

MULTI-SPECIFIC ANTIBODY
WITH BINDING SPECIFICITY FOR HUMAN IL-13 AND IL-17

FIELD OF THE INVENTION

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The present invention relates to a multi-specific antibody having specificity for human IL-13, human IL-17A and/or IL-17F. The invention further relates to methods for producing the multi-specific antibody and to its therapeutic use for the treatment of atopic dermatitis and other diseases.

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BACKGROUND OF THE INVENTION

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Atopic dermatitis (AD), also known as atopic eczema, is an inflammatory disease which results in intensely itchy, red, swollen, weeping and cracked skin which often thickens over time.

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Since the beginning of the twentieth century, many mucosal inflammatory disorders have become more common; atopic dermatitis is a classic example of such a disease. It now affects 15–30% of children and 2–10% of adults in developed countries and in the United States has nearly tripled in the past thirty to forty years. Over 15 million American adults and children have atopic dermatitis.

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Treatments used for AD include systemic immunosuppressants such as cyclosporin, methotrexate, interferon gamma, mycophenolate, mofetil and azathioprine. Antidepressants and naltrexone may be used to control pruritus (itchiness). In 2016, crisaborole, a phosphodiesterase-4 inhibitor, was approved for mild-to-moderate eczema, and in 2017, dupilumab, a monoclonal antibody antagonist of IL-4R α was approved to treat moderate-to-severe eczema.

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Because of the limitations of the existing medications, there is a great need for improved treatment of atopic dermatitis.

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WO2013/102042A2 (Abbvie) describes dual specific binding proteins directed against IL-13 and IL-17 and their potential use in treatment of an extensive list of diseases. The binding proteins did not progress into clinical development.

WO2015/127405A2 (Genentech) describes anti-IL-13/IL-17 bispecific antibodies, and methods of using them for treating moderate to severe asthma and/or eosinophilic asthma. In a phase I clinical trial, BITS7201A was associated with a high incidence of anti-drug antibodies (ADAs) and was withdrawn from clinical development.

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SUMMARY OF THE INVENTION

The present invention provides an improved multi-specific antibody capable of binding human IL-13, human IL-17A and/or human IL-17F.

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The antibodies of the present invention have improved properties in comparison with currently available antibodies, for example lower immunogenicity and/or better pharmacokinetic profiles. In addition, the antibodies of the present invention may be engineered so that they can be more efficiently purified using an improved purification method which comprises fewer steps than currently available methods, which is cost and time effective at the industrial scale. The antibodies of the present invention may therefore have improved manufacturability.

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The invention further provides:

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- ❖ An isolated polynucleotide encoding the multi-specific antibody.
- ❖ An expression vector carrying the polynucleotide.
- ❖ A host cell comprising the vector.
- ❖ A method of producing the multi-specific antibody comprising culturing the host cell and recovering the antibody produced.
- ❖ A pharmaceutical composition comprising the multi-specific antibody.
- ❖ A multi-specific antibody or pharmaceutical composition for use in a method of treatment of the human or animal body by therapy.
- ❖ A method of treating or preventing atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis, comprising administering a therapeutically effective amount of the multi-specific antibody or the pharmaceutical composition to a patient in need thereof.

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BRIEF DESCRIPTION OF THE FIGURES

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Figure 1.

Ab650 humanisation alignments.

Alignments of the rat antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences, together with the designed humanised sequences.

(A) Light chain graft 650:

650 = rat variable light chain sequence.

- 5 650gL8 = humanised graft of 650 variable light chain using IGKV1-39 human germline as the acceptor framework.

CDRs are shown in bold/underlined.

Donor residues are shown in bold/italic and are highlighted: I58 and Y71.

(B) Heavy chain graft 650:

- 10 650 = rat variable heavy chain sequence.

650gH9 = humanised graft of 650 variable heavy chain using IGHV1-69 human germline as the acceptor framework.

CDRs are shown in bold/underlined.

Donor residues are shown in bold/italic and are highlighted: A67, F69 and V71.

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Figure 2.

Amino acid and DNA sequences.

Figure 3.

- 20 Purification of an IL-13/IL-17AF multi-specific antibody.

(A) BEH200 SEC-UPLC analysis of purified multi-specific antibody, detection by FLR.

(B) Protein samples separated by Tris-glycine SDS-PAGE under non-reducing (lane 1) or reducing (lane 2) conditions. The gel was stained with Coomassie quick stain and destained in dH₂O. Mark 12 protein markers (Life Technologies) were used as standards (M).

- 25 Molecular weights (MW) were measured in kilo daltons (kDa).

Figure 4.

Inhibition of STAT6 signalling by IL-13/IL-17AF multi-specific antibody.

- 30 Figure 5.

