

BISPECIFIC MOLECULES AND METHODS OF TREATMENT USING THE SAME

FIELD OF THE INVENTION

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The present invention relates to bispecific antigen binding molecules targeting IL-13 and OX40L, antibodies targeting OX40L, pharmaceutical compositions comprising the same, and methods of treatment using the same, e.g. in treating a dermatological disease or condition such as atopic dermatitis.

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BACKGROUND TO THE INVENTION

Atopic dermatitis (AD) is a chronic, relapsing/remitting, noncontagious, pruritic inflammatory systemic skin disease. It is a common and increasingly prevalent disease and in developed nations it is estimated to affect approximately 15-30% of children and 2-10% of adults, with an estimated 20% of people believe to be affected at some point in their life by AD. Whilst AD may occur at any age, it typically starts in childhood and the disease is more common in children. Although many people outgrow the condition, the disease is nonetheless prevalent in adults where it may occur as a persistent disease from childhood or as adult-onset or recurrent AD. AD is often associated with elevated serum IgE levels and sufferers often have a personal or family history of allergic conditions such as allergic rhinoconjunctivitis, asthma, or food allergies (Boguniewicz *et al.* (2017), *J Allergy Clin Immunol Pract* 5(6):1519-1531). AD is associated with an increased risk of infections and of developing serious clinical conditions such as coronary artery disease, ischemic stroke, and other cardiovascular diseases. Symptoms of AD include erythema, edema, xerosis, erosions/excoriations, oozing and crusting, and lichenification, with pruritus (itching) being considered a hallmark of the condition (Kirchhof *et al.* (2018), *J Cutan Med Surg.*, 22(IS) 6S-9S).

The pathophysiology of AD is considered complex with evidence of there being a role for genetic, environmental, and immunologic factors and for a central role of the type 2 inflammatory pathway (Kirchhof *et al.* (2018)). Unlike psoriasis, which is predominantly

driven by Th17, AD has a multifactorial pathophysiology and, as such, optimal efficacy may require the targeting of multiple pathways.

Bispecific antibodies offer the potential to target multiple pathways and to provide a competitive advantage compared to other biologics currently in development for AD.

Numerous cytokines and other factors have been implicated in AD and antibodies targeting a variety of molecules are currently in clinical trials or development for AD.

T helper type 2 (Th2)-associated cytokines have pleiotropic effects on the innate and adaptive immune system. In synergy with tumour necrosis factor α , IL-4 and IL-13 induce thymic stromal lymphopoietin (TSLP) production in keratinocytes and augment the ongoing Th2 skewing of the immune system. IL-4 and IL-13 downregulate mRNA expression and protein synthesis of several structural barrier proteins including filaggrin, involucrin, and loricrin, thus inducing skin barrier dysfunction and aggravation of keratinocyte-mediated immune activation. Due to the Th2-driven inflammatory characteristics of AD, Th2-related molecules may provide an attractive target in order to reduce inflammation and break the detrimental feedback loop.

However, the pathophysiology of AD is complex. Although type-2 mechanisms are dominant, there is increasing evidence that the disorder involves multiple immune pathways. In AD patients, the numbers of OX40L positive dendritic cells (DCs) are highly increased, and also its partner, OX40, is upregulated at the sites of inflammation on infiltrating lymphocytes. Blocking the OX40-OX40L pathway has been shown to be protective in several animal models of human autoimmune diseases such as AD, asthma, irritable bowel disease, transplant rejection, autoimmune diabetes, GvHD, autoimmune encephalomyelitis. Dual IL-13 plus OX-40L blockade will block type 2 response evoked by IL-13 and will have a broad inhibition of the different Th subsets.

Numerous medications and treatments are available for the management of AD. These generally aim at reducing skin inflammation and itching (pruritus), restoring skin barrier function, and improving health-related quality of life (HRQoL). Available therapies

include moisturizing and basic care (e.g. trigger avoidance), phytotherapy, topical therapies, and systemic therapies. Examples of medications used in the treatment of AD include anti-itch creams, antihistamines, topical corticosteroids (TCS), and calcineurin inhibitors. In more recent years antibody-based treatments have been developed.

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Various drawbacks of available treatment methods have been reported in the art including safety and toxicity concerns, development of resistance to treatment (e.g. resistance to TCSs, or to calcineurin inhibitors), as well as patient tolerability and convenience resulting in poor patient compliance. There thus exists a need for the development of new treatment methods for AD.

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Besides AD, IL-13 and OX40L have been identified as important factors in numerous other diseases and conditions, and antagonism of these cytokines may accordingly be useful in the treatment of these diseases and conditions.

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Examples of diseases and conditions in which IL-13 is implicated include: dermatological diseases (e.g. atopic dermatitis, prurigo nodularis, chronic hand eczema, allergic dermatitis), asthma, allergic rhinitis, COPD, cancer, inflammatory bowel disease, fibrosis, scleroderma, and eosinophilic esophagitis (see e.g. May R and Fung M. *Cytokine* 2015;75:89-116, and Gandhi N. *et al. Nat. Rev. Drug Discover.* 2016;15:35-50).

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Examples of diseases and conditions in which OX40L is implicated include: dermatological diseases (e.g. atopic dermatitis, prurigo nodularis, chronic hand eczema, allergic dermatitis), gastrointestinal autoimmune diseases (e.g. ulcerative colitis or Crohn's Disease), allergic encephalitis, graft-vs-host disease, proliferative lupus nephritis, rheumatoid arthritis, inflammatory muscle diseases, inflammatory vasculitis, asthma, and collagen-induced arthritis (see e.g. Murata *et al. J Immunol* 2002; 169:4628-4636 and Hori, *Internat. J. Hematol.* 2006; 83:17-22)).

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30 SUMMARY OF THE INVENTION

The invention provides a bispecific antigen-binding molecule comprising a first antigen binding domain (A) which is an IL-13 antigen binding domain and a second

antigen binding domain (B) which is an OX40L antigen binding domain and wherein the bispecific antigen-binding molecule specifically binds to both IL-13 and OX40L and antagonises both IL-13 signalling from IL-13R and OX40L signalling from OX40; and wherein:

- 5 B is an antibody or antigen binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are:
- a) CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106;
 - 10 b) CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94; and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112; or
 - c) CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100; and CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118.

- Optionally, A is an antibody or antigen binding fragment thereof comprising the
 15 CDRH1, CDRH2 and CDRH3 of SEQ ID NOs: 1, 2 and 3; and the CDRL1, CDRL2 and CDRL3 of SEQ ID NOs: 13, 14 and 15.

- The bispecific antigen-binding molecule of the invention may preferably be a bispecific antibody, most preferably in a symmetric IgG-like format. The relative positions of A and B are preferably such that A is proximal to the Fc region of the molecule and B is
 20 distal to the Fc region. A preferred format is the FIT-Ig format described in WO2015103072. The bispecific antigen-binding molecule of the invention may therefore consist of three polypeptide chains each with domains arranged as follows (N-C terminus):

- Chain 1: VHB-CH1;
 - Chain 2: VLB-CL-VHA-CH1-Fc; and
 - 25 Chain 3: VLA-CL;
- or alternatively as follows:
- Chain 1: VHA-CH1;
 - Chain 2: VLA-CL-VHB-CH1-Fc; and
 - Chain 3: VLB-CL;

- 30 wherein CH1 = heavy chain constant domain 1 preferably of human IgG1, CL = light chain constant domain, Fc = Fc region of antibody, preferably of human IgG1 comprising heavy chain constant domains 2 and 3 (CH2 and CH3).

Preferred embodiments include a bispecific antibody consisting of:

- a) Chain 1: SEQ ID NO: 36; Chain 2: SEQ ID NO: 37; and Chain 3: SEQ ID NO: 38;
- b) Chain 1: SEQ ID NO: 39; Chain 2: SEQ ID NO: 40; and Chain 3: SEQ ID NO: 41; or
- c) Chain 1: SEQ ID NO: 42; Chain 2: SEQ ID NO: 43; and Chain 3: SEQ ID NO: 44;
- d) Chain 1: SEQ ID NO: 45; Chain 2: SEQ ID NO: 46; and Chain 3: SEQ ID NO: 47
- e) Chain 1: SEQ ID NO: 48; Chain 2: SEQ ID NO: 49; and Chain 3: SEQ ID NO: 50; or
- f) Chain 1: SEQ ID NO: 51; Chain 2: SEQ ID NO: 52; and Chain 3: SEQ ID NO: 53.

The invention also provides an OX40L-specific antibody or antigen-binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are:

- a) CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106;
- b) CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94; and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112; or
- c) CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100; and CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118.

The invention also provides a pharmaceutical composition comprising the bispecific antigen-binding molecule of the invention, or the OX40L-specific antibody or antigen-binding fragment thereof of the invention, and one or more of a pharmaceutically acceptable carrier, diluent, excipient, and/or preservative.

The invention also provides a method of treating a disease or condition in a patient, wherein the disease or condition is associated with or mediated by IL-13 and/or OX40L, and wherein the method comprises administering to the patient the bispecific antigen-

binding molecule of the invention, or the OX40L-specific antibody or antigen-binding fragment thereof of the invention, or the pharmaceutical composition of the invention.

5 The invention also provides the bispecific antigen-binding molecule of the invention, or the OX40L-specific antibody or antigen-binding fragment thereof of the invention, or the pharmaceutical composition of the invention, for use in a method of treating a disease or condition in a patient, wherein the disease or condition is associated with or mediated by IL-13 and/or OX40L.

10 The invention also provides the use of the bispecific antigen-binding molecule of the invention, or the OX40L-specific antibody or antigen-binding fragment thereof of the invention, or the pharmaceutical composition of the invention, in the manufacture of a medicament for use in a method of treating a disease or condition in a patient, wherein the disease or condition is associated with or mediated by IL-13 and/or OX40L.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1: representative results of an experiment to measure affinity of binding to IL-13 of exemplary bispecific molecules

20 Figure 2: representative results of an experiment to measure affinity of binding to OX40L of exemplary bispecific molecules

Figure 3: representative results of an experiment to measure affinity of binding to OX40L of exemplary bispecific molecules

Figure 4: representative results of an experiment to measure affinity of binding to OX40L of exemplary bispecific molecules

25 Figure 5: representative results of an experiment to measure IL-13 blocking potency of exemplary bispecific molecules

Figure 6: representative results of an experiment to measure IL-13 blocking potency of exemplary bispecific molecules

30 Figure 7: representative results of an experiment to measure IL-13 blocking potency of exemplary bispecific molecules

Figure 8: representative results of an experiment to measure OX40L blocking potency of exemplary bispecific molecules

Figure 9: representative results of an experiment to measure OX40L blocking potency of exemplary bispecific molecules

Figure 10: representative results of an experiment to measure OX40L blocking potency of exemplary bispecific molecules

Figure 11: representative results of an experiment to show that both arms of exemplary bispecific molecules are simultaneously functional

- 5 Figure 12: representative results of an experiment to show that both arms of exemplary bispecific molecules are simultaneously functional

Figure 13: representative results of an experiment to show internalisation through the IL-13Ra2 receptor of exemplary bispecific molecules

- 10 Figure 14: representative results of an experiment to show that exemplary bispecific molecules do not have agonistic effect through OX40L

Figure 15: representative results of an experiment to measure stability under heat stress of exemplary bispecific molecules.

Figure 16: representative results of the concentration-time PK profiles in Tg mice of exemplary bispecific molecules.

- 15 Figure 17. representative results of the concentration-time PK profiles in cynomolgus monkey of exemplary bispecific molecules.

DETAILED DESCRIPTION OF THE INVENTION

- 20 It is to be understood that different applications of the disclosed products and methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting.

- 25 In addition, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, the term “an agent” includes a reference to a single agent as well as a plurality of agents (including mixtures of agents).

- 30 As used herein, the term “about” as used in relation to a numerical value means, for example, $\pm 25\%$ of the numerical value, preferably $\pm 15\%$, more preferably $\pm 10\%$, more preferably still $\pm 5\%$, and most preferably $\pm 2\%$ or $\pm 1\%$. Where necessary, the word “about”

may be omitted from the definition of the invention.

The term “polypeptide” is used herein in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogues, or other peptidomimetics. The term “polypeptide” therefore includes short peptide sequences, longer polypeptides and proteins. The term “amino acid” may refer to either natural and/or unnatural or synthetic amino acids, including both D or L optical isomers, as well as amino acid analogues and peptidomimetics.

The term “antibody” as referred to herein includes whole antibodies and any antigen binding fragment thereof. Whilst an antibody may assume a variety of forms and characteristics, an antibody typically refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding fragment thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR).

An antibody may comprise or consist of a complete antibody molecule having full length heavy and light chains or an antigen-binding fragment thereof. The term “antigen-binding fragment” or the like of an antibody includes a reference to a portion of an antibody that retains the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. The antibodies and antigen binding fragments thereof may be, but are not limited to Fab, modified Fab, Fab’, modified Fab’, F(ab’)₂, Fv, single chain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, *Nature Biotech.* 23(9):1126-1136; Adair and Lawson, 2005, *Drug Design Reviews - Online* 2(3), 209-217). Methods for creating and manufacturing these antibody fragments

are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181) and the fragments may be screened for utility in the same manner as intact antibodies. Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in WO 2005/003169, WO 2005/003170 and WO 5 2005/003171 and Fab-dAb fragments described in WO 2009/040562. Multivalent antibodies may comprise multiple specificities or may be monospecific (see for example WO 92/22853 and WO 05/113605 and the DVD-Ig as disclosed in WO 2007/024715, or the so-called (FabFv)₂Fc described in WO 2011/030107). An alternative multi-specific antigen-binding fragment comprises a Fab linked to two scFvs or dsscFvs, each scFv or 10 dsscFv binding the same or a different target (e.g., one scFv or dsscFv binding a therapeutic target and one scFv or dsscFv that increases half-life by binding, for instance, albumin). Such antibody fragments are described in WO 2015/197772, which is hereby incorporated by reference in its entirety and particularly with respect to the discussion of antibody fragments. These antibody fragments may be obtained using conventional 15 techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

The term antibody encompasses monoclonal antibodies, polyclonal antibodies, monospecific antibodies, and multispecific (e.g. bispecific) antibodies, as well as antigen- 20 binding fragments thereof. A multispecific antibody is capable of binding to at least two target epitopes, typically on separate antigens. In the case of a bispecific antigen-binding molecule of the invention, the bispecific antigen-binding molecule is capable of binding to two separate antigens (IL-13 and OX40L). A preferred format of bispecific molecule is a bispecific antibody, and particularly preferred is a symmetric IgG-like format. Examples 25 include Dual Variable Domain (DVD)-Ig, 2+2 CrossMab, and Fabs-in-Tandem (FIT)-Ig molecules.

The term antibody encompasses antibodies of any class (e.g. an IgG, IgE, IgM, IgD, IgA or IgY antibody) or subclass (e.g. IgA1, IgA2, IgG1, IgG2, IgG3 or IgG4). An antibody may, 30 for instance, be a chimeric antibody, a CDR-grafted antibody, a nanobody, a human or humanised antibody, or an antigen-binding fragment of any of the foregoing. Typically, the antibody is a human antibody or a human antibody derivative. The term "human

antibody derivative” and the like refers to any modified form of the human antibody, e.g. a conjugate of the antibody and another agent (e.g. a drug) or antibody. Fully human antibodies are those antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or
5 substantially identical to sequences of human origin, but not necessarily from the same antibody.

The terms "disease", "disorder" and "condition" may be used herein interchangeably, unless the context clearly dictates otherwise.

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All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

IL-13 and OX40L

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The terms “targets” and “directed to” and the like may be used interchangeably herein. The present invention provides bispecific antigen-binding molecules which target (i) IL-13 and (ii) OX40L. The molecules are useful in the methods of the present invention, e.g. to treat a dermatological disease or condition such as AD. The general class of the molecule of the
20 invention may be referred to herein as “an anti-IL-13 and OX40L bispecific antigen-binding molecule”.

A bispecific antigen-binding molecule which targets IL-13 may specifically bind to IL-13 and a bispecific antigen-binding molecule which targets OX40L may specifically bind to OX40L. Specific binding typically means that the molecule does not exhibit
25 significant binding to anything other than the intended target(s). Some binding off-target may be acceptable if it is to another target which is generally expressed in different organs or by different cell types to those expressing IL-13 or OX40L.

By binding to a partner of each of the IL-13/IL-13R and OX40L/OX40 interactions, the bispecific antigen-binding molecule antagonises both IL-13 and OX40L. The bispecific
30 antigen-binding molecule of the invention is therefore an IL-13 antagonist as well as an OX40L antagonist. The term “antagonist” and the like includes a reference to a substance (e.g. a bispecific antigen-binding molecule of the invention) which inhibits or attenuates

one or more biological activities of a ligand molecule (e.g. IL-13 or OX40L), such as intracellular signalling mediated by the ligand molecule when bound to its receptor. An antagonist may inhibit or attenuate the binding or interaction of the ligand with its receptor (by binding to either the target or the receptor) but could also for instance inhibit the dimerization of the receptor without affecting the binding of the ligand to the receptor. In some embodiments, the binding between the ligand and its receptor is completely or substantially blocked. An antagonist may, for instance, be a neutralising antibody.

In some embodiments, an antagonist may inhibit dimerization or multimerization of the receptor without inhibiting or attenuating the binding or interaction of the ligand with one or more of the receptors. For example, in the case of IL-13 antagonism, the antigen-binding molecule may inhibit IL-13 signalling by inhibiting IL4Ra/IL13Ra1 dimerization without inhibiting the binding of IL-13 to IL13Ra1 or IL13Ra2. In some embodiments, the dimerization of the receptor may be completely or substantially inhibited. As another example, in the case of OX40L antagonism, the antigen-binding molecule may inhibit OX40L signalling by inhibiting OX40 multimerization without inhibiting the binding of OX40L to OX40. In some embodiments, the multimerization of the receptor may be completely or substantially inhibited.

The expression "IL-13" (interleukin-13) as used herein includes any native mammalian IL-13 sequence (e.g. human, non-human primate (e.g. monkey) or mouse), preferably human IL-13. The term encompasses full-length, unprocessed IL-13 as well as any form of IL-13 resulting from cellular processing. The term encompasses wild type proteins, naturally occurring variants, e.g. splice variants or allelic variants, as well as any other isoforms and mutant forms, as well as modified and unmodified forms of any of the foregoing. A reference to IL-13 includes proteins which may, for instance, be produced recombinantly or by synthetic methods and which have the same amino acid sequence as a naturally occurring or endogenous mammalian IL-13. Where the corresponding mammal is human, the protein may be referred to as hIL-13. The nucleotide and amino acid sequences of IL-13 from various species have been determined and are readily available from public sequence databases. The term hIL-13 encompasses the exemplary hIL-13 sequences accessible at UniProtKB Accession No. P35225 or as disclosed in WO 2022/079036 (with

and without the signal peptide), as well as biologically active fragments thereof and other hIL-13 sequences that may arise from the cellular processing thereof. In certain instances, the IL-13 sequences may comprise a signal peptide which may optionally be an exogenous, i.e. non-native, signal peptide. In other instances, the IL-13 proteins are mature proteins
5 without a signal peptide.

The expression "IL-13R" (interleukin-13 receptor) as used herein typically refers to the "shared" IL-4 / IL-13 receptor which consists of a complex formed by an IL-4R α chain subunit and an IL-13R α 1 chain subunit but may also include the "private" IL-13 receptor
10 which consists of a single IL-13R α 2 chain subunit. The heterodimerization of IL-4R α and IL-13R α 1 to form IL-13R is induced by IL-13 binding and promotes the activation of the Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway, resulting in phosphorylation of STAT6. Phosphorylated STAT6 acts as a transcription factor activating many genes. IL-13 can also bind with very high affinity to the single
15 chain IL-13R α 2, which is thought to function as a negative regulator of IL-13.

