-10 M or less, such as 3.5E-10 M or less, such as 3.0E-10 M or less, such as 1.7E-10 M or less, such as 1.5E-10 M or less.

Clause 72B. The construct of either clause 70B or 71B wherein the EC50 is established in a chemokine induction assay.

Clause 73B. The construct of clause 72B wherein the chemokine induction is measured with an ELISA.

Clause 74B. The construct of either clause 72B or 73B wherein the chemokine is selected from CCL-2, CCL-5 or CXCL-10.

Clause 75B. The construct of clause 72B wherein the EC50 is established using the assay set out in Example 2.

Clause 76B. The construct of any one of clauses 1B to 75B wherein the construct induces clustering of LTBR.

Clause 77B. The construct of any one of clauses 1B to 76B wherein the LTBR binding agent comprises (a) a heavy chain variable region sharing at least 50% identity with SEQ ID NO: 1 or 37 and (b) a light chain variable region sharing at least 50% identity SEQ ID NO: 2 or 38; such as (a) a heavy chain variable region sharing at least 50% identity with SEQ ID NO: 1 and (b) a light chain variable region sharing at least 50% identity SEQ ID NO: 2.

Clause 78B. The construct of clause 77B wherein the LTBR binding agent comprises (a) a heavy chain variable region sharing at least 70% identity with SEQ ID NO: 1 or 37 and (b) a

light chain variable region sharing at least 70% identity SEQ ID NO: 2 or 38; such as (a) a heavy chain variable region sharing at least 70% identity with SEQ ID NO: 1 and (b) a light chain variable region sharing at least 70% identity SEQ ID NO: 2.

Clause 79B. The construct of any one of clauses 1A to 78B wherein the PD-L1 binding agent comprises (a) a heavy chain variable region sharing at least 50% identity with any one of SEQ ID NOs: 7, 9, 11, 13 and 35 and (b) a light chain variable region sharing at least 50% identity with any one of SEQ ID NOs: 8, 10, 12, 14 and 36; such as (a) a heavy chain variable region sharing at least 50% identity with any one of SEQ ID NOs: 7, 9, 11 and 13 and (b) a light chain variable region sharing at least 50% identity with any one of SEQ ID NOs: 8, 10, 12 and 14.

Clause 80B. The construct of clause 79B wherein the PD-1 binding agent comprises (a) a heavy chain variable region sharing at least 70% identity with any one of SEQ ID NOs: 7, 9, 11, 13 and 35 (b) a light chain variable region sharing at least 70% identity with any one of SEQ ID NOs: 8, 10, 12, 14 and 36; such as (a) a heavy chain variable region sharing at least 70% identity with any one of SEQ ID NOs: 7, 9, 11 and 13 and (b) a light chain variable region sharing at least 70% identity with any one of SEQ ID NOs: 8, 10, 12 and 14.

Clause 81B. The construct of any one of clauses 1B to 80B wherein the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 7 and SEQ ID NO: 8, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 7 and SEQ ID NO: 8, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 82B. The construct of any one of clauses 1B to 80B wherein the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 11 and SEQ ID NO: 12, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 11 and SEQ ID NO: 12, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1

and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 83B. The construct of any one of clauses 1B to 80B wherein the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 9 and SEQ ID NO: 10, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 9 and SEQ ID NO: 10, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 84B. The construct of any one of clauses 1B to 80B wherein the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 13 and SEQ ID NO: 14, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 13 and SEQ ID NO: 14, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 1 and SEQ ID NO: 2, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Clause 85B. The construct of any one of clauses 1B to 80B wherein the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO: 35 and SEQ ID NO: 36, respectively and more suitably, the PD-L1 binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 35 and SEQ ID NO: 36, respectively; and the LTBR binding agent comprises a heavy chain variable region and a light chain variable region sharing at least 50% identity, such as at least 70% identity, such as at least 90% identity, such as at least 95% identity, such at least 99% identity with SEQ ID NO:

37 and SEQ ID NO: 38, respectively and more suitably, the LTBR binding agent comprises a heavy chain variable region and a light chain variable region comprising, such as consisting of, SEQ ID NO: 37 and SEQ ID NO: 38, respectively.

Clause 86B. A method of activating LTBR in a tumour in a subject, the method comprising administering to the subject the construct according to any one of clauses 1B to 85B.

Clause 87B. The construct of any one of clauses 1B to 85B wherein the construct is for use as a medicament.

Clause 88B. A construct according to clause 87B for use in the treatment of cancer.

Clause 89B. A method of treating cancer comprising administering to a subject in need thereof the construct according to any one of clauses 1B to 85B.

Clause 90B. Use of a construct according to any one of clauses 1B to 85B in the manufacture of a medicament for the treatment of cancer.

Clause 91B. The construct for use, method or use according to any one of clauses 88B to 90B wherein the cancer is a solid cancer.

Clause 92B. The construct for use, method or use according to any one of clauses 88B to 90B wherein the cancer is a non-haematological cancer.

Clause 93B. A set of one or more polynucleotides encoding the construct of any one of clauses 1B to 85B.

Clause 94B. A set of one or more expression vectors collectively comprising the polynucleotide of clause 93B.

Clause 95B. A cell comprising the expression vectors or polynucleotides of either clause 93B or 94B.