(A) Inhibition of IL-6 production by IL-13/IL-17AF multi-specific antibody, in response to human or cynomolgus IL-17A in combination with TNF- α . (i) Human IL-17A. (ii) Cynomolgus IL-17A.

- 35 (B) Inhibition of IL-6 production by IL-13/IL-17AF multi-specific antibody, in response to human or cynomolgus IL-17F in combination with TNF- α . (i) Human IL-17F. (ii) Cynomolgus IL-17F.

Figure 6.

Simultaneous neutralisation of IL-13, IL-17A and IL-17F by IL-13/IL-17AF multi-specific antibody in an NHEK CXCL1 release bioassay.

Key:

- 5 circle = anti-IL-13/IL-17AF
- square = anti-IL-17A
- up-pointing triangle = anti-IL-17F
- down-pointing triangle = anti-IL-13

Figure 7.

Diagrammatic illustration of a multi-specific IL-13/IL-17AF antibody according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION**IL-13**

IL-13 is a short-chain cytokine sharing 25% sequence identity with IL-4. It comprises approximately 132 amino acids, forming a secondary structure of four helices spanning residues 10-21 (helix A), 43-52 (helix B), 61-69 (helix C), and 92-110 (helix D), along with two β strands spanning residues 33-36 and 87-90. The solution structure of IL-13 has been solved, revealing the predicted up-up-down-down four-helix-bundle conformation that is also observed with IL-4. (Eisenmesser 2001).

Human IL-13 is a 17kDa glycoprotein and is produced by activated T-cells of the Th2 lineage, although Th0 and Th1 CD4+ T cells, CD8+ T cells, and several non-T cell populations such as mast cells also produce IL-13. The functions of IL-13 include immunoglobulin isotype switching to IgE in human B cells and suppressing inflammatory cytokine production in both humans and mice.

IL-13 binds to its cell surface receptors, IL-13R-alpha1 and IL-13R-alpha2. IL-13R-alpha1 interacts with IL-13 with a low affinity ($K_D \sim 10\text{nM}$), followed by recruitment of IL-4R-alpha to form a high affinity ($K_D \sim 0.4\text{nM}$) signalling heterodimeric receptor complex.

The IL-4R/IL-13R-alpha1 complex is expressed on many cell types, such as B cells, monocytes/macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial

cells, airway epithelial cells, and airway smooth muscle cells. Ligation of the IL-13R-alpha/IL-4R receptor complex results in activation of a variety of signal-transduction pathways, including signal transducer and activator of transcription 6 (STAT6) and insulin receptor substrate 2 (IRS2) pathways.

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The IL-13R-alpha2 chain alone has a high affinity for IL-13 ($K_D \sim 0.25-0.4 \text{ nM}$). It functions both as a decoy receptor that negatively regulates IL-13 binding, and as a signalling receptor that induces TGF- β synthesis and fibrosis via AP-1 pathway in macrophages and possibly other cell types.

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IL-13 is implicated in the pathogenesis of many human disorders and therapeutic strategies have been designed to inhibit or counteract IL-13 activity. In particular, antibodies that bind to and neutralise IL-13 have been sought as a means to inhibit IL-13 activity. However, there exists a need in the art for suitable and/or improved antibodies capable of binding IL-13, especially human IL-13 and in particular, antibodies which are capable of neutralising human IL-13.

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The present invention provides a novel family of binding proteins, CDR grafted antibodies, humanised antibodies and fragments thereof, capable of binding human IL-13, binding with high affinity, and binding and neutralising human IL-13.

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Antibodies that inhibit IL-13 activity may operate via several possible mechanisms of action. Bin 1 represents an antibody that binds to human IL-13 and prevents binding of IL-13R α 1 and as a result also blocks IL-4R from binding. Bin 1 antibodies may also prevent binding of IL-13 to IL-13R α 2. Bin 2 represents an antibody that binds hIL-13 in such a way that it allows binding to IL-13R α 1 but prevents recruitment of IL-4R into the complex. We were selecting antibodies that operated via bin 1.

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In one embodiment, the multi-specific antibody binds to human IL-13 and prevents binding of IL-13R α 1.

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In one embodiment, the multi-specific antibody binds to human IL-13 and prevents binding of IL-13R α 2.

In one embodiment, the multi-specific antibody binds to human IL-13 and prevents binding of IL-13R α 1 and IL-13R α 2.

In one embodiment, the multi-specific antibody binds to human IL-13 with a K_D of <100pM.