The IL-13R is typically mammalian (e.g. human, non-human primate (e.g. monkey) or mouse), preferably human. The term encompasses full-length, unprocessed subunits as well as any form of the subunit resulting from cellular processing. The term encompasses
20 wild type proteins, naturally occurring variants, e.g. splice variants or allelic variants, as well as any other isoforms and mutant forms, as well as modified and unmodified forms of any of the foregoing. A reference to IL-13R includes proteins which may, for instance, be produced recombinantly or by synthetic methods and which have the same amino acid sequence as a naturally occurring or endogenous mammalian IL-13R subunit. Where the
25 corresponding mammal is human, the protein may be referred to as hIL-13R. The nucleotide and amino acid sequences of IL-13R subunits from various species have been determined and are readily available from public sequence databases. Exemplary sequences of hIL-13R are also disclosed in WO 2022/079036. The term hIL-13R encompasses a protein comprising or consisting of these sequences, as well as biologically
30 active fragments thereof and other sequences that may arise from the cellular processing thereof. In certain instances, the above sequences may comprise a signal peptide which

may optionally be an exogenous, i.e. non-native, signal peptide. In other instances, the IL-13R proteins are mature proteins without a signal peptide.

There are different modes of binding able to antagonise IL-13 signalling. For example, lebrizumab inhibits IL-13 signalling by binding to IL-13 with very high affinity and inhibiting dimerization of IL13Ra1 with IL-4R α (Ultsch et al. J Mol Biol. 2013), while tralokinumab prevents IL-13 from binding to both IL-13Ra1 and IL-13Ra2 (Popovic et al. J Mol Biol. 2017). In both cases the phosphorylation of STAT6 and subsequent gene expression consequences are prevented.

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Assays to determine antagonism of IL-13 are known and any suitable assay may be used, such as those described in the Examples. Suitable assays include the use of cell lines which have been designed to assess the activation of the STAT6 pathway induced by IL-13. Suitable cell lines may express a reporter gene under the control of a STAT6 responsive promoter. For example, the cells may be modified to express secreted embryonic alkaline phosphatase (SEAP) under the control of the IFN- β minimal promoter fused to four STAT6 binding sites. In these cells, activation of the STAT6 pathway induces the expression of the reporter gene (e.g., SEAP). Expression of the reporter may be detected using any known method. For example, secretion of SEAP into the supernatant can readily be assessed using a SEAP detection reagent, such as QUANTI-Blue Solution. The cell line used in the assay must have a fully active STAT6 signalling pathway. Therefore, when the cell line is HEK (e.g., HEK293), the human STAT6 gene must be stably transfected. In one embodiment, the HEK cells used in the assay for IL-13 antagonism are HEK-Blue IL-4/IL-13 reporter cells (InvivoGen).

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Alternatively, an IL-13-induced STAT6 phosphorylation assay in human primary keratinocytes (two donors) may be used to assay for IL-13 antagonism. For instance, primary keratinocytes (e.g. NHEK, Adult skin, Lonza) may be cultured in keratinocyte serum-free medium (SFM) supplemented with bovine pituitary extract (25 μ g/ml) and recombinant epidermal growth factor (EGF) (0.25 ng/ml). The keratinocytes are deprived of growth factors prior to stimulation and then stimulated with a serial dilution of human recombinant IL-13. After 10 to 60 stimulation, the intracellular levels of

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pSTAT6 are then determined, e.g., using AlphaLISA SureFire Ultra p-STAT6 (Tyr641) Assay Kit (ALSU-PST6-A500, PerkinElmer).

The expression "OX40L" (OX40 Ligand, Tumor necrosis factor ligand superfamily member 4) as used herein includes any native mammalian OX40L sequence (e.g. human, non-human primate (e.g. monkey) or mouse), preferably human OX40L. The term encompasses full-length, unprocessed OX40L as well as any form of OX40L resulting from cellular processing. The term encompasses wild type proteins, naturally occurring variants, e.g. splice variants or allelic variants, as well as any other isoforms and mutant forms, as well as modified and unmodified forms of any of the foregoing. A reference to OX40L includes proteins which may, for instance, be produced recombinantly or by synthetic methods and which have the same amino acid sequence as a naturally occurring or endogenous mammalian OX40L. Where the corresponding mammal is human, the protein may be referred to as hOX40L. The nucleotide and amino acid sequences of OX40L from various species have been determined and are readily available from public sequence databases. The term hOX40L encompasses the full-length, unprocessed 183 amino acid sequence of OX40L in WO 2022/079036 or accessible at UniProtKB accession number P23510, as well as biologically active fragments and other hOX40L sequences that may arise from the cellular processing thereof such as by proteases or alternative splicing (e.g. residues 51-183 of the full length hOX40L protein). An exemplary hOX40L sequence comprises residues 51-183 of the said full length sequence and is also disclosed in WO 2022/079036.

The expression "OX40" (OX40, Tumor necrosis factor receptor superfamily member 4) as used herein includes any native mammalian OX40 sequence (e.g. human, non-human primate (e.g. monkey) or mouse), preferably human OX40. The term encompasses full-length, unprocessed OX40 as well as any form of OX40 resulting from cellular processing. The term encompasses wild type proteins, naturally occurring variants, e.g. splice variants or allelic variants, as well as any other isoforms and mutant forms, as well as modified and unmodified forms of any of the foregoing. A reference to OX40 includes proteins which may, for instance, be produced recombinantly or by synthetic methods and which have the same amino acid sequence as a naturally occurring or endogenous mammalian OX40.

Where the corresponding mammal is human, the protein may be referred to as hOX40. The nucleotide and amino acid sequences of OX40 from various species have been determined and are readily available from public sequence databases. The term hOX40 encompasses a
5 protein comprising or consisting of the exemplary OX40 sequence accessible at UniProtKB Accession No P43489 or as set forth in SEQ ID NO: 8 of WO 2022/079036, as well as biologically active fragments thereof and other sequences that may arise from the cellular processing thereof. In certain instances, the sequence may comprise a signal peptide which may optionally be an exogenous, i.e. non-native, signal peptide. In other
10 instances, the OX40 protein is a mature protein without a signal peptide.

OX40L is a member of the tumor necrosis factor superfamily that arranges forming a functional homotrimer. Three copies of OX40 bind to the trimeric ligand to form the OX40-OX40L complex. Receptor clustering is required for full activation of the signalling
15 pathway. Antibodies binding to both OX40 and to OX40L have been described as able to antagonise intracellular signalling induced by OX40L-OX40 complex formation (Compaan et al. Structure 2006; Croft et al. Immunol Rev. 2009; Webb et al. Review. Clinic Rev Allerg Immunol. 2016; Guttman-Yassky et al. J Allergy Clin Immunol.

20 Assays to determine antagonism of OX40L are known and any suitable assay may be used, such as those described in the Examples. Suitable assays include those designed to detect bioactive OX40L by monitoring the activation of the NF- κ B and AP-1 pathways. For example, the assays may use cell lines which express a reporter gene under the control of a NF- κ B/AP-1-responsive promoter. According to these assays, binding of human OX40L to
25 the homotrimeric OX40 receptor on the surface of these cells triggers a signalling cascade leading to the activation NF- κ B and the subsequent production of the reporter gene.

Alternatively, OX40L-induced IL2 and IFN γ expression in CD3 positive T cells or PBMCs (from two donors) treated with suboptimal concentrations of anti-CD3 (primed) may be
30 used to assay for OX40L-OX40 antagonism. For instance, PBMCs may be purified from fresh whole human blood from donors by density gradient centrifugation. The isolated PBMCs may then be plated in RPMI 1640 supplemented with 10% FBS, 2 mM L-

glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin and the cells then incubated with suboptimal concentrations of anti-CD3 and a serial dilution of human recombinant OX40L. The cells are incubated overnight and then IL2 and IFN γ levels in the culture supernatant may be measured by ELISA (R&D Systems, #D2050 and #DY285) according to the manufacturer's instructions.

Alternatively, OX40L back-signalling in OX40L expressing cells can be used to assay for OX40L-OX40 antagonism. For instance, cells of the THP-1 cell line may be primed by LPS and incubated in the presence of OX40 (ECD) and/or benchmark and identified antibodies. IL-6 levels may be measured by ELISA (R&D Systems, #D6050) according to the manufacturer's instructions. The internalization of OX40L may also be measured by FACS in the same conditions using serial dilutions of biotin-conjugated human OX40 protein or with biotin-conjugated anti-OX40L antibodies and stained with streptavidin-allophycocyanin for example.

The content of all database entries recited in the preceding paragraphs or elsewhere herein are hereby incorporated by reference in their entireties.

The terms IL-13, IL-13R, OX40L and OX40 as used herein typically refer to hIL-13, hIL-13R, hOX40L and hOX40, respectively. Accordingly, unless the context clearly indicates otherwise, references herein to IL-13, IL-13R, OX40L and OX40 are to be understood as being a reference to the human version thereof.

Anti-OX40L antibodies

The invention provides an OX40L-specific antibody or antigen-binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are:

- a) CDRH1: SEQ ID NO: 4, CDRH2: SEQ ID NO: 5, CDRH3: SEQ ID NO: 6; and CDRL1: SEQ ID NO: 16, CDRL2: SEQ ID NO: 17, CDRL3: SEQ ID NO: 18;
- b) CDRH1: SEQ ID NO: 7, CDRH2: SEQ ID NO: 8, CDRH3: SEQ ID NO: 9; and CDRL1: SEQ ID NO: 19, CDRL2: SEQ ID NO: 20, CDRL3: SEQ ID NO: 21; or

c) CDRH1: SEQ ID NO: 10, CDRH2: SEQ ID NO: 11, CDRH3: SEQ ID NO: 12;
and CDRL1: SEQ ID NO: 22, CDRL2: SEQ ID NO: 23, CDRL3: SEQ ID NO: 24.

Each of the above CDRs is defined according to the Chothia system.

The same three OX40L-specific antibodies or antigen-binding fragments thereof
5 may alternatively be described by CDRs defined according to the Kabat system:

a) CDRH1: SEQ ID NO: 83, CDRH2: SEQ ID NO: 84, CDRH3: SEQ ID NO: 85;
and CDRL1: SEQ ID NO: 101, CDRL2: SEQ ID NO: 102, CDRL3: SEQ ID NO: 103;

b) CDRH1: SEQ ID NO: 89, CDRH2: SEQ ID NO: 90, CDRH3: SEQ ID NO: 91;
and CDRL1: SEQ ID NO: 107, CDRL2: SEQ ID NO: 108, CDRL3: SEQ ID NO: 109; or

10 c) CDRH1: SEQ ID NO: 95, CDRH2: SEQ ID NO: 96, CDRH3: SEQ ID NO: 97;
and CDRL1: SEQ ID NO: 113, CDRL2: SEQ ID NO: 114, CDRL3: SEQ ID NO: 115.

The same three OX40L-specific antibodies or antigen-binding fragments thereof
may alternatively be described by CDRs defined according to the IMGT system:

a) CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88;
15 and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106;

b) CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94;
and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112; or

c) CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100;
and CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118.

20

The different CDR systems may be used interchangeably, but the IMGT system is typically preferred.

The OX40L-specific antibody may be monospecific. Binding and other characteristics of
25 the OX40L-specific antibody may otherwise be as described herein in connection with
bispecific molecules of the invention, limited where appropriate to the OX40L-binding
parts of the bispecific molecules.

The OX40L-specific antibody or antigen-binding fragment thereof of the invention may
30 comprise a heavy chain variable region sequence (VH_B) and a light chain variable region
sequence (VL_B), wherein:

- a) VH_B is a polypeptide comprising or consisting of SEQ ID NO: 27, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VL_B is a polypeptide comprising or consisting of SEQ ID NO: 28, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto;
- 5 b) VH_B is a polypeptide comprising or consisting of SEQ ID NO: 29, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VL_B is a polypeptide comprising or consisting of SEQ ID NO: 30, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; or
- 10 c) VH_B is a polypeptide comprising or consisting of SEQ ID NO: 31, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VL_B is a polypeptide comprising or consisting of SEQ ID NO: 32, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto;
- optionally wherein variation in amino acid identity is not permitted in the CDR sequences of the said VH_B and VL_B sequences, optionally as defined according to the any one of the IMGT, Kabat, or Chothia system, preferably the IMGT system. That is, variation is permitted in the framework regions only.
- 15

The OX40L-specific antibodies of the present invention specifically bind OX40L. As used herein, the term “specifically binds” and the like may refer to where there is a binding

20 affinity which is characterised by a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM.

Accordingly, an OX40L-specific antibody of the invention may specifically bind OX40L

25 with a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM.

Various methods exist in the art for determining the value of the dissociation constant K_D ,

30 such as surface plasmon resonance (SPR). In the context of the present invention, the K_D value is preferably determined using Surface Plasmon Resonance at 25°C and/or 37°C e.g. with a Biacore™ system.

The OX40L-specific antibody or antigen-binding fragment thereof of the invention may be a chimeric, humanized or human or antibody. The OX40L-specific antibody or antigen-binding fragment thereof may comprise an IgG1, IgG2, IgG3 or IgG4 constant region, optionally a human IgG1, IgG2, IgG3 or IgG4 constant region. The OX40L-specific antibody or antigen-binding fragment thereof may comprise a human IgG1 Fc (Fragment crystallizable) region sequence, said sequence comprising a modification of the human wildtype sequence to reduce Fc receptor binding (optionally the L234A/L235A (LALA) modification) and/or a modification of the human wildtype sequence to increase serum half-life (optionally the M252Y/S254T/T256E (YTE) modification).

Also disclosed herein is a reference anti-OX40L antibody. Sequences of this reference anti-OX40L antibody are described in the Examples.

15

Anti-IL-13 antibodies

A preferred example of an IL-13 specific binding domain is an antibody or antigen binding fragment thereof specific for IL-13, comprising the CDRH1, CDRH2 and CDRH3 of SEQ ID NOs: 1, 2 and 3; and the CDRL1, CDRL2 and CDRL3 of SEQ ID NOs: 13, 14 and 15.

Said antibody or antigen binding fragment thereof may comprises a heavy chain variable region sequence (VH_A) and a light chain variable region sequence (VL_A), wherein VH_A is a polypeptide comprising or consisting of SEQ ID NO: 25, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VL_A is a polypeptide comprising or consisting of SEQ ID NO: 26, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; optionally wherein variation in amino acid identity is not permitted in the CDR sequences of the said VH_A and VL_A sequences, optionally as defined according to the any one of the IMGT, Kabat, or Chothia system, preferably the Kabat system. That is, variation is permitted in the framework regions only.

Said antibody may be Lebrikizumab.

Also disclosed herein is a reference anti-IL13 antibody. Sequences of this reference anti-IL-13 antibody are described in the Examples.

Bispecific antigen-binding molecules

5 As used herein the term “bispecific antigen-binding molecule” encompasses any antigen binding construct which has the ability to bind, and preferably neutralize biological function of, two different antigens/targets (in the invention IL-13 and OX40L). A bispecific antigen-binding molecule can take a variety of forms and may for instance be a protein, polypeptide or molecular complex. A bispecific antigen-binding molecule may, for
10 instance, be a single multifunctional polypeptide, or it may be a multimeric complex of two or more covalently or non-covalently associated polypeptides. In an exemplary embodiment the bispecific antigen-binding molecule is a bispecific antibody.

The bispecific binding molecules of the present invention are preferably superior in at least
15 one characteristic relative to mono-specific drugs (“monads”) each directed to one of the two targets (e.g. when administered as a combination) and/or relative to a different bispecific antigen-binding molecule which has an IL-13 binding domain and an OX40L binding domain. An exemplary molecule of this type in FIT-Ig format is disclosed herein and may be referred to as BsAb007 or as a prototype.

20 The bispecific binding molecules of the present invention may advantageously display higher potency and/or efficacy and/or suitability for use in treating the disease or condition associated with or mediated by IL-13 and/or OX40L (e.g. AD) relative to treatment regimens which use a combination of mono-specific drugs each directed to one of the two
25 targets, and/or relative to a treatment regimen using a different bispecific antigen-binding molecule which has an IL-13 binding domain and an OX40L binding domain. The bispecific binding molecules of the present invention may advantageously display higher stability or lower immunogenicity than such comparisons. Characteristics of the bispecific antigen binding molecules of the invention may include superior efficacy to monads or to a
30 different bispecific antigen-binding molecule, and/or they may provide comparable or superior PK values (permitting reduced dosing frequency) and/or comparable or superior safety profiles to monads or to a different bispecific antigen-binding molecule. The

bispecific antibodies of the present invention may be more suitable for clinical use, for example because it is more convenient and less painful to inject a single drug than to provide two separate injections of two monads (which may lead to increased patient compliance), or because it is more challenging (in terms of acceptable viscosity / stability) or more costly to formulate two monads as a single injection. Characteristics of exemplary bispecific antigen binding molecules of the invention are demonstrated in the Examples.

The bispecific antigen-binding molecules of the present invention include anti-IL-13/OX40L binding molecules which specifically bind IL-13 and OX40L. As used herein, the term “specifically binds” and the like may refer to where there is a binding affinity which is characterised by a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM.

Accordingly, an anti-IL-13/OX40L bispecific antigen-binding molecule of the invention may specifically bind IL-13 with a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM and/or may specifically bind OX40L with a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM.

Various methods exist in the art for determining the value of the dissociation constant K_D , such as surface plasmon resonance (SPR). In the context of the present invention, the K_D value is preferably determined using Surface Plasmon Resonance at 25°C and/or 37°C e.g. with a Biacore™ system. Another preferable method for determining the value of the dissociation constant K_D is KinExA (Kinetic Exclusion Assay) technology.

The anti-IL-13/OX40L bispecific antigen-binding molecule of the invention preferably binds IL-13 with affinity which is at least 0.1x the affinity for IL-13 of the antibody lebrikizumab, when measured in the same assay; and/or preferably binds OX40L with affinity which is at least 0.1x the affinity for OX40L of the antibody amltelimab, when

measured in the same assay. The anti-IL-13/OX40L bispecific antigen-binding molecule of the invention more preferably binds IL-13 with affinity which is at least 0.5x the affinity for IL-13 of the antibody lebrikizumab, when measured in the same assay; and/or preferably binds OX40L with affinity which is at least 0.5x the affinity for OX40L of the antibody amltelimab, when measured in the same assay.

The anti-IL-13/OX40L bispecific antigen-binding molecule of the invention preferably binds IL-13 with affinity which is increased, not reduced or not significantly reduced relative to the affinity for IL-13 of a monospecific antibody comprising the same anti-IL-13 variable regions, when measured in the same assay; and/or preferably binds OX40L with affinity which is increased, not reduced or not significantly reduced relative to the affinity for OX40L of a monospecific antibody comprising the same anti-OX40L variable regions, when measured in the same assay

The bispecific binding molecules of the present invention comprise a first antigen binding domain (A) which is an IL-13 antigen binding domain and a second antigen binding domain (B) which is an OX40L antigen binding domain as described herein wherein the bispecific antigen-binding molecule specifically binds to both IL-13 and OX40L and antagonises both IL-13 signalling from IL-13R and OX40L signalling from OX40. In some embodiments, B is an antibody or antigen binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106.

In some embodiments, B is an antibody or antigen binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94; and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112.

In some embodiments, B is an antibody or antigen binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are

CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100; and
CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118

In some embodiments, A is an antibody or antigen binding fragment thereof comprising
the CDRH1, CDRH2 and CDRH3 of SEQ ID NOs: 1, 2 and 3; and the CDRL1, CDRL2
5 and CDRL3 of SEQ ID NOs: 13, 14 and 15.

In some embodiments, B comprises a heavy chain variable region sequence (VHB) and a
light chain variable region sequence (VLB), wherein VHB is a polypeptide comprising or
consisting of SEQ ID NO: 27, or a sequence having at least 80, 90, 95, or 99% amino acid
10 identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 28, or
a sequence having at least 80, 90, 95, or 99% amino acid identity thereto.

In some embodiments, B comprises a heavy chain variable region sequence (VHB) and a
light chain variable region sequence (VLB), wherein VHB is a polypeptide comprising or
consisting of SEQ ID NO: 29, or a sequence having at least 80, 90, 95, or 99% amino acid
15 identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 30, or
a sequence having at least 80, 90, 95, or 99% amino acid identity thereto.