Clause 96B. A method of producing a construct according to any one of clauses 1B to 85B, the method comprising culturing the cell of clause 95B under suitable conditions such that the polynucleotide is expressed, and the construct is produced.

A further series of clauses setting out embodiments of the invention are as follows.

Clause 1C. A construct comprising (i) an LTBR binding agent and (ii) a PD-1 binding agent or a PD-L1 binding agent.

Clause 2C. The construct according to clause 1, wherein the construct comprises an antibody or fragment thereof.

Clause 3C. The construct according to either clause 1 or 2, wherein the LTBR binding agent is a variable domain and the PD-1 binding agent or PD-L1 binding agent is a variable domain.

Clause 4C. The construct according to any one of clauses 1 to 3 wherein the LTBR binding agent specifically binds to LTBR.

Clause 5C. The construct according to any one of clauses 1 to 4 wherein the PD-1 binding agent specifically binds to PD-1.

Clause 6C. The construct according to any one of clauses 1 to 4 wherein the PD-L1 binding agent specifically binds to PD-L1.

Clause 7C. The construct according to any one of clauses 1 to 6 wherein the LTBR binding agent is an LTBR agonist.

Clause 8C. The construct according to any one of clauses 1 to 7 wherein the LTBR binding agent is capable of binding LTBR on a first cell and the PD-1 binding agent or PD-L1 binding agent is capable of binding PD-1 or PD-L1 on a second cell simultaneously.

Clause 9C. The construct according to any one of clauses 1 to 8 wherein the LTBR binding agent is capable of binding LTBR on a cell and the PD-1 binding agent or PD-L1 binding agent is capable of binding PD-1 or PD-L1 on the same cell simultaneously.

Clause 10C. The construct according to any one of clauses 1 to 9 wherein the construct is a multivalent, bispecific antibody.

Clause 11C. The construct according to clause 10 wherein the construct is a bivalent, bispecific antibody.

Clause 12C. The construct according to any one of clauses 1 to 11 wherein the construct binds to LTBR with an affinity (K_D) of 1.0E-08 M or less.

Clause 13C. The construct according to any one of clauses 1 to 12 wherein the construct binds to PD-1 or PD-L1 with an affinity (K_D) of 9.0E-10 M or less.

Clause 14C. A construct according to any one of clauses 1 to 13 for use in the treatment of cancer.

Clause C15. A polynucleotide encoding the construct of any one of clauses 1 to 13.

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CLAIMS

1. A construct comprising (i) an LTBR binding agent and (ii) a PD-1 binding agent or a PD-L1 binding agent.
2. The construct according to claim 1, wherein the construct comprises an antibody or fragment thereof.
3. The construct according to either claim 1 or 2, wherein the LTBR binding agent is a variable domain and the PD-1 binding agent or PD-L1 binding agent is a variable domain.
4. The construct according to any one of claims 1 to 3 wherein the LTBR binding agent specifically binds to LTBR.
5. The construct according to any one of claims 1 to 4 wherein the PD-1 binding agent specifically binds to PD-1.
6. The construct according to any one of claims 1 to 4 wherein the PD-L1 binding agent specifically binds to PD-L1.
7. The construct according to any one of claims 1 to 6 wherein the LTBR binding agent is an LTBR agonist.
8. The construct according to claim 7 wherein the LTBR agonist is an agonist of NFKB pathways.
9. The construct according to claim 8 wherein the LTBR agonist induces clustering of LTBR.
10. The construct according to either claim 8 or 9 wherein the LTBR agonist activates the classical NFKB pathway (such as leading to expression of adhesion molecules such as ICAMs, such as ICAM-1, VCAM-1 and/or MAdCAM-1).
11. The construct according to any one of claims 8 to 10 wherein the LTBR agonist activates the alternative NFKB pathway (such as leading to expression of chemokines, such as CCL-2, CCL-5, CCL-19, CCL-21, CXCL-10 and/or CXCL-13).
12. The construct according to any one of claims 1 to 11 wherein the PD-1 binding agent blocks interaction of PD-1 and PD-L1.
13. The construct according to any one of claims 1 to 11 wherein the PD-L1 binding agent blocks interaction of PD-1 and PD-L1.
14. The construct according to any one of claims 1 to 13 wherein the LTBR binding agent is capable of binding LTBR on a first cell and the PD-1 binding agent or PD-L1 binding agent is capable of binding PD-1 or PD-L1 on a second cell simultaneously.

15. The construct according to any one of claims 1 to 14 wherein the LTBR binding agent is capable of binding LTBR on a cell and the PD-1 binding agent or PD-L1 binding agent is capable of binding PD-1 or PD-L1 on the same cell simultaneously.
16. The construct according to any one of claims 1 to 15 wherein the construct is a multivalent, bispecific antibody.
17. The construct according to claim 16 wherein the construct is a bivalent, bispecific antibody.
18. The construct according to any one of claims 1 to 17 wherein the construct binds to LTBR with an affinity (K_D) of 1.0E-08 M or less.
19. The construct according to any one of claims 1 to 18 wherein the construct binds to PD-1 or PD-L1 with an affinity (K_D) of 9.0E-10 M or less.
20. A construct according to any one of claims 1 to 19 for use in the treatment of cancer.
21. A polynucleotide encoding the construct of any one of claims 1 to 19.