In some embodiments, B comprises a heavy chain variable region sequence (VHB) and a
light chain variable region sequence (VLB), wherein VHB is a polypeptide comprising or
consisting of SEQ ID NO: 31, or a sequence having at least 80, 90, 95, or 99% amino acid
20 identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 32, or
a sequence having at least 80, 90, 95, or 99% amino acid identity thereto.

In some embodiments of the bispecific binding molecules of the present invention, A
comprises a heavy chain variable region sequence (VHA) and a light chain variable region
sequence (VLA), wherein VHA is a polypeptide comprising or consisting of SEQ ID NO:
25 25, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLA
is a polypeptide comprising or consisting of SEQ ID NO: 26, or a sequence having at least
80, 90, 95, or 99% amino acid identity thereto.

In some embodiments of the bispecific binding molecules of the present invention, A
and/or B comprises or consists of an antibody or antigen binding fragment thereof, and the
30 antibody is preferably a chimeric, humanized or human or antibody.

In some embodiments, the bispecific antigen-binding molecule is a bispecific antibody, e.g. a bivalent, trivalent or tetravalent bispecific antibody. In certain embodiments the bispecific antigen-binding molecule is a symmetric-IgG-like molecule, such as a bispecific antibody in the DVD-Ig format, 2+2 CrossMab format or FIT-IG format. The FIT-Ig
5 format is particularly preferred. This format is described in WO2015103072.

In some embodiments, the bispecific antigen-binding molecule is a polypeptide complex comprising variable domains of an antibody and T cell receptor (TCR) constant regions, wherein the TCR constant regions are capable of forming a dimer comprising at least one
10 non-native interchain bond (a complex of this type is described WO2019057122 and may be referred to herein as a WuXibody).

Antigen binding domains and other components

15 Bispecific antigen-binding molecules of the present invention include bispecific antigen-binding molecules which comprise a first antigen binding domain (A) which is an IL-13 antigen binding domain and a second antigen binding domain (B) which is an OX40L antigen binding domain; such a bispecific antigen-binding molecule may be referred to generally herein as “an anti-IL-13 and OX40L bispecific antigen-binding molecule”. In
20 other words, the bispecific antigen-binding molecule is a molecule wherein A specifically binds to IL-13 and B specifically binds to OX40L.

The anti-IL-13 and OX40L bispecific antigen-binding molecules of the invention may comprise one or more further IL-13 binding domains. Thus, embodiments are envisaged
25 where there are e.g. two or three IL-13 binding domains. In embodiments where there is more than one IL-13 binding domain, the two or more of the IL-13 binding domains may be identical, substantially identical or different to one another.

The anti-IL-13 and OX40L bispecific antigen-binding molecules of the invention may
30 comprise one or more further OX40L binding domains. Thus, embodiments are envisaged where there are e.g. two or three OX40L binding domains, or e.g. two or three OX40 binding domains. In embodiments where there is more than one OX40L binding domain,

the two or more of the OX40L binding domains may be identical, substantially identical or different to one another.

5 The terms “antigen-binding domain” and “binding domain” and the like may be used interchangeably herein. An antigen-binding domain is typically capable of specifically binding a particular antigen of interest (e.g. IL-13, OX40L), such as specifically binding with a K_D value of ≤ 250 nM, ≤ 100 nM, ≤ 50 nM, ≤ 25 nM, ≤ 10 nM, ≤ 5 nM, ≤ 2.5 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.5 nM, ≤ 0.4 nM, ≤ 0.25 nM, ≤ 0.1 nM, ≤ 0.05 nM, ≤ 0.01 nM, ≤ 0.005 nM, or ≤ 0.001 nM.

10

Examples of antigen-binding domains that may be used in the present invention include immunoglobulin-based antigen-binding domains and non-immunoglobulin-based antigen-binding domains. Thus, examples of antigen-binding domains include binding domains derived from an immunoglobulin or antibody or from a source other than an immunoglobulin or antibody (e.g. from a proteinaceous binding molecule with immunoglobulin-like binding properties). As used herein, the term “derived from” and the like includes a reference to where a given entity (e.g. an antigen-binding domain, an antibody) may be obtained from a particular source, whether directly or indirectly, and optionally with one or more modifications such as with one or more mutations.

20

An antigen-binding domain (e.g. an IL-13 antigen-binding domain or an OX40L antigen-binding domain) may for example comprise or consist of an antibody or an antigen-binding fragment thereof, e.g. Fabs, scFabs, Fvs, and scFvs. Non-limiting examples of antigen-binding fragments which may be used in the practice of the present invention include Fab fragments, $F(ab')_2$ fragments, Fab' fragments, Fd fragments, Fv fragments, dAb fragments, isolated complementarity determining region (CDR)s, single chain antibodies such as scFv and heavy chain antibodies such as VHH and camel antibodies as well as other antigen-binding fragments disclosed elsewhere herein. Typically, an antigen-binding fragment of an antibody comprises one or more CDRs (e.g. CDRH3 optionally in combination with one of further CDRs (e.g. a set of six CDRs from an HCVR/LCVR pair)).

30

Antibodies for use in the present invention may be obtained by any suitable means. For instance, antibodies may be obtained by administering polypeptides to an animal, e.g. a non-human animal, using well-known and routine protocols, see for example *Handbook of Experimental Immunology*, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable. Antibodies can also be obtained by administering polypeptides to a transgenic animal, e.g. an animal that is genetically modified to generate antibodies derived from human heavy and/or light chain-encoding genes, such as a mouse derived from a AlivaMab system (WO 2010/039900 and WO 2011/123708). Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today*, 4:72) and the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, pp77-96, Alan R Liss, Inc., 1985). Antibodies may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by for example the methods described by Babcook, J. *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93(15): 7843-7848; WO 92/02551; WO 2004/051268 and WO 2004/106377. Antibodies can also be generated using various phage display methods known in the art and include those disclosed by Brinkman *et al.* (in *J. Immunol. Methods*, 1995, 182: 41-50), Ames *et al.* (*J. Immunol. Methods*, 1995, 184:177-186), Kettleborough *et al.* (*Eur. J. Immunol.* 1994, 24:952-958), Persic *et al.* (*Gene*, 1997 187 9-18), Burton *et al.* (*Advances in Immunology*, 1994, 57:191-280) and WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108. Methods for obtaining and identifying antibodies which may be used in the practice of the present invention also include those described in the ImmunoQure patent applications WO2013/098419 (“*Methods of Providing Monoclonal Auto-Antibodies with Desired Specificity*”), WO2013/098420 (“*Method of Isolating Human Antibodies*”), and

WO2015/001407 (*“Method of Providing Anti-Human Cytokine Antibodies for Pharmaceutical Use”*).

5 Non-antibody antigen-binding domains are also contemplated for use in the practice of the present invention. Accordingly, an antigen-binding domain (e.g. an IL-13 antigen-binding domain or an OX40L antigen binding domain) may, for example, be derived from, or comprise or consist of, a non-antibody scaffold protein, a DARPIn (designed ankyrin repeat proteins), an anticalin or a lipocalin, an affibody, an avimer, an adnectin, an atrimer, or an evasin etc.

10

Combinations of different types of antigen-binding domains described herein are contemplated within a bispecific antigen-binding molecule of the invention. Thus, for instance, the antigen-binding domains can each independently comprise or consist of an antibody or an antigen-binding fragment of an antibody or be derived from, or comprise or consist of, a non-antibody scaffold protein, a DARPIn (designed ankyrin repeat proteins), an anticalin or a lipocalin, an affibody, an avimer, an adnectin, an atrimer, or an evasin etc.

20

In certain embodiments, A and/or B may comprise or consist of an antibody (e.g. an IgG antibody such as IgG1 or IgG4).

In certain embodiments, A and/or B may comprise or consist of an antigen-binding fragment of an antibody (e.g. an Fv fragment (e.g. scFv), a Fab fragment).

25 In certain embodiments, A and/or B may be derived from, or comprise or consist of, a non-antibody scaffold protein, a DARPIn (designed ankyrin repeat proteins), an anticalin or a lipocalin, an affibody, an avimer, an adnectin, an atrimer, or an evasin etc.

30 In certain embodiments, A comprises or consists of an antibody (e.g. an IgG antibody such as IgG1 or IgG4) and B comprises or consists of an antigen-binding fragment of an antibody (e.g. an Fv fragment (e.g. scFv), a Fab fragment).

In certain embodiments, A comprises or consists of an antigen-binding fragment of an antibody (e.g. an Fv fragment (e.g. scFv), a Fab fragment) and B comprises or consists of an antibody (e.g. an IgG antibody such as IgG1 or IgG4).

- 5 In certain embodiments both A and B comprise or consist of an antigen-binding fragment of an antibody (e.g. an Fv fragment (e.g. scFv), a Fab fragment).

A bispecific antigen-binding molecule of the invention may be produced by any suitable means. For example, all or part of the molecule may be expressed as a fusion protein by a
10 cell comprising a nucleotide which encodes said molecule. Alternatively, parts of the molecule may be produced separately, for example by expression from separate nucleotides optionally in separate cells, and then subsequently joined together.

In addition to the at least two antigen binding domains, the bispecific antigen-binding
15 molecules may optionally comprise one or more further components. Such one or more further components may, for instance, facilitate the association or binding of the antigen binding domains with each other. Non-limiting examples of one or more further components which may be incorporated into a bispecific antigen-binding molecule of the invention include linkers (e.g. peptide linkers and hinge regions), heavy chain constant
20 regions (such as human CH1, CH2 or CH3), light chain constant regions (CL) such as human kappa or lambda CL regions, and Fc domains (which typically include CH2 and CH3). Thus, in certain embodiments a bispecific antigen-binding molecule of the invention comprises an Fc domain, preferably a human Fc domain, or a fragment thereof. A human Fc domain may be a native or variant human Fc domain.

25 An Fc domain is composed of two polypeptide chains, each referred to as a heavy chain Fc region, which dimerize to form the Fc domain. An Fc domain may be a native or variant Fc domain (e.g. with one or more amino acid insertions, deletions, or substitutions). An Fc domain may for instance be modified or engineered to render it better suited for its
30 intended pharmacological use, e.g. to alter (e.g. increase) half-life and/or to alter effector function.

Preferably an Fc domain is a human Fc domain. An Fc domain or region may be from any suitable class of an antibody, e.g. IgA, IgD, IgE, IgG, or IgM, or subclass thereof (e.g. IgA1, IgA2, IgG1, IgG2, IgG3 or IgG4). Preferably, the Fc domain is human and/or is an IgG domain, e.g. IgG1 or IgG4, more preferably an IgG1. In a native antibody the Fc regions within an Fc domain are typically identical, but for the purpose of the present invention the two Fc regions within an Fc domain, if present, may be the same or different e.g. from different antibody classes, or subclasses (e.g. from two different IgG classes).

Preferred Fc region sequences are human IgG1 sequences comprising one or more modification of the human wildtype sequence to reduce Fc receptor binding (e.g. N297G, N297A or N297Q modification, L234A/L235A (LALA) modification, or L234A/L235A/P329G (LALAPG) modification) and/or one or more modification of the human wildtype sequence to increase serum half-life (e.g. M428L/N434S (LS) modification, T250Q/M428L (QL) modification, or M252Y/S254T/T256E (YTE) modification). More preferably, the human IgG1 Fc region sequences comprise a modification of the human wildtype sequence to reduce Fc receptor binding (L234A/L235A (LALA) modification) and/or a modification of the human wildtype sequence to increase serum half-life (M252Y/S254T/T256E (YTE) modification).

The term "linker" and the like as used herein includes a reference to any molecule or entity that joins two or more different components of a bispecific antigen-binding molecule of the invention. Examples of linkers include peptide linkers, and non-immunoglobulin polypeptides such as albumin (e.g. two or more antigen-binding domains may be linked to albumin (e.g. HSA) to form a bispecific antigen-binding molecule comprising the two or more antigen binding domains each bound to an albumin molecule). A hinge region may also be used to link components of the antigen-binding molecules of the invention e.g. to bind an antigen-binding domain (e.g. in the form of an antigen-binding fragment of an antibody such as a Fab fragment) to an Fc region. Hinge regions are typically found at the N-termini of Fc regions. A hinge region may be a native or modified /variant hinge region.

Components of the antigen-binding molecules of the invention (e.g. A and B) may be connected to one another by any suitable means. Components may be directly connected to

one another, or indirectly connected to one another by one or more suitable molecules (e.g. by a linker or hinge region). Thus, for example, a bispecific antigen-binding molecule of the invention may comprise or consist of a fusion protein comprising A and B, optionally joined by a peptide linker. Various combinations of direct and/or indirect means are
5 envisaged within the practice of the present invention, and thus a variety of direct and/or indirect means may be employed to connect the components of a bispecific antigen-binding molecule of the invention. In some embodiments, the antigen binding domains are connected to one another through an Fc domain or a fragment thereof. Typically, Fc domains require the use of hinge regions and therefore the antigen binding domains may,
10 for example, be connected through an Fc domain via one or more hinge regions.

A bispecific antigen-binding molecule of the invention may optionally be linked directly or indirectly to a further moiety e.g. a therapeutic moiety. Thus, in some embodiments the bispecific antigen-binding molecule (e.g. bispecific antibody) is conjugated to one or more
15 additional therapeutic agents.

Bispecific Antibodies

The bispecific antigen-binding molecule of the invention may be a bispecific antibody.
20 Antibodies of the invention are typically monoclonal antibodies. An antibody of the invention may for instance be a chimeric antibody, a CDR-grafted antibody, a nanobody, a human or humanised antibody or an antigen-binding fragment of any of the foregoing. Typically, the antibody is a human antibody. As discussed above, an antibody may comprise a complete antibody molecule having full length heavy and light chains or an
25 antigen-binding fragment thereof. Accordingly, an antibody of the invention may comprise or consist of a complete antibody molecule having full length heavy and light chains, or it may comprise or consist of an antigen-binding fragment thereof.

The constant region domains of the bispecific antibody molecule of the present invention,
30 if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. Typically,

the constant region domains are human. In particular, human IgG (i.e. IgG1, IgG2, IgG3 or IgG4) constant region domains may be used, e.g. a human IgG1 or IgG4 constant region domain. The light chain constant region may be either lambda or kappa.

- 5 The bispecific antibody of the invention may be a human antibody. The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. A human
10 antibody may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been
15 grafted onto human framework sequences.

- It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such
20 modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995).

- 25 Fully human antibodies are those antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, but not necessarily from the same antibody. Examples of fully human antibodies may include antibodies produced, for
30 example by the phage display methods described above and antibodies produced by mice in which the murine immunoglobulin variable and optionally the constant region genes have been replaced by their human counterparts e.g. as described in general terms in EP

0546073, US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,661,016, US 5,770,429, EP 0438474 and EP 0463151.

The term “humanized antibody” is intended to refer to CDR-grafted antibody molecules in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences. As used herein, the term ‘CDR-grafted antibody molecule’ refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine or rat monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, *Nature Biotechnology*, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, *Methods*, 36, 25-34). When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the CDR-grafted antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs or specificity determining residues.

An antigen binding portion of an antibody may be described as a binding domain. A binding domain will generally comprise 6 CDRs (3 in case of VHH), three from a heavy chain and three from a light chain. The CDRs are typically in a framework and together form a variable region. Thus, in one embodiment an antibody or binding fragment comprises a binding domain specific for the relevant target comprising a light chain variable region and a heavy chain variable region. The residues in antibody variable regions are conventionally numbered according to a number of different systems which are well known in the art. These include the systems known as Chothia, Kabat, and IMGT. Where a variable region is described using three CDR sequences defined according to one of these systems, it will be understood that these can be replaced with the corresponding

three CDR sequences defined according to another system, although the IMGT definition is typically preferred.

Antibodies of the invention may be “isolated” antibodies. An isolated antibody is an
5 antibody which is substantially free of other antibodies having different antigenic specificities.

A wide variety of bispecific antibody formats and production methods are known in the art and any suitable format and production method may be used in the practice of the present
10 invention. There are many available bispecific antibody formats but, generally speaking, bispecific antibodies can be divided into IgG-like and non-IgG like bispecific antibodies. IgG-like bispecific antibodies comprise an Fc domain and may be further categorized into symmetric IgG-like bispecific antibodies (e.g. dual variable domain (DVD)-Igs, or Fabs in tandem (FIT)-Igs) and non-symmetric IgG-like bispecific antibodies. Non-IgG-like
15 bispecific antibodies lack an Fc domain and may for instance be made by fusing two different antigen-binding antibody fragments to a non-immunoglobulin protein (e.g. human serum albumin (HSA)), by directly fusing two antigen-binding antibody fragments, or by chemical conjugation of two different antibodies or smaller antigen-binding antibody fragments. Any of these an IgG-like and non-IgG-like formats and production techniques
20 may be employed in the practice of the present invention. For a review of bispecific antibodies including exemplary bispecific antibody formats and production methods which may be used in the practice of the present invention (such as those outlined in the discussion below), reference is made to Kontermann and Brinkmann (2017), “The making of bispecific antibodies”, *Mabs*, Feb-Mar 9(2): 182-199; Kontermann and Brinkmann (July
25 2015), “Bispecific antibodies”, *Drug Discovery Today*, 20(7): 838-847; Sedykh *et al.* (2018), “Bispecific antibodies: design, therapy, perspectives”, *Drug Design, Development and Therapy*, 12: 195-208; and Fan *et al.* (2015), “Bispecific antibodies and their applications”, *Journal of Hematology and Oncology*, 8:130.

30 Exemplary bispecific antibodies and techniques according to the present invention include but are not limited to asymmetric IgG-like (such as Duobody bispecific antibodies), symmetric IgG-like (e.g. comprising two Fab regions and an Fc domain, such as a DVD-Ig

bispecific antibody or FIT-Ig bispecific antibody), non-IgG-like, quadromas, WuXibodies, knob-in-hole (kih), IgG-scFv fusions, two-in-one or dual action Fab (DAF) antibodies, half molecule exchange, $\kappa\lambda$ -bodies, Duobodies, CrossMab, CrossFab, Triomab, (SEED)body, leucine zipper, common light chain (e.g. kih IgG common LC), ortho-Fab IgG, 2 in 1-IgG, 5 scFv2-Fc, triabodies, scFv-based or diabody bispecific formats, tandem scFvs, single-chain diabodies, nanobodies, dock-and-lock (DNL) method, bi-Nanobody, bispecific T-cell engagers (BiTEs), tandem diabodies (tandAbs), chemically linked Fabs, bivalent and trivalent scFvs, Dual affinity retargeting (DARTs), DART-Fc, scFv-HSA-scFv, and DNL-Fab3 bispecific antibodies.

10

A bispecific antibody of the present invention may be a WuXibody. A detailed description of WuXibodies may be found in WO 2019/057122 (WuXi Biologics). The term “WuXiBody” includes bispecific antibodies comprising soluble chimeric protein with variable domains of an antibody and T cell receptor (TCR) constant regions, wherein the 15 TCR constant regions are capable of forming a dimer comprising at least one non-native interchain bond; such WuXibodies are described in more detail in WO 2019/057122 along with methods for their production and various possible WuXibody formats.

In a preferred embodiment, the bispecific antibody is an IgG-like bispecific antibody that is 20 a symmetric IgG-like bispecific antibody (e.g. a DVD-Ig bispecific antibody or FIT-Ig bispecific antibody) or a non-symmetric IgG-like bispecific antibody. In a more preferred embodiment, the bispecific antibody is of a symmetric IgG-like format. In a preferred embodiment of the present invention, the relative position of A and B of the symmetric IgG-like bispecific antibody are such that A is proximal to the Fc region of the molecule 25 and B is distal to the Fc region. In another preferred embodiment of the present invention, the relative position of A and B of the symmetric IgG-like bispecific antibody are such that B is proximal to the Fc region of the molecule and A is distal to the Fc region.

An example of a symmetric IgG-like format is a dual-variable domain immunoglobulin (DVD-Ig). A DVD-Ig can be generated from two parental mAbs by placing two variable 30 domains from one of the parental mAbs onto the heavy chain and the light chain of the other parental antibody, instead of one variable domain, to yield a tetravalent IgG-like molecule. Another example of symmetric IgG-like format, also tetravalent IgG-like, is 2+2

CrossMab, which can be generated from two parental mAbs fusing the Fabs of a first parental mAb to the Fabs of a second parental mAb (as described in WO2010145792 or WO2010145793). A particularly preferred symmetric IgG-like format is the Fabs-in-tandem immunoglobulin (FIT-Ig) format which is described in WO2015103072. In one embodiment of this format, the binding molecule comprises three polypeptide chains, wherein the first polypeptide chain comprises, from amino to carboxyl terminus, a VH_B and a $CH1$, the second polypeptide chain comprises, from amino to carboxyl terminus, a VL_B , a CL , a VH_A , a $CH1$ and Fc region (wherein CL is fused directly to VH_A), and the third polypeptide chain comprises from amino to carboxyl terminus, a VL_A and CL . In one embodiment of this format, the binding molecule comprises three polypeptide chains, wherein the first polypeptide chain comprises, from amino to carboxyl terminus, a VH_A and a $CH1$, the second polypeptide chain comprises, from amino to carboxyl terminus, a VL_A , a CL , a VH_B , a $CH1$ and Fc region (wherein CL is fused directly to VH_B), and the third polypeptide chain comprises from amino to carboxyl terminus, a VL_B and CL . The pairing of the various VH/VL and $CH1/CL$ pairs results in assembly of a tetravalent IgG-like molecule. In this embodiment, VH_B is a OX40L binding heavy chain variable region sequence, VL_B is a OX40L binding light chain variable region sequence, VH_A is an IL-13 binding heavy chain variable region sequence, and VL_A is an IL-13 binding light chain variable region sequence. $CH1$ is a heavy chain constant domain 1, CL is a light chain constant domain, and Fc comprises heavy chain constant domains 2 and 3 ($CH2$ and $CH3$). VH_A/VL_A pairs and VH_B/VL_B pairs are preferably selected from those disclosed here. Any suitable CL , $CH1$, and Fc domain may be used, but preferably human domains are used. Human CL may be lambda or kappa. In a preferred embodiment human CL is kappa. An exemplary CL kappa sequence is provided as SEQ ID NO: 35. An exemplary CL lambda sequence is provided as SEQ ID NO: 119. $CH1$ and Fc are preferably IgG1. An exemplary $CH1$ sequence is provided as SEQ ID NO: 33. Fc may optionally include one or more modifications relative to human wild type sequences to increase half-life and/or disrupt Fc receptor binding. Suitable modifications are described herein. In a preferred embodiment, the bispecific antibody comprises a human IgG1 Fc region sequence, said sequence comprising a modification of the human wildtype sequence to reduce Fc receptor binding (optionally the L234A/L235A (LALA) modification) and/or a modification of the human wildtype sequence to increase serum half-life (optionally the M252Y/S254T/T256E (YTE)

- modification). An exemplary Fc sequence is provided as SEQ ID NO: 34. Exemplary FIT-Ig molecules of the invention are described further in the Examples and the sequences of Chains 1, 2 and 3 are provided. In some embodiments, a bispecific antibody of the present invention consists of three polypeptide chains each with domains arranged as follows (N-C
- 5 terminus):
- Chain 1: VHB-CH1;
- Chain 2: VLB-CL-VHA-CH1-Fc; and
- Chain 3: VLA-CL,
- wherein VHB, CH1, VLB, CL, VHA, FC and VLA are as described herein.
- 10 In a preferred embodiment Chain 1 is as provided in SEQ ID NO: 36; Chain 2 is as provided in SEQ ID NO: 37; and Chain 3 is as provided in SEQ ID NO: 38.
- In a preferred embodiment Chain 1 is as provided in SEQ ID NO: 39; Chain 2 is as provided in SEQ ID NO: 40; and Chain 3 is as provided in SEQ ID NO: 41.
- In a preferred embodiment Chain 1 is as provided in SEQ ID NO: 42; Chain 2 is as
- 15 provided in SEQ ID NO: 43; and Chain 3 is as provided in SEQ ID NO: 44.

Pharmaceutical compositions

- Any molecule of the invention (e.g. a bispecific antibody of the invention or anti-OX40L
- 20 antibody) may be formulated for administration as a pharmaceutical composition.
- Accordingly, a bispecific antigen-binding molecule of the invention may be provided in the form of a pharmaceutical composition comprising the bispecific antigen-binding molecule and a pharmaceutically acceptable carrier, diluent, excipient, or preservative.
- 25 As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for parenteral, e.g. intravenous, intramuscular or subcutaneous administration (e.g., by injection or infusion), topical or oral administration. Preferred pharmaceutically
- 30 acceptable carriers comprise aqueous carriers or diluents. Examples of suitable aqueous carriers that may be employed in the compositions of the invention include water, buffered water and saline. Examples of other carriers include ethanol, polyols (such as glycerol,

propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

5

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

- 10 The pharmaceutical compositions of the invention may comprise one or more additional active ingredients as well as a molecule of the invention.

Therapeutic uses

- 15 In a further aspect of the invention, there is provided any molecule of the invention (e.g. bispecific antigen-binding molecule or anti-OX40L antibody) for use as a medicament. Methods of treatment of a disease or condition are also provided and comprise administering a molecule of the invention to a subject in need thereof to thereby treat the disease or condition.

20

As discussed above, IL-13 and OX40L have been identified as important factors in a variety of diseases and conditions and, as such, the bispecific antigen-binding molecules of the invention may be used to treat such diseases and conditions. Accordingly, one aspect of the present invention provides a method of treating a disease or condition which is

- 25 associated with or mediated by IL-13 and/or OX40L in a patient, the method comprising administering to the patient an anti-IL-13 and OX40L bispecific antigen-binding molecule (e.g. a bispecific antibody) of the invention. The invention also provides an anti-IL-13 and OX40L bispecific antigen-binding molecule of the invention for use in a method of treating a disease or condition which is associated with or mediated by IL-13 and/or
- 30 OX40L. The invention also provides an anti-IL-13 and OX40L bispecific antigen-binding molecule of the invention for use in the manufacture of a medicament for the treatment of a disease or condition which is associated with or mediated by IL-13 and/or OX40L.

The phrase “a disease or condition which is associated with or mediated by” and the like in relation to a particular cytokine (here IL-13 or OX40L) includes a reference to a disease or condition which is associated with or mediated by expression, signalling or activity of the cytokine, or treatable by antagonism of the cytokine e.g. by blocking the interaction
5 between the cytokine and a ligand for the cytokine or by otherwise inhibiting the activity and/or signalling of the cytokine. Examples of diseases or conditions which are associated with or mediated by IL-13 and/or OX40L include dermatological diseases (e.g., atopic dermatitis, prurigo nodularis, chronic hand eczema, allergic dermatitis, psoriasis, lichen
10 planus, hidradenitis suppurativa), asthma, allergic diseases (e.g., allergic rhinitis), cardiovascular diseases (e.g., myocardial infarction, cardiac hypertrophy-related diseases), atherosclerosis, musculoskeletal diseases (rheumatoid arthritis), COPD, age-related macular degeneration, periodontitis uveitis, cancer, inflammatory bowel disease, fibrosis, scleroderma, or eosinophilic esophagitis.

15

The molecules of the invention may be useful in treating a dermatological disease or condition e.g. atopic dermatitis, prurigo nodularis, chronic hand eczema, allergic dermatitis, psoriasis, lichen planus or hidradenitis suppurativa. In a preferred embodiment, the molecules of the invention may be useful for the treatment of atopic dermatitis.
20 Accordingly, the invention provides a method of treating a dermatological disease or condition in a patient comprising administering to the patient a molecule (e.g. a bispecific antibody) of the invention. The invention also provides a molecule of the invention for use in a method of treating a dermatological disease or condition. The invention also provides a molecule of the invention for use in the manufacture of a medicament for the treatment of
25 a dermatological disease or condition.

In the present invention, treatment may be in respect of a patient with the disease or condition, or may be in respect of a patient in whom the disorder is to be prevented such as patient which is prone to, or at risk of, having the disease or condition. Thus, the term
30 "treatment" and the like as used herein encompasses therapeutic and prophylactic treatment. The term “treatment” may for instance refer to preventing the disease or condition from occurring, delaying the onset of the disease or one or more symptoms

- thereof, causing regression of the disease or medical condition in a patient, suppressing the disease or medical condition (e.g. slowing the development of the disease or medical condition, or reducing the severity and/or frequency of flares), or alleviating to some extent one or more of the symptoms of the disease or medical condition in a patient. In the case of
- 5 AD, symptoms include pruritus, erythema, edema, xerosis, erosions/excoriations, oozing and crusting, lichenification, impaired skin barrier, and redness. The term "treatment" and the like does not necessarily entail complete treatment or prevention and the term may therefore encompass varying degrees of treatment or prevention.
- 10 In therapeutic applications, administration is to a subject already suffering from the disease or condition. Such therapeutic treatment may for instance cure, alleviate or partially arrest the disease or condition or one or more of its symptoms. Accordingly, therapeutic administration may result in a decrease in symptom severity, or an increase in frequency or duration of symptom-free periods. An amount adequate to accomplish a therapeutically
- 15 useful effect may be referred to as a "therapeutically effective amount".

- In prophylactic applications, administration is to a subject not yet, or not currently, exhibiting symptoms of the disease or condition. Such prophylactic treatment may for instance prevent, delay, or reduce in severity the development of the disease or condition
- 20 or one or more of its symptoms. An amount adequate to accomplish a prophylactically useful effect may be referred to as a "prophylactically effective amount". The subject may have been identified as being at risk of developing the disease or condition by any suitable means. The patient may be prone to, or at risk of, having the disease or condition (e.g. a patient with a family or individual history of the disease or condition) or in whom
- 25 the disorder is to be prevented.

- Therapeutically and prophylactically effective amounts will depend on the severity of the disease or condition as well as the weight and general state of the subject. It will be understood that determining an appropriate dosage may be achieved using routine
- 30 experimentation, by constructing a matrix of values and testing different points in the matrix, which is all within the ordinary skills of a trained physician.

The term “treatment” as used herein may refer to an improvement in the severity of the disease or condition or quality of life (QoL). Various test procedures and scoring systems are available for assessing disease severity (e.g. mild, moderate, moderate-to-severe, or severe) and quality of life and any one or more suitable measures may be used. An overview of disease severity and quality of life measures for AD may be found in e.g. Rehal and Armstrong (2011), Plos ONE 6(4):e17520, and in Gooderham *et al.* (2018), *J Cutan Med Surg.*, 22(IS) 10S-16S). One common measure of disease severity in AD patients is the Eczema Area Severity Index (EASI). Other examples of suitable disease severity and QoL measures for AD include: SCORing Atopic Dermatitis (SCORAD), the Body Surface Area (BSA) assessment, the Physician’s Global Assessments (PGA), Investigator Global Assessment (IGA), Dermatitis Severity Index (ADSI), Six Area, Six Sign Atopic Dermatitis (SASSAD), Investigators’ Global Atopic Dermatitis Assessment (IGADA), the Pruritus – Visual Analogue Scale (Pruritus-VAS), 5-D Itch (Pruritis) Scale, Dermatology Life Quality Index (DLQI), Children’s Dermatology Life Quality Index (CDLQI), Dermatitis Family Impact (DFI), and Infant’s Dermatology, and Quality of Life (IDQOL), and the Medical Outcome Sleep Study (MOSS).

A bispecific antigen binding molecule of the invention may, for example, be used to treat acute or chronic AD. A bispecific antigen binding molecule of the invention may be used to treat mild, moderate, moderate-to-severe, or severe AD. Disease severity can easily be determined by a skilled person using standard test procedures such as by using one or more of the above-mentioned measures of disease severity or quality of life measures for AD, e.g. EASI.

The terms "patient" and "subject" are used interchangeably herein and the terms include a reference to any human or non-human animal (preferably a mammal). The term "mammal" as used herein refers to any member of the class Mammalia, including, without limitation, humans and non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic / companion animals such as dogs and cats; as well as rabbits and rodents such as mice, rats, and guinea pigs, and the like. Typically, the invention relates to administration to a human patient. The human patient may be an adult patient (18 years or older). Alternatively, the human patient

may be a paediatric patient (less than 18 years old). In some instances, the patient may be less than 12 years old. In certain embodiments, the subject is a human patient who has been identified as having a disorder or condition likely to respond to a bispecific antigen-binding molecule of the invention.

5

Administration

The molecules and pharmaceutical compositions of the present invention may be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Routes of administration may include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection. In some embodiments a bispecific antigen-binding molecule of the invention (e.g. a bispecific antibody of the invention) is administered to the patient by injection, preferably by subcutaneous or intravenous injection. Alternatively, a bispecific antigen-binding molecule of the invention can be administered via a non-parenteral route, such as by topical or oral administration.

A suitable dosage of a molecule of the invention may be determined by a skilled medical practitioner. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular bispecific antigen-binding molecule employed, the route of administration, the time of administration, the rate of excretion of the bispecific antigen-binding molecule, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight,

condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

5 A suitable dose of a molecule of the invention may be, for example, in the range of from about 0.1 µg/kg to about 100mg/kg body weight of the patient to be treated. For example, a suitable dosage may be from about 1 µg/kg to about 10mg/kg body weight per day or from about 10 µg/kg to about 5 mg/kg body weight per day. It may be possible to administer a bispecific molecule of the invention at a lower dose than the combined quantity of two monospecific molecules binding to the same targets.

10

The initial dose may be followed by administration of a second or plurality of subsequent doses. The second and subsequent doses may be separated by an appropriate time. Dosage and frequency may vary depending on the half-life of the bispecific antigen-binding molecule in the patient and the duration of treatment that is desired. The dosage and frequency of administration can also vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage may be administered at relatively infrequent intervals over a long period of time. In therapeutic applications, a relatively high dosage may be administered, for example until the patient shows partial or complete amelioration of symptoms.

20

Additional medications/separate treatment methods

A molecule of the invention (e.g. a bispecific antibody) may be administered in combination with an additional medication and/or treatment method for the prevention or treatment of the disease or condition. Combined administration of two or more agents may be achieved in a number of different ways. In one embodiment, the molecule of the invention and the other agent may be administered together in a single composition. In another embodiment, the molecule of the invention and the other agent may be administered in separate compositions as part of a combined therapy. For example, the molecule of the invention may be administered prior to, concurrent with, or after the other agent. The separate compositions may be administered by the same route, or by different routes.

30

Examples of suitable medications and/or treatments are described in the art. For instance, in relation to AD see e.g. Dhadwal *et al.* (2018), *J Cutan Med Surg.*, 22(IS) 21S-29S). In the case of AD examples include topical treatments (such as topical corticosteroids, and
 5 topical calcineurin inhibitors), phototherapy, and systemic treatments (such as systemic corticosteroids, methotrexate, cyclosporine A, mycophenolate, and azathioprine).

Examples

10 Example 1 – OX40L binding antibody discovery

8 AlivaMab (Ablexis) transgenic mice were each immunized with a stabilized human OX40L-Fc fusion protein, leading to the generation of approximately 11,500 candidate antibody-expressing hybridomas. 96 hybridomas were selected based on screening by (i) target blocking by blocking ELISA (measurement of inhibition of the
 15 OX40-OX40L interaction when OX40L is fixed and OX40 is soluble); (ii) target blocking by soluble ELISA (measurement of inhibition of the OX40-OX40L interaction when OX40L is soluble and OX40 is fixed); and (iii) target binding by flow cytometry (assessment of binding to OX40L-expressing cells). A secondary round of screening selected 24 hybridomas that were optimal in both (i) target blocking by blocking ELISA;
 20 and (ii) target blocking by soluble ELISA. These were then assessed by sequencing of heavy chain and light chain variable regions, and analysis of sequences for diversity; developability assessment (check for low/zero non-specific binding by ELISA – to Baculonavirus particles (BVP) and to polyspecificity reagent (PSR), plus a check for favourable thermal properties (T_m/Tagg measured by Uncle) and low hydrophobic
 25 character measured using HPLC); target binding by flow cyotmetry (determination of K_D for both human and cynomolgus OX40L expressing cell lines); and functionality assays with a soluble competition ELISA versus the reference antibody Ab009 as a comparator.

The 12 leading OX40L binding sequences were selected based on having the best performance for binding to and blocking of OX40L; high sequence diversity (6 heavy
 30 chain V genes, 5 kappa and 3 lambda light chain genes used); and no concerns regarding developability (thermal stability, hydrophobicity). 4 candidates which expressed with titers below 30mg/L were then excluded to leave 8 leading candidates.

Example 2 –sequences of the 12 leading OX40L binding domains

Table Ex2

Hybridoma code	SEQ ID	Heavy chain variable region (VH) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (IMGT system)	SEQ ID	Light chain variable region (VL) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (IMGT system)
10L15A	27	QVQLVQSGAEVKKPGASVKVSCAASGYTFTGY YMHVVRQAPGGGLEWMGWINPNSGGTHYVQKF QGRVTMTRDTSISTAYMELSLRSDDTAVYYC ARSDYDSSGYYYGNSFDYWGGTTLTVTVSS	28	EIVLTQSPGTLSPGERATLSCRASQSVNSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYFCQHYSRSPWTFGGTKV EIK
10E23A	29	QVQLQESGPGLVKPKSETLSLTCTVSGGSISNY FWSWIRQAPGKGLEWIGRIYKSWRTNYPNPSLK SRVTMSVDTSKNQFSLKSSVTAADTAVYYCA RERENRNDAYDAFDIWGGTMTVTVSS	30	DIQLTQSPSFLSASVGDRTITCRASQGISSYLAW YQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTE FTFTISSLPQEDFVITYCQQLNSYPPTFGGQTKVE IK
05P04A	31	EVQLVESGGGLVLPKGGSLRLSCAASGFTFNSY NLHWVRQAPGKGLEWVSSIIISTSTYKDYADSV KGRFTISRDNNAKNSLFLQMNSLRAEDTAVYYC ARGTFFDYWGQGTTLTVTVSS	32	QLVLTQSPSASASLGASVKLTCTLS SCRSSYA IAW HQQRPEKGPRLYLMKNSDGSRGDGIPTDRFSGSS SGTERYLTISSLQAEDADYYCQTWVTGIVQVFGGG TKLTVL
06C09A	65	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSY AMTWVRQAPGKGLEWVSIISGSGGLTYADSV KGRFTISRDNKNTLYLQMNSLRAEDTAIYYC ARDLQWEPLGYWGQGTTLTVTVSS	66	AIQMTQSPSSLASVGDRTITCRASQGIRDALGW YQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTD FTLTISSLPQEDFATYYCQDYNYPYTFGGQTKLE IK
08N01A	67	EVQLVESGGGLVLPKGGSLRLSCAASGFTFNSA WMNWVRQAPGKGLEWVGRIKSIDGRSIDYAA PVKGRFTISRDDSKHTLYLQMNSLKTEDTAVY YCTTGGRSYGTDFEWGGTTLVAVSS	68	SFELTQPPSVSVSPQGTARITCFGDALPKQYAYWY QQKPGQAPVLIYKDSKRPSGIPERFSGSGSGTTV TLTISGVQAEDEADYYCQSDSSGPVYVFGGQTKV TVL
12K10A	69	EVQLVESGGGLVLRPGGSLRLSCAASGFTFSSY NMNWVRQAPGKGLEWVSIISRNTRYKDYADSV MGRFTISRDNNAKNSLYLQMNSLRAEDTAVYYC ARGTFFDYWGQGTTLVSVSS	70	QFVLTQSPSASASLGASVSLTCTLSGRSSYAIW HQQQPEKGPRLYLMKNSDGSYKGGDIPTDRFSGSS SGTERHLTISSLQSEDEANYYCQTWGSIGIVFGGG TKLTVL
13C22A	71	EVQLVESGGGLVLPKGGSLRLSCAASGFTFNSA LMSWVRQAPGKGLEWVGRIKSKTDGTTDYGA PVKGRFTISRDDSRNTLYLQMNSLKTEDTAVY YCTLDQHYGMDFEWGQGTTVTVSS	72	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNN KNYLAWYQQKPGQPPKLLIYWASTRESGVDRFSG SGSGTDFTLTISSLQAEADVAVYYCQYYNLRITFG QGTKEVIEIK
13M16A	73	EVQLVESGGGLVLPKGGSLRLSCAASGFTFNSA GMNWVRQAPGKGLEWVGRIKTKTDGTTDYAA PVKARFTISRDDSKNTLYLQMNSLKTEDTAVY YCTTGGRTPDFEWGGTTLTVTVSS	74	QSVLTQPPSASGTPGQRTVITSCSGSSNIGSYVY WYQHLPGTAPKLLIYSNNKRPSPGVDRFSGSKSGT SASLAISGLRSEDEADYYCAAWDDSLSGWVFGGGT KLTVL
07C16A	75	QVQLQESGPGLVKPSQTLSTCTVSGDSINRG GYFWSWIRKHPGKGLEWIGIYHSGRTYYNPS LKSRTVISVDTSKKQFSLKLISVSAADTAVY CARDGRDGGFDIWGGTMTVTVSS	76	DIQMTQSPSTLSAFVGDRTITCRASQGISNWLAW YQQKPGIAPKLLIYKASTLESVPSRFSGSGSGTE FTLTISSLPDDFATYYCQQYDSYTFGGQTKLDI K
07E10A	77	EVQLLESGGDLVQPGGSLRLSCAASGFAFSSY AMNWVRQAPGKGLEWVSATSGSGRSTLFADSV KGRFTVSRDNKNTLYLQINSLRAEDTAVYYC TKVQLGFDGGFDIWGGTMTVTVSS	78	DIQLTQSPSFLSASVGDRTITCRASQGISSYLAW YQQKPGKAPNLLISAASLTQSGVPSRFSGSGSGTE FTLTISSLPQEDFATYYCQHLNSYPYTFGGQTKLE IK
08F15A	79	EVQLVESGGGFVKPGGSLRLSCAASGFTFSIA WMSWVRQAPGKGLEWVGREKSKTDGTTDYAA PVKGRFTISKDDSKNTLYLHMNSLKTEDTAVY YCTVAHWGFIDEFWGGTTLTVTVSS	80	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNN KKYLAWYQQKPGQPPKLLIYWASTRESGVDRFSG SGSGTDFTLTISSLQAEADVAVYYCQYYSTPPTFG PGTKVDIK
14P23A	81	QVQLQESGPGLVKPKSETLSLTCTVSGGSISY YWSWIRQSAGKLEWIGRIYSTGRNNYPNPSLT SRVTMSINTSQNRFSKLSSVTAADTAVYYCA RERFSRSYRDAFDIWGGTMTVIVSS	82	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQYGSPLTFGGGQTKV EIK

The top three rows of the above table are the three preferred candidate OX40L-binding sequences. The bottom four rows are the candidates excluded from further analysis due to expression titers below 30 mg/L.

The following table provides the CDR sequences for each of three preferred candidate OX40L-binding sequences, determined via each of the Chothia, Kabat and IMGT systems. The systems may be used interchangeably herein. For example, where a given OX40L-binding region is defined herein by reference 3 heavy chain CDRs determined according to Chothia, these may be switched for the corresponding 3 heavy chain CDRs defined according to Kabat, or the corresponding 3 heavy chain CDRs defined according to IMGT; and where a given OX40L-binding region is defined herein by reference 3 light chain CDRs determined according to Chothia, these may be switched for the corresponding 3 light chain CDRs defined according to Kabat, or the corresponding 3 light chain CDRs defined according to IMGT. For example, based on the table below, the OX40L binding region labelled 10L15A may be defined as comprising CDRH1: SEQ ID NO: 4, CDRH2: SEQ ID NO: 5, CDRH3: SEQ ID NO: 6; and CDRL1: SEQ ID NO: 16, CDRL2: SEQ ID NO: 17, CDRL3: SEQ ID NO: 18, which are all determined according to Chothia. It is to be understood that these sequences could be substituted for CDRH1: SEQ ID NO: 83, CDRH2: SEQ ID NO: 84, CDRH3: SEQ ID NO: 85; and CDRL1: SEQ ID NO: 101, CDRL2: SEQ ID NO: 102, CDRL3: SEQ ID NO: 103, which are all determined according to Kabat, or these sequences could be substituted for CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106, which are all determined according to IMGT. The same applies in general for the CDRs of binding regions described herein, including the other two OX40L-binding regions shown in the table below.

	CDRH1	SEQ	CDRH2	SEQ	CDRH3	
10L15A	GYTFTGY (Chothia)	4	PNSG (Chothia)	5	DYDSSGGYYGNSFD (Chothia)	6
	GYVMH (Kabat)	83	WINPNSGGTHYVQKFQG (Kabat)	84	SDYDSSGGYYGNSFDY (Kabat)	85
	GYTFTGYY (IMGT)	86	INPNSGGT (IMGT)	87	ARSDYDSSGGYYGNSFDY (IMGT)	88
10E23A	GGISISNY (Chothia)	7	KSW (Chothia)	8	RFNRNDAYDAFD (Chothia)	9
	NYFWS (Kabat)	89	RIYKSWRTNYPNLSKS (Kabat)	90	ERFNRNDAYDAFDI (Kabat)	91
	GGISISNYF (IMGT)	92	IYKSWRT (IMGT)	93	ARERFNRNDAYDAFDI (IMGT)	94
05P04A	GFTFNSY (Chothia)	10	STST (Chothia)	11	TFED (Chothia)	12

	SYNLH (Kabat)	95	SIISTSTYKDYADSVKG (Kabat)	96	GTFFFDY (Kabat)	97
	GFTFNSYN (IMGT)	98	IISTSTYK (IMGT)	99	ARGTFFDY (IMGT)	100
	CDRL1		CDRL2		CDRL3	
10L15A	SQSVSNSY (Chothia)	16	GAS (Chothia)	17	YSRSPW (Chothia)	18
	RASQSVSNSYLA (Kabat)	101	GASSRAT (Kabat)	102	QHYSRSPWT (Kabat)	103
	QSVSNSY (IMGT)	104	GAS (IMGT)	105	QHYSRSPWT (IMGT)	106
10E23A	SQGISSY (Chothia)	19	AAS (Chothia)	20	LNSYPP (Chothia)	21
	RASQGISSYLA (Kabat)	107	AASTLQS (Kabat)	108	QQLNSYPPT (Kabat)	109
	QGISSY (IMGT)	110	AAS (IMGT)	111	QQLNSYPPT (IMGT)	112
05P04A	LSSGRSSYA (Chothia)	22	LNSDGSH (Chothia)	23	WVTGIQ (Chothia)	24
	TLSSGRSSYAIA (Kabat)	113	LNSDGSHSRGD (Kabat)	114	QTWVTGIQV (Kabat)	115
	SGRSSYA (IMGT)	116	LNSDGSH (IMGT)	117	QTWVTGIQV (IMGT)	118

For comparison, the following are the variable region sequences for the reference anti-OX40L antibody (Ab009).

SEQ ID	Heavy chain variable region (VH) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (Kabat system)	SEQ ID	Light chain variable region (VL) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (Kabat system)
60	EVQLVESGGGLVQPGGSLRLSCAASGFTFSN <u>YAMN</u> <u>WVRQAPGKGLEWVS</u> <u>TI</u> <u>SGSGGATRYADSVK</u> <u>GRFTISRDNSRNTVY</u> <u>LMNSLRVEDTAVFYCTK</u> <u>DLIMATVRGPYYGMDV</u> WGQTTTVTVSS	61	DIQMTQSPSSLSASVGDRTITC <u>RASQSISSYL</u> NWY QQKPGKAPNLLIY <u>AASSLQ</u> GVPSRFSGSGSETDFT LTISSLQPEDFATYYC <u>QQSHSVSFT</u> FGPGTKVDIK

- 5 The following table also provides the CDR sequences for the reference anti-OX40L antibody (Ab009) determined according to the Kabat system:

	CDR1	SEQ	CDR2	SEQ	CDR3	SEQ
HC	NYAMN (Kabat)	54	TI SGSGGATRYADSVKG (Kabat)	55	DRLIMATVRGPYYGMDV (Kabat)	56
LC	RASQSISSYL (Kabat)	57	AASSLQ (Kabat)	58	QQSHSVSFT (Kabat)	59

The following are the variable region sequences for the reference anti-IL-13 antibody (Ab008).

SEQ ID	Heavy chain variable region (VH) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (Kabat system)	SEQ ID	Light chain variable region (VL) sequence shown N-C terminus, with CDRs 1, 2 and 3 appearing in order, underlined (Kabat system)
25	QVTLRESGPAVKPTQTLTLCTVSGFSLSAYS VNWIRQPPGKALEWLA MIWGDGKIVYNSALKSR LTISKDTSKNQVLTMTNMDPVDATYYCAGDG YYPYAMD NWGQGS LVTVSS	26	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSF MHWYQQKPGQPPKLLIY LASNLES GVPDFSGSGG TDFTLTISSLQAEDVAVYYC QQNNEDPRT FGGGTKV EIK

The following table also provides the CDR sequences for the reference anti-IL-13 antibody (Ab008) determined according to the Kabat system:

	CDR1	SEQ	CDR2	SEQ	CDR3	SEQ
HC	AYSVN (Kabat)	1	MIWGDGKIVYNSALKS (Kabat)	2	DGYYPYAMDN (Kabat)	3
LC	RASKSVDSYGNS FMH (Kabat)	13	LASNLES (Kabat)	14	QQNNEDPRT (Kabat)	15

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Example 3 – sequences of bispecific molecules

The sequences shown below are for chains 1, 2 and 3 of bispecific antibodies, formatted in the FIT-Ig format as described in WO2015103072. Each was recombinantly expressed using standard methods. BsAb001, BsAb003 and BsAb002 are bispecific antibodies comprising, respectively, the top three OX40L binding sequences identified in the previous example. Each is formatted such that the OX40L binding part of the bispecific is distal to the Fc region of the molecule. BsAb004, BsAb005 and BsAb006 are “Fab switched” versions of BsAb001, BsAb002 and BsAb003, formatted such that the OX40L binding part of the bispecific is proximal to the Fc region of the molecule.

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Summary of the exemplary bispecific antibodies:

Name code for bispecific molecule	Reference for OX40L part - hybridoma code or other identifier	OX40L part proximal or distal to Fc
BsAb001	10L15A	Distal
BsAb002	05P04A	Distal
BsAb003	10E23A	Distal
BsAb004	10L15A	Proximal
BsAb005	05P04A	Proximal
BsAb006	10E23A	Proximal
BsAb007	From Amlitelimab	Distal

Sequence information for the exemplary bispecific molecules:

BsA b	Chain 1 VH _B -CH1	SEQ	Chain 2 VL _B -CL-VH _A -CH1-Fc	SEQ	Chain 3 VL _A -CL	SEQ
001	QVQLVQSGAEVKKPGASVKVCKAS GYTFTGYMHVWRQAPGQGLEWMGW INPNSGGTHYVQKFQGRVTMRDTS ISTAYMELSRLSDDTAVYYCARSD YDSSGYYYGNSFDYWGGTTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSST LGTQTYICNVNHKPSNTKVDKRVEP KSC	36	EIVLTQSPGTLSPGERATLSCRASQSVSN SYLAWYQQKPGQAPRLLIYGASSRATGI PDRFSGSGSGTDFTLTISRLEPEDFAV FYCQHYSRSPWTFGGGTKVEIKRTVA APSVFI FPPSDEQLKSGTASVVL CLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFN RGECQVTLRESGPALVKPTQTLTLT CTVSGFSLAYSVNWIRQPPGKALEW LAMIWGDGKIVYNSALKSRLTISKD TSKNQVVLMTNMDPVDATYYCAGD GYYPYAMDNWGQSSLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCP PCPAPAEAGGPSVFLFPPKPKDT LYITREPEVTCVVVDVSHEDPEVKEN WYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSTM HEALHNHYTQKSLSLSPGK	37	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSEFMHWYQQKPGQ PPKLLIYLASNLESGVPDRFSGS GSGTDFTLTISSLQAEDVAVYYC QQNNEDPRTFGGGTKVEIKRTVA APSVFI FPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFN RGEC	38
003	QVQLQESGPGLVKPSETLSLTCTVS GGISNRYFWSWIRQPAKGLEWIGR IYKSWRTNYPNPSLKSRTMSVDTSK NQFSLKLSVTAADTAVYYCARERF NRNDAYDAFDIWGQGTMTVSSAST KGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSC	39	DIQLTQSPSFLSASVGDRTITCRASQGISS YLAWYQQKPGKAPKLLIYAASLTQSGVPSRF SGSGSGTEFTFTISSLQPEDFVITYCQQLNS YPPTFGGTKEIKRTVAAPSVFI FPPSDEQ LKSGTASVCLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDYSLSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFN RGECQVTLRESGPALVKPTQTLTLT CTVSGFSLAYSVNWIRQPPGKALEW LAMIWGDGKIVYNSALKSRLTISKD TSKNQVVLMTNMDPVDATYYCAGD GYYPYAMDNWGQSSLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCP PCPAPAEAGGPSVFLFPPKPKDT LYITREPEVTCVVVDVSHEDPEVKEN WYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSTM HEALHNHYTQKSLSLSPGK	40	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSEFMHWYQQKPGQ PPKLLIYLASNLESGVPDRFSGS GSGTDFTLTISSLQAEDVAVYYC QQNNEDPRTFGGGTKVEIKRTVA APSVFI FPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFN RGEC	41

002	EVQLVESGGGLVQPGGSLRLSCAAS GFTFNSYNLHWVRQAPGKGLEWVSS IISTSTYKDYADSVKGRFTISRDN KNSLFLQMNSLRAEDTAVYYCARGT FFDYWGQGLTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNV NHHKPSNTKVDKRVKPKSC	42	QLVLTQSPSASASLGASVKLTCTLSSGRSSY AIAWHQQRPEKGPRLMKLNSDGSLSRGDGI PDRFSGSSSGTERYLTISSLQAEDVAVYYCQ TWVTGTIQVFGGGTKLTVLGQPKAAPSVTLF PSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTPSKQSNKKAASSYLSLT PEQWKSHRYSQCQVTHEGSTVEKTVAPTECS QVTLRESGPALVKPTQTLTLCTVSGFSLSA YSVNWIRQPPGKALEWLAIWGDGKIVYNSA LKSRLTISKDTSKNQVLTMTNMDPVDATY YCAGDGYYPYAMDNDWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVTPSSSLGTQTYICNVNHHKPSNTKVDKRV PKSCDKTHTCPPCPAPEAAGGPSVFLFPPK KDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLT HQLDNLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK	43	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSEFMHWYQQKPGQ PPKLLIYLASNLESGLVPDRFSGS GSGTDFTLTISSLQAEDVAVYYC QQNNEPDRTPFGGGTKVEIKRTVA APSVFIFFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKSDSTYLSSTLT LSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC	44
007	EVQLVESGGGLVQPGGSLRLSCAAS GFTFNSYAMNWRQAPGKGLEWVST ISGSGGATRYADSVKGRFTISRDN RNTVYLQMNSLRAEDTAVFYCTKDR LIMATVRGPPYYGMDVWGQGTITVTV SSASTKGPSPVFLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHHKPSNTKVDKRV EPKSC	62	DIQMTQSPSSLSASVGDRTITCRASQSISS YLNWYQQKPGKAPNLLIYAASLQSGVPSRF SGSGSEDTFTLTISLQPEDFATYYCQSHS VSFTFGPGTKVDIKRTVAAPSVFIFFPSDEQ LKSGTASVCLLNNTFYPREAKVQWKVDNALQ SGNSQESVTEQDSKSDSTYLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGECQVT LRESGPALVKPTQTLTLCTVSGFSLSA YSVNWIRQPPGKALEWLAIWGDGKIVYNSA LKSRLTISKDTSKNQVLTMTNMDPVDATY YCAGDGYYPYAMDNDWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVTPSSSLGTQTYICNVNHHKPSNTKVDKRV PKSCDKTHTCPPCPAPEAAGGPSVFLFPPK KDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLT HQLDNLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK	63	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSEFMHWYQQKPGQ PPKLLIYLASNLESGLVPDRFSGS GSGTDFTLTISSLQAEDVAVYYC QQNNEPDRTPFGGGTKVEIKRTVA APSVFIFFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKSDSTYLSSTLT LSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC	64
BsA b	Chain 1 VH _A -CH1	SEQ	Chain 2 VL _A -CL-VH _B -CH1-Fc	SEQ	Chain 3 VL _B -CL	SEQ
004	QVTLRESGPALVKPTQTLTLCTV GSLSAYSVNWIRQPPGKALEWLA IWGDGKIVYNSALKSLRTISKDT SKNQVLTMTNMDPVDATYTCAGDGY YPYAMDNDWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYI CNVNHHKPSNTKVDKRVKPKSC	45	DIVMTQSPDLSVSLGERATINC RASKSVDSYGNSEFMHWYQQKPGQPPKLLIYLASNLESGLV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QQNNEPDRTPFGGGTKVEIKRTVAAPSVFIFFP SDEQLKSGTASVCLLNNTFYPREAKVQWKVD NALQSGNSQESVTEQDSKSDSTYLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGE CQVQLVQSGAEVKKPGASVKVSCKASGYTFT GYMHWRQAPGQGLEWMGWINPNSGGTHYV QKFGQGRVTMRDTSISTAYMELSRRLSDDTA VYYCARSDYDSSGGYYGNSEFDYWGQGLTVTV SSASTKGPSPVFLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHHKPSN TKVDKRVKPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLYITREPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQLDNLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFPYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC FSVMHEALHNHYTQKSLSLSPGK	46	EIVLTQSPGTLSSLSPGERATLSC RASQSVSNLYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYFCQHY SRSPWTFGGGTKEIKRTVAAPS VFIFFPSDEQLKSGTASVCLLN NTFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKSDSTYLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	47

006	QVTLRESGPALVKPTQTLTLTCTVS GFSLSAISVNWIRQPPGKALEWLAM IWGDGKIVYNSALKSRLTISKDTSK NQVVLMTNMDPVDATATYYCAGDGY YPYAMDNWGGSLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVKPKSC	48	DIVMTQSPDLSVSLGERATINCRASKSVDS YGNSEFMHWYQQKPGQPPKLLIYLASNLESGV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QNNEDPRTFGGGTKVEIKRTVAAPSVEIFPP SDEQLKSGTASVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDYSLSSLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGE CQVQLQESGPGLVKPSETLSLTCTVSGGSIS NYFWSWIRQAPAGKLEWIGRIYKSWRTNYP SLKSRVTMSVDTSKNQFSLKLSVTAADTAV YYCARERFNRNDAYDAFDIWGGTMVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPEAAGGFSVFL FPPKPKDTLYITREPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGGSFLYSLKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK	49	DIQLTQSPSFLSASVGDRTITC RASQGISSYLAWYQQKPGKAPKL LIYAASTLQSGVPSRFSGSGSGT EFTFTISSLQPEDFVITYYCQQLN SYPPTFGGQTKVEIKRTVAAPS FIFFPSDEQLKSGTASVCLLNN FYPREAKVQWKVDNALQSGNSQES SVTEQDSKDYSLSSLTLSKA DYEKHKVYACEVTHQGLSSPVTK SFNRGEC	50
005	QVTLRESGPALVKPTQTLTLTCTVS GFSLSAISVNWIRQPPGKALEWLAM IWGDGKIVYNSALKSRLTISKDTSK NQVVLMTNMDPVDATATYYCAGDGY YPYAMDNWGGSLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVKPKSC	51	DIVMTQSPDLSVSLGERATINCRASKSVDS YGNSEFMHWYQQKPGQPPKLLIYLASNLESGV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QNNEDPRTFGGGTKVEIKRTVAAPSVEIFPP SDEQLKSGTASVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDYSLSSLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGE CEVQLVESGGGLVKKPGGSLRLSCAASGFTFN SYNLHWVRQAPAGKLEWVSSIIISTYKDYA DSVKGRTISRDNKNSLFLQMNSLAEDTA VYICARGTFFDYWGQGLTVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVTV VPSSSLGTQTYICNVNHKPSNTKVDKRVKPK SCDKTHTCPPCPAPEAAGGFSVFLFPPKPKD TLYITREPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSGSF FLYSLKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	52	QLVLTQSPSASASLGASVKLTCT LSSGRSSYAIAWHQQRPEKGPRI LMKLNLDGSHSRGDGIFDRFSGS SSGTERYLTISSLQAEDADYYC QTWVTGIQVFGGGTKLTVLGGPK AAPSVTLFPPSSEELQANKATLV CLISDFYPGAVTVAWKADSSPVK AGVETTTPSKQSNKKAASSYLS LTPEQWKSRSYSCQVTHEGSTV EKTVPATECS	53

VH_B = OX40L binding heavy chain variable region

VL_B = OX40L binding light chain variable region

VH_A = IL-13 binding heavy chain variable region

VL_A = IL-13 binding light chain variable region

CH1 = heavy chain constant domain 1 (human IgG1) (e.g. SEQ ID NO: 33)

CL = light chain constant domain (human kappa (e.g. SEQ ID NO: 35) or human lambda (e.g. SEQ ID NO: 119))

Fc = comprises heavy chain constant domains 2 and 3 (human IgG1) optionally including one or more modifications to increase half-life and/or disrupt Fc receptor binding (e.g. SEQ ID NO: 34)

The following are examples of constant region sequences suitable for use in bispecific molecules of the invention.

IgG CH1	SEQ	IgG Fc	SEQ	IgG Kappa CL	SEQ
ASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPPSSSL GTQTYICNVNHKPSNTKVD KRVEPKSC	33	DKTHTCPPCPAPEAAGGPSVFLFPPK PKDTLYITREPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDG SFFLYSKLTVDKSRWQQGNVFCFSVM HEALHNHYTQKSLSLSPGK	34	RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSYSL STLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFENRGE	35
				IgG Lambda CL	119
				GQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADS SPVKAGVETTTPSKQSNKYAAS SYLSLTPEQWKSHRYSYSCQVTHE GSTVEKTVAPTECS	

Example 4 – binding affinity of bispecific molecules

5 1) Binding affinity of IL-13 arm by SPR

The affinity towards human IL-13, expressed as the equilibrium dissociation constant (K_D) of the antibodies, was quantified using Multiple Cycle kinetics SPR (Biacore 8K running Biacore Insight Evaluation Software Instrument V 3.0.12.15655). Antibody diluted to 1.0 $\mu\text{g/mL}$ in running buffer (HBS-P with BSA) was captured to ~500 RU on Protein A Sensor chip (Cat No. 29127555). IL-13 (Sino Biological 10369-HNAC) was used as analyte in 7 point 2-fold dilution series from 0.391 nM to 25 nM at room temperature. Analysis was done using 1:1 fitting model with double reference subtraction. Results are shown in Figure 1 and in the table below.

Sample No.	K_a (1/Ms)	K_d (1/s)	K_D (M)	R_{MAX} (RU)	χ^2 (RU ²)
BsAb001	7.21E+05	3.36E-08	<1.00E-11	67.3	5.51
BsAb002	9.46E+05	9.68E-09	<1.00E-11	47.4	0.65
BsAb003	1.07E+06	1.83E-07	<1.00E-11	62.0	4.24
BsAb004	2.06E+06	1.84E-08	<1.00E-11	53.4	3.22
BsAb005	1.91E+06	3.48E-08	<1.00E-11	56.3	3.93
BsAb006	2.54E+06	2.75E-09	<1.00E-11	55.4	3.72
BsAb007	6.51E+05	1.53E-07	<1.00E-11	65.3	4.55
Ab008	2.26E+06	5.03E-08	<1.00E-11	88.3	1.73
Ab009	-	-	NB	-	-

2) Binding affinity of IL-13 arm by Kinexa

The bispecific antibodies were profiled for binding affinity to human IL-13 by Kinetic Exclusion Assay (KinExA) using a KinExA 3200 S/N: 5000 instrument and recombinant IL-13 from Sino Biological as a binding partner (10369-HNAC). Samples were prepared in PBS containing 1 mg/mL BSA and 0.1% NaN₃. The assay is designed to estimate experimentally K_{on} , while the rate of dissociation, K_{off} , is calculated based on the following equation: $K_{off} = K_d \times K_{on}$. K_d analysis requires the immobilization of recombinant IL-13 to a solid phase (microbead), which is used as a probe to capture the different antibody molecules, which are labelled with a fluorescent secondary molecule for their detection. The signal generated from the captured labelled antibody to the solid phase is used to determine the K_d value. The KinExA Pro software performs a least squares analysis on the measured data to analyze if the values fit to a curve representative of a 1:1 reversible bi-molecular interaction. The results for the binding affinity (K_d), association and dissociation rates (K_{on} & K_{off}) for antibodies to IL-13 are shown in the table below.

BsAb002 was used to determine the activity of the IL-13. IL-13 was then used as the concentration reference for the other antibodies (mAbs), which allows for directly comparing the K_d 's of each antibody. All bispecific molecules showed a higher affinity to IL-13 compared to comparator Ab008 according to K_d measurements. Especially, antibodies BsAb004, BsAb005 and BsAb006 showed a K_d around ten times lower than the comparator. Of note, reference antibody Ab008 does not fit a standard 1:1 binding model and it is fit instead with a cooperative binding analysis that provides an "effective" K_d . BsAb005 also displays non-standard binding which could not be corrected with the cooperative model. In these cases, the lower bound of the K_d does resolve when the parameters are adjusted to a 90% confidence interval instead of a 95% confidence interval.

	BsAb001	BsAb002	BsAb003	BsAb004	BsAb005	BsAb006	Ab008
K_d	1.06 pM	2.51 pM	1.33 pM	388 fM	315 fM	306 fM	3,73 pM (Effective)
95% Confidence Interval	560 fM- 1.68 pM	1.47 pM – 3.86 pM	550 fM- 2.47 pM	69.0 fM – 1.02 pM	106 fM – 623 fM *	22.1 fM - 845 fM	3.24 pM – 4.28 pM
Concentration calculated for:	BsAb001	IL13	BsAb003	BsAb004	BsAb005	BsAb006	Ab008

Activity	74.6%	54.8%	77.8%	64.2%	71.9%	87.5%	75.8%
95% Confidence interval	69.4%-80.0%	50.2%-59.9%	66.4%-89.3%	58.0%-70.7%	61.6%-83.6%	76.0%-100%	63.3%-89.4%
Hill Coefficient	-	-	-	-	-	-	1.98
95% Confident interval	-	-	-	-	-	-	1.80-2.00
On Rate ($M^{-1} s^{-1}$)	8.28×10^5	4.40×10^5	5.57×10^5	5.34×10^6	5.71×10^6	2.42×10^6	2.23×10^6
95% Confident interval	7.74×10^5 -8.84×10^5	4.25×10^5 – 4.55×10^5	5.50×10^5 – 5.63 $\times 10^5$	4.94×10^6 – 5.83×10^6	5.22×10^6 – 6.30×10^6	2.16×10^6 – 2.72×10^6	1.75×10^6 – 2.84×10^6
Off Rate (s^{-1})	8.77×10^7	1.11×10^6	7.40×10^7	2.07×10^6	1.79×10^6	7.41×10^7	8.33×10^6

* This curve was resolved with a 90 confidence interval

Hill coefficient; commonly used as an empirical measure of the degree of Cooperativity and 95% confident interval were estimated for reference antibody Ab008.

5 3) Binding affinity of OX40L arm by SPR

The affinity towards human OX40L, expressed as the equilibrium dissociation constant (K_D) of the antibodies, was quantified using Multiple Cycle kinetics SPR (Biacore 8K running Biacore Insight Evaluation Software Instrument V 3.0.12.15655). Antibody diluted to 1.0 $\mu g/mL$ in running buffer (HBS-P with BSA) was captured to ~300 RU on Protein A Sensor chip (Cat No. 29127555). The extracellular domain of OX40L (Acro Biosystems OXL-H52Q8) was used as analyte in 7 point 2-fold dilution series from 0.781 nM to 50 nM at room temperature. The experiment was repeated 3 independent times. Analysis was done using 1:1 fitting model with double reference subtraction. Results are shown in Figure 2 and in the table below.

Sample No.	n=1*			n=2			n=3		
	$K_D(M)$	RMAX (RU)	Rel. K_D	$K_D(M)$	RMAX (RU)	Rel. K_D	$K_D(M)$	RMAX (RU)	Rel. K_D
Ab009	4.15E-10	93.7	-	3.47E-10	89.6	-	3.25E-10	88.9	-
BsAb001**	1.38E-09	43.5	1.03	1.59E-09	45.1	1.21	1.54E-09	45.7	1.31
BsAb002	1.09E-08	40.4	8.13	1.19E-08	33.7	9.08	1.30E-08	39.5	11.02
BsAb003**	1.85E-09	47.5	1.38	1.37E-09	44.6	1.05	2.34E-09	49	1.98
BsAb004	1.98E-09	38.1	1.48	2.49E-09	47	1.90	2.51E-09	47.8	2.13
BsAb005**	3.90E-09	50.4	2.91	2.90E-09	54.3	2.21	2.89E-09	53.6	2.45
BsAb006	7.10E-09	42.7	5.30	8.65E-09	38.4	6.60	1.08E-08	49.2	9.15
BsAb007	1.34E-09	49.5	1.00	1.31E-09	49	1.00	1.18E-09	49	1.00

* n=1 data

** Data does not fit a 1:1 binding kinetics model closely

Relative K_D s are shown compared to BsAb007.

- 5 4) Binding affinity of the OX40L arm by FACS assay with CHO cells expressing OX40L.

The affinity, expressed as the equilibrium dissociation constant (K_D), of the bispecific antibodies towards human OX40L expressed on a cell membrane was quantified by flow cytometry using CHO cells expressing human OX40L. To this aim, 100.000 cells
 10 per well in 180 μ l medium were seeded in a 96 well plate. To reduce unspecific signal, 2 μ l of FcR blocking reagent (130-059-901, Miltenyi) were added during 10 min at room temperature (RT). Then, 20 μ l of dose response (range from 90 μ M to 13.7 pM, final concentrations) of antibodies were added for 30 minutes at RT. The cells were washed, and the antibodies were detected with a polyclonal anti-IgG H+L antibody conjugated with
 15 AF647 for 30 minutes. Cells were washed and analysed in a flow cytometer using propidium iodide for cell viability. The experiment was repeated 3-4 independent times. K_D calculation was done using the one site specific binding equation (GraphPad). BsAb001, BsAb002, BsAb003, and BsAb005 had the highest binding affinity with the lowest K_D s (1-2 nM) (see Figure 3 and table below). Figure 3 shows K_D of bispecific
 20 antibodies when bound to membrane OX40L expressed by CHO cells. Each dot shows data from an independent experiment. Bars show arithmetic mean of three to four independent experiments $\pm$ SD. Numbers above bars show the arithmetic mean.

BsAb001, BsAb002, BsAb003 and BsAb005 K_D s were similar to the prototype
 25 BsAb007 (1-2 nM) and ~7-fold higher than reference antibody Ab009 (see Figure 3 and table below). BsAb004 and BsAb006 had the lowest affinities and highest K_D s (Figure 3 and Table below).

BsAb004 has K_D of ~8 nM, ~3.6-fold higher than the prototype BsAb007 and ~46-
 fold higher than Ab009 (Figure 3 Bar chart and Table below). No K_D could be calculated
 30 for BsAb006 as no binding saturation was observed (see Figure 3 and table below.).

	K _D (nM) CHO-OX40L Flow			
	n=1	n=2	n=3	n=4
Ab009	0.09	0.12	0.26	0.14
BsAb001	2.2	1.8	1.35	
BsAb002	1.39	1	0.82	
BsAb003	1.44	1.4	1.13	
BsAb004		6	5.2	14
BsAb005		2	1.02	0.97
BsAb007	3.2	0.46	3.5	

5) Binding affinity of OX40L arm by FACS assay with HUVEC cells

The affinity, expressed as the equilibrium dissociation constant (K_D), of BsAb001, BsAb002 and BsAb003 towards human OX40L expressed naturally on the HUVEC cell line (Human umbilical vein endothelial cells) was quantified by flow cytometry. Methods: Cells in 180 µl medium were seeded in a 96 well plate. To reduce unspecific signal, 2.5 µl of FcR blocking reagent (130-059-901, Miltenyi) were added during 10 min at room temperature (RT). Then, 20 µl of dose response (range from 90 µM to 13.7 pM final concentration) of antibodies were added for 30 min at RT. The cells were washed, and the antibodies were detected with a polyclonal anti-IgG H+L antibody conjugated with AF647 for 30 minutes. Cells viability was assessed with the live/dead staining reagent eFluor780. The experiment was repeated two to three independent times. K_D calculation was done using the one site specific binding equation (GraphPad). Results are shown in Figure 4: K_D of bispecific antibodies when bound to membrane OX40L naturally expressed by HUVEC cells. Each dot shows data from an independent experiment. Bars show arithmetic mean of two to three independent experiments ±SD. Numbers above bars show the arithmetic mean. BsAb001, BsAb002 and BsAb003 K_Ds when binding to membrane OX40L are similar to the prototype BsAb007 (0.5 - 2 nM).

20

Example 5 – blocking potency of bispecific molecules

1) IL-13 blocking potency by the bispecific antibodies.

The IL-13 blocking potency of the bispecific antibodies was evaluated with a reporter gene assay using the HEK-Blue™ IL4/IL-13 cells (Invivogen, cat. # hkb-il413) stimulated with rhIL-13 (recombinant human IL-13 from Sino Biological, cat. # LS10369-HNAC) to activate the STAT6 pathway. 25.000 HEK-Blue™ IL4/IL-13 cells/well in 24 µl

25

of assay media were seeded in 384 well plates and incubated for 2h. Then 20 μ l of dose response (range from 2 nM to 4 pM final concentrations) of bispecific antibodies pre-incubated 30 min with rhIL-13 (at its EC80) or rhIL-13 alone were added to the cells. After incubating the cells overnight, 10 μ l of the culture supernatants were harvested and

5 incubated 2h with QUANTI-Blue reagent. STAT6 activity was measured by absorbance (at 635nm). The blocking potency of IL-13 signaling (absolute IC₅₀) was calculated from the curves of the percentage of IL-13 signaling inhibition obtained at the different antibody concentrations (see Figure 5). Figure 5 is a graph of % inhibition. It shows representative % inhibition of IL-13 potency curves obtained for the different BsAb with the IL-13-

10 STAT6 gene reporter assay. Data shown are from one (n1) out of three independent experiments. The percentages of IL-13 signaling inhibition were calculated relative to the activity observed with IL-13 alone. The IL-13 blocking potency (IC₅₀s) of all the bispecific antibodies was in the in the 50-250 pM range (table below and Figure 5). The IL-13 blocking potency of the bispecific antibodies with external anti-IL-13 arm (BsAb004,

15 BsAb005 and BsAb006) was very similar compared to reference antibody Ab008 (~100 pM) (Table below and Figure 6). Figure 6 is a bar chart showing absolute IL-13 blocking IC₅₀s of the antibodies calculated from % inhibition curves carried out in three independent experiments. Each dot shows data from an independent experiment. Bars show arithmetic mean of three independent experiments $\pm$ SD.

20 The IL-13 blocking potency of the bispecific antibodies with internal anti-IL-13 arm was ~2-fold lower compared to reference antibody Ab008 (table below and Figure 6).

Antibody	Absolute IC ₅₀ (pM) or % of inhibition at 2nM				
	n1	n2	n3	Mean	SD
Ab008	66	139	79	95	39
Ab009	6%	-	10%	8%	3%
Isotype control IgG4	13%	22%	3%	13%	10%
Isotype control of BsAb	16%	25%	1%	14%	12%
BsAb001	135	171	208	171	37
BsAb002	170	218	254	214	42
BsAb003	139	189	231	186	46
BsAb004	72	115	121	103	27
BsAb005	68	106	114	96	25
BsAb006	58	82	75	72	12
BsAb007	144	253	281	226	72

Absolute IC₅₀s or the % of inhibition at the top concentration (2nM) of the antibodies calculated from % inhibition curves carried out in three independent experiments (n1, n2 and n3) and their arithmetic means and standard deviation (SD).

2) IL-13 blocking potency by the bispecific antibodies after saturation of the anti-OX40L arm.

5 The IL-13 blocking potency of the bispecific antibodies after prior binding to soluble OX40L was assessed with a reporter gene assay as described above using the HEK-Blue™ IL4/IL-13 cells (Invivogen, cat. # hkb-il413) stimulated with rhIL-13 (recombinant human IL-13 from Sino Biological, cat. # LS10369-HNAC) to activate the STAT6 pathway.

10 The assay was carried out as described above including a prior incubation of 5 minutes with the bispecific antibodies at the top concentration (2 nM) with either 200 nM or 20 nM soluble human OX40L (Sigma, cat #SRP0571) before carrying out the dilutions for the dose response. 200 nM OX40L was predicted to effectively saturate both OX40L arms of 2 nM of BsAb within 5 minutes by simulating binding kinetics of the BsAb to OX40L.

15 The IL-13 blocking potency of the bispecific antibodies was unaffected by prior binding to OX40L at predicted saturating concentrations (Figure 7). Figure 7 is a bar chart showing absolute IL-13 blocking IC₅₀s of the antibodies previously bound to OX40L at the indicated concentrations. IC₅₀s were calculated from IL-13 potency inhibition curves carried out with the IL-13-STAT6 gene reporter assay. Each dot shows data from an independent experiment. Bars depict arithmetic mean of two (OX40L 200 nM) or three (no OX40L and OX40L at 20 nM) independent experiments ±SD.

3) OX40L blocking potency (OX40 reporter assay).

25 The OX40L blocking potency of the bispecific antibodies was evaluated using the OX40 Bioassay from Promega (JA2195) consistent of OX40 effector Jurkat T cells stimulated with CHO cells overexpressing human OX40L.

20.000 OX40 effector cells were seeded per well in 20 µl of assay media-5% FBS in 384 well plates and incubated overnight. Next day 20 µl of dose response (range from

400 nM to 0.2 pM, final concentrations) of antibodies pre-incubated for 30 minutes with CHO cells overexpressing OX40L (at its EC80) were added to the effector cells. After 5h incubation, 20 µl of Bio-Glo luminescence Reagent were added to the assay to detect OX40 activity in the effector cells.

- 5 The blocking potency of OX40L (absolute IC₅₀) was calculated from the curves of the percentage of OX40 signaling inhibition obtained at the different antibody concentrations. See Figure 8, which is a % inhibition graph showing representative % inhibition of OX40 potency curves obtained for the different BsAb with the OX40 gene reporter assay. Data shown are from one (n1) out of three independent experiments. The
10 percentages of OX40 signaling inhibition were calculated relative to the activity observed with CHO cells overexpressing OX40L alone.

- All the bispecific antibodies, except BsAb006, had an OX40 blocking potency of an absolute IC₅₀ in the 3 – 5 nM range, similar to reference antibody Ab009 (table below and Figure 9). Figure 9 is a bar chart showing absolute OX40L blocking IC₅₀s of
15 antibodies calculated from % inhibition curves carried out in two or three independent experiments. Each dot shows data from an independent experiment. Bars show arithmetic mean of the independent experiments ±SD. BsAb006 had a clear reduction in OX40 blockade potency compared to reference antibody Ab009. BsAb004 showed some level of blockade potency decay compared to Ab009.

	Absolute IC ₅₀ (nM) or % of inhibition at 400nM				
	n1	n2	n3	Mean	SD
Isotype control IgG4	27%	17%	N/A	22.00%	7.07%
Isotype control of BsAb	-33%	-9%	N/A	-21.00%	16.97%
BsAb001	3.42	4.27	2.53	3.41	0.87
BsAb002	4.56	3.52	N/A	4.04	0.74
BsAb003	3.26	4.24	2.40	3.30	0.92
BsAb004	5.40	9.59	3.77	6.25	3.00
BsAb005	5.21	4.32	N/A	4.77	0.63
BsAb006	9.08	46.40	6.56	20.68	22.31
BsAb007	2.90	3.86	1.87	2.88	0.99
Ab008	-19%	1%	N/A	-9%	14%
Ab009	4.36	5.55	3.34	4.42	1.11
Absolute IC ₅₀ s or the % of inhibition at the top concentration (400 nM) of the antibodies calculated from % inhibition curves carried out in three independent					

experiments (n1, n2 and n3) and their arithmetic means and standard deviation. N/A means not applicable as the n3 experiment was carried out only with some of the antibodies.

The isotype control of BsAb is a FIT-Ig format molecule with the same human IgG1 constant region sequences (i.e. including YTE and LALA mutations) as the candidates, but with antigen binding domains that are known to not bind to any human protein.

4) OX40L blocking potency after saturation of IL-13 arm.

The OX40L blocking potency of the bispecific antibodies after prior binding to IL-13 was assessed with the using the OX40 Bioassay from Promega reporter gene assay as described above.

The assay was carried out as described above including a prior incubation of 5 minutes with the bispecific antibodies at the top concentration (400 nM) with either 120 nM or 1200 nM human IL-13 before carrying out the dilutions for the dose response. 1200 nM IL-13 was predicted to completely saturate both IL-13 arms of 400 nM of BsAb within 5 minutes by modelling the binding kinetics of the BsAb to IL-13.

The OX40L blocking potency of the bispecific antibodies was unaffected by prior binding to IL-13 at predicted saturating concentrations (see Figure 10). Figure 10 is a bar chart showing absolute OX40L blocking IC₅₀s of the antibodies previously bound to IL-13 at the indicated concentrations. IC₅₀s were calculated from OX40 potency inhibition curves carried out with the OX40 gene reporter assay. Each dot shows data from an independent experiment. Bars depict arithmetic mean of two independent experiments $\pm$ SD.

Example 6: Binding affinity and blocking potency of bispecific molecules to cynomolgus proteins

The bispecific antibodies were profiled for binding affinity to cynomolgus IL-13 (Sino Biological, 11057-CNAH) by Kinetic Exclusion Assay (KinExA) and recombinant human IL-13 from Sino Biological as a binding partner (10369-HNAC) using a KinExA 4000 S/N:4004 instrument (Sapidyne Instruments GmbH, Hanover, Germany), similarly as previously explained in example 4.2. Samples were prepared in PBS containing 1 mg/mL BSA and 0.02% NaN₃. Data was analysed with the least squares analysis on the measured

data to fit optimal solutions for the K_D and the activity of the Antibody to a curve representative of a 1:1 reversible bi-molecular interaction. Results are shown in the table below and compared with results for human IL-13.

The affinity towards cynomolgus OX40L, expressed as the equilibrium dissociation constant (K_D) of the antibodies, was quantified using Multiple Cycle kinetics SPR (Biacore 8K running Biacore Insight Evaluation Software Instrument V 3.0.12.15655). Antibody diluted to 1.0 $\mu\text{g/mL}$ in running buffer (HBS-P with BSA) was captured to ~ 300 RU on Protein A Sensor chip (Cat No. 29127555). The extracellular domain of OX40L (Acro Biosystems OXL-H52Q8 for human protein and OXL-C5243 for Cyno) was used as analyte. An eight point, two-fold dilution in running buffer was used (range from 50 nM to 0.39 nM of human OX40L, and range from 100 nM to 0.78 nM of cynomolgus OX40L). For each concentration, the association phases were monitored for 240 seconds, and the dissociation phase was measured for 900 seconds and cropped to 500 secs for improved fitting. The experiment was repeated 3 independent times. Analysis was done using 1:1 fitting model with double reference subtraction. Results are shown in the table below and compared with results for human OX40L.

	K_D (M) (measured by Kinexa)		K_D (M) (measured by SPR)	
	hIL-13	cyno IL-13	hOX40-L	cyno OX40-L
BsAb001	1.06E-12	<1.13E-12	1.38E-09	3.9E-09

BsAb001 binds with similar potency (within 10-fold) human and *Cynomolgus sp* IL-13 and OX40-L.

The IL-13 blocking potency of the bispecific antibodies was evaluated with a reporter gene assay using the HEK-Blue™ IL4/IL-13 cells (Invivogen, cat. # hkb-il413) stimulated with (recombinant human IL-13 from Sino Biological, cat. # LS10369-HNAC) or rcIL-13 (recombinant cynomolgus IL-13 from Sino Biological, cat. # 11057-CNAH) to activate the STAT6 pathway. In both cases, bispecific antibodies were pre-incubated with rhIL-13 or rcIL-13 for 30 min, then 24 μL of the mix was added onto 24 μL of 25,000 HEK-Blue™ IL4/IL-13 cells/well previously seeded in 384-well plates and incubated for 24h at 37°C, 5% CO₂. 10 μL of the supernatant was then incubated with 40 μL of QUANTI-Blue™

Solution for 2h at 37°C, 5% CO₂ and SEAP levels then determined using a spectrophotometer at 635 nm (EnSight/Revvity). The concentration range for dose response of the bispecific antibodies was 2 nM to 3 pM.

The results from two independent experiments are shown in the table below.

5

Antibody	Absolute IC ₅₀ (pM) or % of inhibition at 2 nM			
	hIL13		cIL13	
	Mean	SD	Mean	SD
Ab008	100.0	25.4	159.0	55.2
Ab009	2%	1%	1%	2%
BsAb002	213.6	69.9	194.3	78.6
BsAb003	190.0	31.2	149.7	96.7
BsAb001	214.9	45.9	142.1	81.9
BsAb004	107.1	26.0	143.8	59.7
BsAb006	63.3	5.8	111.7	60.1
BsAb005	93.4	33.5	144.0	60.4
Absolute IC ₅₀ s or the % of inhibition at the top concentration (2 nM) of the antibodies calculated from % inhibition curves carried out in two independent experiments. The arithmetic means and standard deviation (SD) are shown.				

The OX40L blocking potency of the bispecific antibodies was evaluated using the OX40 Bioassay from Promega (JA2195) consistent of OX40 effector Jurkat T cells stimulated with CHO cells overexpressing human OX40L (CHO-W3789F2 medium from Wuxi) or with HEK-293 cells overexpressing cynomolgus OX40L (Flpin293-W3789 cynomolgus from Wuxi). In that case 20 µl of 20,000 OX40 Effector cells were seeded in 384-well plates and incubated for 24h at 37°C, 5%CO₂. The next day, bispecific antibodies were pre-incubated with CHO-hOX40L cells or HEK-cOX40L cells for 30 min, then 20 µl of the mix was added onto 20 µl of the Effector cells and incubated for 5 hours at 37°C, 5%CO₂. 20 µl of Bio-Glo reagent was then added to the assay and luminescence was read in Luminoskan (Thermo). The addition of OX40L induces the OX40 pathway-activated luminescence that can be detected in a dose-dependent manner.

The results from two independent experiments are shown in the table below.

Antibody	Absolute IC ₅₀ (nM) or % of inhibition at 400 nM			
	hOX40L		cOX40L	
	Mean	SD	Mean	SD
Ab008	3%	7%	-16%	1%
Ab009	2.7	0.6	2.5	0.0
BsAb002	2.9	0.1	3.9	0.1
BsAb003	2.8	0.7	3.6	0.1
BsAb001	3.2	1.7	2.8	0.1
BsAb004	4.9	3.0	5.6	1.0
BsAb006	7.3	4.9	8.5	0.4
BsAb005	4.4	1.7	4.2	0.1
Absolute IC ₅₀ s or the % of inhibition at the top concentration (400 nM) of the antibodies calculated from % inhibition curves carried out in two independent experiments. The arithmetic means and standard deviation (SD) is shown.				

Example 7 – Simultaneous functionality of both target-binding arms in bispecific molecules

- Simultaneous functionality of both arms (IL-13 and OX40L) was assessed in a cellular system where PBMCs from allergic donors responsive to DerP1 were co-cultured with MRC5 (fibroblast) and A549 (epithelial) cells. The cultures were stimulated with Dermatophagoides pteronyssinus (DerP) that contain the DerP1 protein. DerP stimulation induced IL-13 and IL-5 production in these cultures by the PBMCs. IL-5 production is controlled by the OX40L-OX40 axis in this system. IL-5 production inhibition by the bispecific antibodies was measured to assess the functionality of the OX40L arm. IL-13 triggers Eotaxin-3 production by the epithelial cells. Eotaxin-3 production inhibition by the bispecific antibodies was measured to assess the functionality of the IL-13 arm.
- Methods: 12.500 MRC5 cells/well and 12.500 A549 cells/well in 100µl of Serum Free Dendritic cell base media were seeded in 96 well plates and incubated overnight. Next day, 20µl of dose response (range from 0.1 µM to 0.01 pM final concentrations) of antibodies were added to the plates for 30 min. Then, 200.000 allergic PBMC/well were added in 40 µl of assay media followed by 20 µl of containing the DerP extract. The culture was incubated for 6 days and the supernatants were collected for the subsequent

analysis of Eotaxin-3 and IL-5 by ELISA. Each condition was assayed in technical triplicates. Data from of 2-3 donors is shown.

Results: The results are shown in Figures 11 and 12. Figure 11 shows Eotaxin-3 and IL-5 inhibition profiles of BsAb (BsAb001, BsAb002 and BsAb003) and reference antibodies Ab008 (aIL-13) and Ab009 (aOX40L) in triculture system. The percentages of inhibition were calculated relative to unstimulated (basal) and DerP stimulated (top) control samples which did not receive antibodies. Data represented are the arithmetic mean $\pm$ standard error of mean (SEM) of triplicates assayed with each donor. Data shown are from 2-3 different donors. Figure 12 shows the Eotaxin-3 production blockade potency (IC₅₀) of the antibodies in the triculture system. Each dot shows data from an independent experiment. Bars depict arithmetic mean of three independent experiments $\pm$ SD.

BsAb001, BsAb002 and BsAb003 show complete Eotaxin-3 inhibition with similar level of functionality as reference antibody Ab008, Ab008/Ab009 combination or the Bispecific antibody BsAb007. Selective inhibition IL-5 by blockade of OX40L but not blockade of IL-13 is confirmed by treatment with Ab009 vs Ab008 (Figure 11). BsAb001, BsAb002 and BsAb003 show similar IL-5 inhibition compared with Ab009, Ab009/Ab008 combination or Bispecific antibody BsAb007. These data showed that the OX40L arm was functional for all 3 tested bispecific antibodies.

In conclusion, these results demonstrate that BsAb001, BsAb002 and BsAb003 have similar level of functionality compared to reference antibodies Ab008 and Ab009 blocking IL-13 and OX40L and subsequently inhibiting Eotaxin-3 and IL-5 production in a complex cellular assay with human allergic PBMCs co-cultured with Epithelial and fibroblast cells. These results confirm the therapeutic potential for the treatment of atopic dermatitis, as well as a broad range of immunological disease indications.

Example 8 - Internalization of bispecific molecules through the IL13Ra2 receptor

Internalization of the BsAbs through the IL13Ra2 receptor when bound to IL-13 was assessed using A375 cells. Antibodies were labeled with the Incucyte® Human Fabfluor-pH Red Antibody Labeling Reagent (Sartorius, 4722) and pre-incubated with the same amount of IL-13 (Sinobiological, 10369-HNAC). A dose response was carried out from 50 nM to 22 pM. Internalization in A375 cells was analyzed in a Incucyte® Live-Cell Analysis System. Internalization efficiencies were analyzed after 48h of culture. All the

- BsAb showed similar internalization efficiencies (EC_{50}) that were comparable to that of reference antibody Ab008 (Table below and Figure 13). None of the BsAbs were internalized when no previous preincubation with IL-13 was carried out. Figure 13 shows results of antibody internalization assay + IL-13 (same concentration as sample) (Total Red Object Area). Data were recorded for 48 hours, and then automatically analyzed by data processing software installed on Incucyte. The data represents Total Red Object Area ($\mu m^2/Image$) $\pm$ SEM (n = 2) at 48h.

log(agonist) vs. response -- Variable slope (four parameters)							
Best-fit values							
	BsAb001	BsAb002	BsAb003	BsAb004	BsAb005	BsAb006	Ab008
Bottom	-1712	-1398	-1689	-2212	-1139	-1148	-1638
Top	403101	475941	466829	438235	329039	387222	238135
LogEC ₅₀	0.5911	0.578	0.5515	0.481	0.5556	0.5494	0.6701
HillSlope	2.215	2.372	2.344	2.294	2.677	2.37	2.315
EC ₅₀	3.9	3.785	3.561	3.027	3.594	3.543	4.679
Span	404813	477339	468518	440447	330179	388370	239773
Best-fit values summary for the internalization study derived from the Total Red Object Area measurements shown in Figure 13							

10 **Example 9 – safety assessment of bispecific molecules**

1) Reverse signaling through OX40L

A potential agonist activity in inducing OX40L signaling of the bispecific antibodies was evaluated in THP-1 cells (ATCC, TIB-202) stimulated with LPS for macrophage differentiation. IL-6 production was analyzed to assess OX40L activation.

- 15 THP-1 cells were incubated with 100 ng/ml LPS or vehicle during 24h. After centrifugation at 300 g for 5 minutes and 2 PBS washing steps, 100 μ l medium + 1 μ l Fc blocking reagent (Invitrogen, 31245) were added to each well for 1 h. After plate centrifugation and washing, dose response (ranging from 2 μ M to 31 nM final concentration) of the antibodies +/- polyclonal anti-IgG antibodies used as surrogate for anti-drug antibodies (ADA) (Biorad, 5211-8004) were added to specific wells. After 24 h of incubation the medium was collected by centrifugation and IL-6 levels were evaluated by ELISA.

None of the BsAb induced OX40L mediated signalling in THP-1 cells resulting in IL-6 production even in the presence of polyclonal anti-IgG antibodies (used as surrogate for ADAs). In contrast efficient IL-6 production was observed with recombinant OX40 Fc molecules used as positive controls (Figure 14). Figure 14 shows stimulation of the THP1
 5 cell line measured as increase of IL-6 production over LPS alone. Data shown is the mean $\pm$ SD from values obtained per triplicate in two independent experiments.

2) Cytokine release assay on healthy donor PBMCs.

10 To assess a potential risk of acute Cytokine Release Syndrome, human PBMCs' from 10 healthy human blood donors were co-cultured with the antibodies and after 48 hours cytokine release was measured.

Methods: Human PBMC (100.000 cells/well) from 10 healthy blood donors were cultured in 100 μ l of media with 50 μ l of dose response (range from 0.2 nM to 2 μ M, final
 15 concentrations) of each BsAb, reference antibodies Ab008 and Ab009, positive/negative controls (anti-CD3 OKT3 plate bound and soluble at one concentration at 2 μ g/mL and OX40-Fc at the same concentrations as the BsAb) or negative controls (PBMCs alone and a Fab-in-Tandem format isotype). After 48h, supernatants were collected for subsequent analysis of cytokines by Luminex (ProcartaPlex™ Panel 21 Storm: G-CSF, GM-CSF,
 20 IFN α , IFN γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-18, IP-10, MCP-1, MIP-1 α , MIP-1 β , TNF α , TNF β)

Results: Cytokine amounts detected in PBMCs incubated with the BsAb001-006 were always below the amounts detected for reference antibodies Ab008 and Ab009. In addition, a positive correlation between BsAb001-006 concentration and cytokine release
 25 was not observed in any of the samples. Therefore, these data did not predict a high risk for acute Cytokine Release syndrome.

Example 10 – thermal stress / stability assessment of bispecific molecules

30 Test samples were subjected to heat stress in an incubator at 50°C for 7 days and analyzed by SEC-UPLC and cIEF to monitor aggregation and charge variants, after 0, 1, 2, 4 and 7 days. Representative results are shown in Figure 15. Different structural changes were observed for BsAb004-006 during thermal treatment (i.e. a shoulder forming towards

HMWS) which can be either structural refolding or dimerization. This tendency to refolding/dimerization was not observed in BsAb001-003. The most stable candidate in the thermal treatment was BsAb003.

5 **Example 11. Pharmacokinetics and Pharmacodynamics Study in Tg mice**

A total of three bispecific molecules of the present invention were administered by single IV bolus to male transgenic mice expressing the human FcRn receptor (scid FcRn/-hFcRn [32] Tg mice, The Jackson Laboratory, USA) at the dose level of 10 mg/kg (n=4 animals per compound).

10

Serial blood sampling, applying microsampling technique, over an extended period of time were collected to capture the full PK profile from a given animal since it delivers more robust PK parameter calculations and facilitates the detection of accelerated anti-drug antibody-related systemic clearance. A total of 13 serum samples were taken from each animal over 28 days and the concentrations of the candidates were quantified with a Ligand Binding Assay method. The concentration-time profiles are shown in Figure 16 (means are shown). The corresponding PK parameters were calculated using non-compartmental analysis.

15

20 *Bioanalytical method*

A ligand binding assay was developed for the determination of the candidates in mouse serum. The assay in sandwich format was set up on a MesoScale Discovery (MSD) platform relying on detection of the complexed analyte by electrochemiluminescence which afforded superior sensitivity and wider dynamic range compared to conventional ELISA with photometric detection. Taking advantage of the two functionalities in the bispecific structure, a target-specific assay was developed for measuring free drug by coating the microtiter plate with recombinant human OX40L. Following capture of the analyte in the serum sample, biotinylated recombinant human IL-13 was added for complexation. Streptavidin-labeled Sulfo-tag was then added, and the plate was loaded onto the MSD for reading. The assay was qualified assessing the following performance parameters: assay range, intra- and inter-assay accuracy, limits of quantification, selectivity, dilution linearity & hook effect, and long-term stability of the analyte in matrix.

25

30

Results

As it is shown in Figure 16 and the table below, none of the candidates displayed accelerated clearance and similar half-lives were obtained for the three bispecific molecules.

Compound	Stats	C _{max}	AUC _{0-inf}	CL	V _{ss}	t _{1/2}
		(µg/mL)	(day*µg/mL)	(mL/day/kg)	(mL/kg)	(day)
BsAb002	Mean	249	2030	5.0	113	16
	SD	50	269	0.77	12	2.1
BsAb003	Mean	290	2770	3.6	94	17
	SD	36	299	0.44	14	0.8
BsAb001	Mean	257	2760	3.7	94	17
	SD	52	392	0.47	18	3.6

Mouse PK data was fitted into a compartmental model and allometric scaling was done to predict a human half-life of 18-25 days.

Example 12. Pharmacokinetics and Pharmacodynamics Study in NHPs

For the non-clinical studies, one of the bispecific molecules of the present invention was selected as the therapeutic candidate (hereafter referred to as the candidate).

The objectives of this study were to evaluate the toxicity and to determine the pharmacokinetic and pharmacodynamic properties of the candidate when administered intravenously (30-minute infusion), once weekly for 4 consecutive weeks to Cynomolgus monkey (*Macaca fascicularis*). Non-human primate cynomolgus was chosen as the species for pharmacology and non-clinical safety testing based on the high sequence identity between human and cynomolgus IL-13 and OX40L proteins (94,7% and 98,91% respectively), and the cross-reactivity to cynomolgus monkey proteins of the bispecific molecules of the present invention (as shown in example 6).

In addition, to assess the effects of the candidate on the immune system functionalities, the cellular and humoral immune responses were evaluated through the in vivo T-cell Dependent Antibody Response (TDAR) and Delayed-Type Hypersensitivity (DTH) tests.

The study design is shown in the table below:

Group No.	Test Material	Dose Level (mg/kg/adm)	Days of Dosing	Main Study	
				No. of Animals	
				Males	Females
1	Vehicle Control	0	1, 8, 15, 22	4	-
2	Candidate	3	1	3	-
3		0.1	1, 8, 15, 22	4	-
4		1		4	-
5		10 ^b		4	-
6		30 ^b		1	1
7		100 ^b		1	1

- = Not applicable;

b staggered dosing start

The following toxicity parameters and endpoints were evaluated in all or selected groups:

- 5 mortality, clinical observations, local reactions at injection site, body weights, food consumption, electrocardiology, clinical pathology parameters (hematology, coagulation, clinical chemistry), organ weights, and macroscopic and microscopic examinations.

Toxicokinetics parameters were evaluated in all groups.

- 10 The immune response to KLH and TTx immunization was analyzed in Groups 1 and 3 to 5 by TDAR (anti-KLH and anti-TTx IgG and IgM), immunophenotyping of PBMCs, ELISPOT (IFN- γ and IL-4), DTH including clinical scoring, histopathology, immunohistochemistry and RNA sequencing on biopsies of KLH- and TTx-challenged skin sites.

- 15 There was no unscheduled mortality, no clinical signs, local reactions at the injection site, body weight, food consumption, electrocardiology, clinical pathology parameters, organ weights, macroscopic or microscopic changes attributed to the candidate administration at any dose level.

- 20 After administration, the candidate was detected and quantified in serum in all treated groups, with the exception of all samples taken on Day 22 from Group 3 (0.1 mg/kg) that were < LLOQ. Therefore, the serum levels measured in this toxicokinetic study confirmed

the systemic exposure of the animals to the drug during the study period. Serum concentrations were < LLOQ in all analysed samples from the control group.

Following once weekly 30-min intravenous infusion (Groups 3 to 7), maximum serum concentrations of the candidate occurred in most of the animals at the end of the infusion time (0.5 h), although in some animals t_{max} was slightly delayed. The increase of the exposure parameters C_{max} and $AUC_{(0-t)}$ was, in general, dose proportional between 1 and 100 mg/kg. Some accumulation was observed after repeated administration since mean (Groups 3 to 5) or individual (Groups 6 and 7) accumulation ratios ranged between 1.3 and 3.2.

In conclusion, administration of the candidate by intravenous infusion over 30 minutes on Days 1, 8, 15 and 22 in the cynomolgus monkey at levels of 0.1, 1, 10, 30 or 100 mg/kg/adm was well tolerated.

The most relevant pharmacological effects were the following. The doses of 1 and/or 10 mg/kg/adm were associated with decrease on the humoral response as observed with a dose-related decrease of anti-KLH IgM and IgG with treatment at doses of 1 and 10 mg/kg/adm, as well as a decrease in TTx specific IgM and IgG serum levels at doses of 1 and 10 mg/kg/adm at days 21 and 28 after immunisation.

Treatment with the candidate, at doses of 1 and 10 mg/kg/adm, resulted in a reduction of the immune cellular response that was seen by different indicators. First, the frequency of several immune cell populations in blood decreased as a consequence of the treatment. This was the case for activated and proliferating NKT cells, activated and proliferating NK cells, proliferating helper T central memory (hTCM) cells, proliferating helper T effector memory (hTEM), proliferating cytotoxic T central memory (cTCM), activated cytotoxicity T effector memory cells, regulatory T cells and memory B cells. Ex vivo T cell activation assays confirmed this tendency for a lower T cell reactivity as release of IFN γ and IL-4 on ELISPOT decreased after *ex vivo* challenge of PBMCs with KLH and TTx.

The DTH provided insights on the effect of the candidate treatment on the effector immune response in the skin. At microscopic level, skin of animals from 1 and 10 mg/kg treatment groups showed lower scores for epidermal hyperplasia and cell infiltration, (reduction of CD68+, CD3+, CD4+ and CD8+ cells) compared to skin from control group animals. At transcriptomic level and in the KLH challenged skin, contrast of the candidate 10 mg/kg

dose vs control revealed an effect on pathways related to epidermal differentiation, keratinization and skin barrier function. The IL-13 signature (generated in vitro from IL-13 stimulated keratinocytes) was analysed in the DTH dataset, results suggested that the IL-13 signature was down-modulated in 1mg/kg and 10 mg/kg groups vs control.

- 5 In conclusion, treatment with the candidate at 1 and 10 mg/kg doses effectively modulated humoral and cellular responses induced by KLH/TTx immunization in cynomolgus monkey.

10 The pharmacokinetics of the dose of 3 mg/kg is shown for three animals in Figure 17 and the table below.

Parameter	Unit	Mean	SD
C_{max}	µg/mL	95.5	15.7
t_{max}*	h	0.5	-
t_{1/2}	h	281	59.6
λ_z	h ⁻¹	0.003	0.001
AUC(0-t)	µg/mL*h	23721	6650
AUC	µg/mL	24187	7225
CL	mL/h/kg	0.13	0.040
V_{ss}	mL/kg	39.5	2.20
V_z	mL/kg	51.3	6.53

- 15 For Group 2 of the previous study (single dose and longer sampling for pharmacokinetic evaluation), serum concentrations seemed to decline in a bi-exponential manner. Mean±SD estimate of the elimination half-life was 11.7±2.5 days (individual values from 8.9 to 13.4 days).

The allometric scaling from NHP predicts a half-life in humans of approximately 18 days, which is consistent with the allometric scaling from Tg mouse, predicting a half-life in humans of 18-25 days (example 6).

BRIEF DESCRIPTION OF SEQUENCES

- SEQ ID NOs: 1, 2, 3 = respectively CDRH1, CDRH2 and CDRH3 of Lebrikizumab (Kabat)
- SEQ ID NOs: 4, 5, 6 = respectively CDRH1, CDRH2 and CDRH3 of 10L15A (Chothia)
- 5 SEQ ID Nos: 7, 8, 9 = respectively CDRH1, CDRH2 and CDRH3 of 10E23A (Chothia)
- SEQ ID NOs: 10, 11, 12 = respectively CDRH1, CDRH2 and CDRH3 of 05P04A (Chothia)
- SEQ ID NOs: 13, 14, 15 = respectively CDRL1, CDRL2 and CDRL3 of Lebrikizumab (Kabat)
- 10 SEQ ID NOs: 16, 17, 18 = respectively CDRL1, CDRL2 and CDRL3 of 10L15A (Chothia)
- SEQ ID NOs: 19, 20, 21 = respectively CDRL1, CDRL2 and CDRL3 of 10E23A (Chothia)
- SEQ ID NOs: 22, 23, 24 = respectively CDRL1, CDRL2 and CDRL3 of 05P04A (Chothia)
- 15 SEQ ID NOs: 25, 26 = respectively VH and VL of Lebrikizumab
- SEQ ID NOs: 27, 28 = respectively VH and VL of 10L15A
- SEQ ID NOs: 29, 30 = respectively VH and VL of 10E23A
- SEQ ID NOs: 31, 32 = respectively VH and VL of 05P04A
- 20 SEQ ID NO: 33 = exemplary human IgG CH1
- SEQ ID NO: 34 = exemplary human IgG Fc
- SEQ ID NO: 35 = exemplary human light chain kappa constant region
- SEQ ID NOs: 36 – 38, respectively FIT-Ig chains 1, 2, 3 of BsAb001
- SEQ ID NOs: 39 – 41, respectively FIT-Ig chains 1, 2, 3 of BsAb003
- 25 SEQ ID NOs: 42 – 44, respectively FIT-Ig chains 1, 2, 3 of BsAb002
- SEQ ID NOs: 45 – 47, respectively FIT-Ig chains 1, 2, 3 of BsAb004
- SEQ ID NOs: 48 – 50, respectively FIT-Ig chains 1, 2, 3 of BsAb006
- SEQ ID Nos: 51 – 53, respectively FIT-Ig chains 1, 2, 3 of BsAb005
- SEQ ID NOs: 54, 55, 56 = respectively CDRH1, CDRH2 and CDRH3 of Amlitelimab (Kabat)
- 30 SEQ ID NOs: 57, 58, 59 = respectively CDRL1, CDRL2 and CDRL3 of Amlitelimab (Kabat)

- SEQ ID NOs: 60, 61 = respectively VH and VL of Amlitelimab
- SEQ ID NOs: 62 – 64, respectively FIT-Ig chains 1, 2, 3 of 179
- SEQ ID NOs: 65 – 82 are alternative anti-OX40L VH and VL sequences shown in Example 2
- 5 SEQ ID NOs: 83, 84, 85 = respectively CDRH1, CDRH2 and CDRH3 of 10L15A (Kabat)
- SEQ ID NOs: 86, 87, 88 = respectively CDRH1, CDRH2 and CDRH3 of 10L15A (IMGT)
- SEQ ID NOs: 89, 90, 91 = respectively CDRH1, CDRH2 and CDRH3 of 10E23A (Kabat)
- SEQ ID NOs: 92, 93, 94 = respectively CDRH1, CDRH2 and CDRH3 of 10E23A (IMGT)
- 10 SEQ ID NOs: 95, 96, 97 = respectively CDRH1, CDRH2 and CDRH3 of 05P04A (Kabat)
- SEQ ID NOs: 98, 99, 100 = respectively CDRH1, CDRH2 and CDRH3 of 05P04A (IMGT)
- SEQ ID NOs: 101, 102, 103 = respectively CDRL1, CDRL2 and CDRL3 of 10L15A (Kabat)
- 15 SEQ ID NOs: 104, 105, 106 = respectively CDRL1, CDRL2 and CDRL3 of 10L15A (IMGT)
- SEQ ID NOs: 107, 108, 109 = respectively CDRL1, CDRL2 and CDRL3 of 10E23A (Kabat)
- 20 SEQ ID NOs: 110, 111, 112 = respectively CDRL1, CDRL2 and CDRL3 of 10E23A (IMGT)
- SEQ ID NOs: 113, 114, 115 = respectively CDRL1, CDRL2 and CDRL3 of 05P04A (Kabat)
- SEQ ID NOs: 116, 117, 118 = respectively CDRL1, CDRL2 and CDRL3 of 05P04A (IMGT)
- 25 SEQ ID NO: 119 = exemplary human light chain lamdba constant region

CLAIMS

1. A bispecific antigen-binding molecule comprising a first antigen binding domain (A) which is an IL-13 antigen binding domain and a second antigen binding domain (B) which is an OX40L antigen binding domain and wherein the bispecific antigen-binding molecule specifically binds to both IL-13 and OX40L and antagonises both IL-13 signalling from IL-13R and OX40L signalling from OX40; and wherein B is an antibody or antigen binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are:
- a) CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106;
- b) CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94; and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112; or
- c) CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100; and CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118
2. The bispecific antigen-binding molecule of claim 1, wherein B comprises a heavy chain variable region sequence (VHB) and a light chain variable region sequence (VLB), wherein:
- a) VHB is a polypeptide comprising or consisting of SEQ ID NO: 27, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 28, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto;
- b) VHB is a polypeptide comprising or consisting of SEQ ID NO: 29, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 30, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; or

c) VHB is a polypeptide comprising or consisting of SEQ ID NO: 31, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 32, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto.

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3. The bispecific antigen-binding molecule of claim 1 or 2, wherein A is an antibody or antigen binding fragment thereof comprising the CDRH1, CDRH2 and CDRH3 of SEQ ID NOs: 1, 2 and 3; and the CDRL1, CDRL2 and CDRL3 of SEQ ID NOs: 13, 14 and 15.

10 4. The bispecific antigen-binding molecule of claim 3, wherein A comprises a heavy chain variable region sequence (VHA) and a light chain variable region sequence (VLA), and wherein VHA is a polypeptide comprising or consisting of SEQ ID NO: 25, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLA is a polypeptide comprising or consisting of SEQ ID NO: 26, or a sequence having at least 80,
15 90, 95, or 99% amino acid identity thereto.

5. The bispecific antigen-binding molecule according to any one of the preceding claims, wherein A and/or B comprises or consists of an antibody or antigen binding fragment thereof, and wherein the antibody is preferably a chimeric, humanized or human
20 or antibody.

6. The bispecific antigen-binding molecule according to any one of the preceding claims which is a bispecific antibody which (a) comprises an IgG1, IgG2, IgG3 or IgG4 constant region, optionally a human IgG1, IgG2, IgG3 or IgG4 constant region; and/or (b)
25 is: i) an IgG-like bispecific antibody; or ii) a non-IgG like bispecific antibody.

7. The bispecific antibody of claim 6, which comprises a human IgG1 Fc (Fragment crystallizable) region sequence, said sequence comprising a modification of the human wildtype sequence to reduce Fc receptor binding (optionally the L234A/L235A (LALA)
30 modification) and/or a modification of the human wildtype sequence to increase serum half-life (optionally the M252Y/S254T/T256E (YTE) modification).

8. The bispecific antibody according to claim 6 or 7 which is an IgG-like bispecific antibody that is: i) a symmetric IgG-like bispecific antibody (e.g. a DVD-Ig bispecific antibody or FIT-Ig bispecific antibody); or ii) a non-symmetric IgG-like bispecific antibody.

5

9. The bispecific antibody according to claim 8 which is:

(a) a symmetric IgG-like bispecific antibody in which the relative positions of A and B are such that A is proximal to the Fc region of the molecule and B is distal to the Fc region; or

(b) a symmetric IgG-like bispecific antibody in which the relative positions of A and B are such that B is proximal to the Fc region of the molecule and A is distal to the Fc region; wherein (a) is preferred.

10. The bispecific antibody according to claim 9(a), consisting of three polypeptide chains each with domains arranged as follows (N-C terminus):

15 Chain 1: VHB-CH1;

Chain 2: VLB-CL-VHA-CH1-Fc; and

Chain 3: VLA-CL;

or the bispecific antibody according to claim 9(b), consisting of three polypeptide chains each with domains arranged as follows (N-C terminus):

20 Chain 1: VHA-CH1;

Chain 2: VLA-CL-VHB-CH1-Fc; and

Chain 3: VLB-CL;

wherein VHA, VLA, VHB and VLB are each polypeptides comprising or consisting of sequences as defined in any one of the preceding claims; CH1 is a polypeptide comprising

25 or consisting of the sequence of any heavy chain constant domain 1 (preferably of human IgG1), CL is a polypeptide comprising or consisting of the sequence of any light chain constant domain (preferably human kappa or lambda), Fc is a polypeptide comprising or consisting of the sequence of any Fc region of an antibody (preferably of human IgG1) comprising heavy chain constant domains 2 and 3 (CH2 and CH3), and optionally

30 including one or more modifications as defined in claim 7.

11. The bispecific antibody of 10, comprising:

- a) Chain 1: SEQ ID NO: 36; Chain 2: SEQ ID NO: 37; Chain 3: SEQ ID NO: 38;
- b) Chain 1: SEQ ID NO: 39; Chain 2: SEQ ID NO: 40; Chain 3: SEQ ID NO: 41;
- c) Chain 1: SEQ ID NO: 42; Chain 2: SEQ ID NO: 43; Chain 3: SEQ ID NO: 44;
- d) Chain 1: SEQ ID NO: 45; Chain 2: SEQ ID NO: 46; Chain 3: SEQ ID NO: 47
- 5 e) Chain 1: SEQ ID NO: 48; Chain 2: SEQ ID NO: 49; Chain 3: SEQ ID NO: 50; or
- f) Chain 1: SEQ ID NO: 51; Chain 2: SEQ ID NO: 52; Chain 3: SEQ ID NO: 53.

12. The bispecific antigen-binding molecule according to claim 5 wherein the bispecific antibody comprises variable domains of an antibody and T cell receptor (TCR)
 10 constant regions, wherein the TCR constant regions are capable of forming a dimer comprising at least one non-native interchain bond.

13. An OX40L-specific antibody or antigen-binding fragment thereof comprising three heavy chain complementarity determining region sequences (CDRs: CDRH1, CDRH2 and
 15 CDRH3) and three light chain CDRs (CDRL1, CDRL2, CDRL3) which are:

- a) CDRH1: SEQ ID NO: 86, CDRH2: SEQ ID NO: 87, CDRH3: SEQ ID NO: 88; and CDRL1: SEQ ID NO: 104, CDRL2: SEQ ID NO: 105, CDRL3: SEQ ID NO: 106;
- b) CDRH1: SEQ ID NO: 92, CDRH2: SEQ ID NO: 93, CDRH3: SEQ ID NO: 94; and CDRL1: SEQ ID NO: 110, CDRL2: SEQ ID NO: 111, CDRL3: SEQ ID NO: 112; or
- 20 c) CDRH1: SEQ ID NO: 98, CDRH2: SEQ ID NO: 99, CDRH3: SEQ ID NO: 100; and CDRL1: SEQ ID NO: 116, CDRL2: SEQ ID NO: 117, CDRL3: SEQ ID NO: 118.

25 14. The OX40L-specific antibody or antigen-binding fragment thereof of claim 13, which comprises a heavy chain variable region sequence (VHB) and a light chain variable region sequence (VLB), wherein:

- a) VHB is a polypeptide comprising or consisting of SEQ ID NO: 27, or a sequence
 30 having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 28, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto;

- b) VHB is a polypeptide comprising or consisting of SEQ ID NO: 29, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 30, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; or
- 5 c) VHB is a polypeptide comprising or consisting of SEQ ID NO: 31, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto; and VLB is a polypeptide comprising or consisting of SEQ ID NO: 32, or a sequence having at least 80, 90, 95, or 99% amino acid identity thereto.
- 10 15. The OX40L-specific antibody or antigen-binding fragment thereof according to claim 13 or 14, which is a chimeric, humanized or human or antibody.
16. The OX40L-specific antibody or antigen-binding fragment thereof according to any one of claims 13 to 15 which comprises an IgG1, IgG2, IgG3 or IgG4 constant region,
- 15 optionally a human IgG1, IgG2, IgG3 or IgG4 constant region.
17. The OX40L-specific antibody or antigen-binding fragment thereof according to any one of claims 13 to 16, which comprises a human IgG1 Fc (Fragment crystallizable) region sequence, said sequence comprising a modification of the human wildtype sequence to
- 20 reduce Fc receptor binding (optionally the L234A/L235A (LALA) modification) and/or a modification of the human wildtype sequence to increase serum half-life (optionally the M252Y/S254T/T256E (YTE) modification).
18. A pharmaceutical composition comprising the bispecific antigen-binding molecule
- 25 of any one of claims 1 to 11 or the OX40L-specific antibody or antigen-binding fragment thereof of any one of claims 13 to 17 and one or more of a pharmaceutically acceptable carrier, diluent, excipient, and/or preservative.
19. A method of treating a a disease or condition in a patient, wherein:
- 30 (i) the disease or condition is associated with or mediated by IL-13 and/or OX40L, and wherein the method comprises administering to the patient the bispecific antigen-binding molecule of any one of claims 1 to 12, the OX40L-specific antibody or antigen-binding

fragment thereof of any one of claims 13 to 17, or a pharmaceutical composition according to claim 18.

20. The method according to claim 19, wherein the disease or condition is selected
5 from the group consisting of: a dermatological disease (e.g., atopic dermatitis, prurigo nodularis, chronic hand eczema, allergic dermatitis, psoriasis, lichen planus, hidradenitis suppurativa), asthma, allergic diseases (e.g., allergic rhinitis), cardiovascular diseases (e.g., myocardial infarction, cardiac hypertrophy-related diseases), atherosclerosis, musculoskeletal diseases (rheumatoid arthritis), COPD, age-related macular degeneration,
10 periodontitis uveitis, cancer, inflammatory bowel disease, fibrosis, scleroderma, and eosinophilic esophagitis.

21. The method according to claim 19 or 20 wherein: (i) the disease or condition is a
dermatological disease such as atopic dermatitis; (ii) the bispecific antigen binding
15 molecule is administered to the patient by injection, optionally by subcutaneous injection; and/or (iii) the patient is a human patient.

22. The method according to any one of claims 19 to 21, wherein the method further
comprises administering an additional medication and/or carrying out a separate treatment
20 method for the treatment of the disease or condition.

23. The bispecific antigen-binding molecule of any one of claims 1 to 12, the OX40L-specific antibody or antigen-binding fragment thereof of any one of claims 13 to 17, or a pharmaceutical composition according to claim 18, for use in a method of treating a
25 disease or condition in a patient, wherein the disease or condition is associated with or mediated by IL-13 and/or OX40L, optionally wherein the method is as defined in any one of claims 19 to 22.

24. Use of the bispecific antigen-binding molecule of any one of claims 1 to 12, the
30 OX40L-specific antibody or antigen-binding fragment thereof of any one of claims 13 to 17, or a pharmaceutical composition according to claim 18, in the manufacture of a medicament for use in a method of treating a disease or condition in a patient, wherein the

disease or condition is associated with or mediated by IL-13 and/or OX40L, optionally wherein the method is as defined in any one of claims 19 to 22.