IL-17

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The IL-17 family of cytokines consists of 6 members based on structural similarities, with a molecular mass of 23-36 kDa and a dimer structure. The founding member IL-17A (often still referred to in the literature as simply IL-17) shares 16% - 50% amino acid sequence identity with other members: IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. IL-17A and IL-17F share the greatest homology (50%) and bind to the same receptor complex, thus shared biological activities have been noted between these 2 cytokines. In addition, IL-17A and IL-17F exist not only as homodimers, but also as an IL-17A/F heterodimer. IL-17E (IL-25) has the least similarity with IL-17A. Of significance and relevance to the biological activity of IL-17A and IL-17F is the finding that they share the same IL-17RA/IL-17RC receptor complex, with IL-17A having greatest affinity for IL-17RA, whereas IL-17F binds more strongly to IL-17RC. The other family member to utilise IL-17RA is IL-17E, which signals via the IL-17RA/IL-17RB receptor complex.

IL-17A and IL-17F are produced by the Th17 subset of CD4+ T cells. In addition, other T cell subsets produce IL-17A and IL-17F including cytotoxic CD8+ T cells (Tc17), gdT cells and NK T cells. Other cell populations reported to secrete IL-17A include neutrophils, monocytes, NK cells, lymphoid tissue inducer-like (LTi-like) cells, intestinal paneth cells and even B cells and mast cells. In addition, epithelial cells have been reported to secrete IL-17F.

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The cell types which respond to IL-17 cytokines are reflected by the expression of the different receptors. IL-17RA is ubiquitously expressed, with particularly high levels in haematopoietic tissues whereas IL-17RC is more highly expressed in non-immune cells of joints, liver, kidney, thyroid and prostate. This differential expression could explain differences in IL-17A and IL-17F biological activity as cells expressing high levels of IL-17RC could be more responsive to IL-17F whereas cells with higher expression of IL-17RA than IL-17RC may respond more readily to IL-17A. Specific cell types that are responsive to IL-17A and F include fibroblasts, epithelial cells, keratinocytes, synoviocytes and endothelial cells with IL-17A also reported to act on T and B cells and macrophages.

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The multi-specific antibody of the present invention is capable of binding to human IL-17A and/or IL-17F. Thus, the antibody can bind to IL-17A homodimer, IL-17F homodimer and/or IL-17AF heterodimer.

- 5 In one embodiment, the multi-specific antibody binds to human IL-17A. In one embodiment, the multi-specific antibody binds to human IL-17F. In one embodiment, the multi-specific antibody binds to human IL-17A and IL-17F.

In one embodiment the multi-specific antibody binds to human IL-17A with a K_D of <50pM.

- 10 In one embodiment the multi-specific antibody binds to human IL-17A with a K_D of <25pM. In one embodiment the multi-specific antibody binds to human IL-17A with a K_D of <10pM.

In one embodiment the multi-specific antibody binds to human IL-17F with a K_D of <200pM.

- 15 In one embodiment the multi-specific antibody binds to human IL-17F with a K_D of <100pM.

Albumin

- The high specificity and affinity of antibodies make them ideal diagnostic and therapeutic agents, particularly for modulating protein:protein interactions. However, antibodies may suffer from an increased rate of clearance from serum, especially when they lack the Fc domain that imparts a long lifetime *in vivo* (Medasan *et al.*, 1997, J. Immunol. 158:2211-2217).
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- Means to improve the half-life of antibodies are known. One approach has been to conjugate the fragment to polymer molecules. Thus, the short circulating half-life of Fab', F(ab')₂ fragments in animals has been improved by conjugation to polyethylene glycol (PEG; see, for example, WO98/25791, WO99/64460 and WO98/37200). Another approach has been to modify the antibody fragment by conjugation to an agent that interacts with the FcRn receptor (see, for example, WO97/34631). Yet another approach to extend half-life has been to use polypeptides that bind serum albumin (see, for example, Smith *et al.*, 2001, Bioconjugate Chem. 12:750-756; EP0486525; US6267964; WO04/001064; WO02/076489; and WO01/45746).
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- Serum albumin is an abundant protein in both vascular and extravascular compartments with a half-life in man of about 19 days (Peters, 1985, Adv Protein Chem. 37:161-245). This is
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similar to the half-life of IgG1, which is about 21 days (Waldeman & Strober, 1969, Progr. Allergy, 13:1-110).

Anti-serum albumin binding single variable domains have been described along with their
5 use as conjugates to increase the half-life of drugs, including NCE (chemical entity) drugs, proteins and peptides, see for example, Holt *et al.*, Protein Engineering, Design & Selection, vol 21, 5, pp283-288, WO04003019, WO2008/096158, WO05118642, WO2006/0591056 and WO2011/006915. Other anti-serum albumin antibodies and their use in multispecific antibody formats have been described in WO2009/040562, WO2010/035012 and
10 WO2011/086091. In particular, we have previously described an anti-albumin antibody with improved humanisation in WO2013/068571.

The multi-specific antibodies of the present invention may be engineered to bind to human serum albumin, in order to extend their *in vivo* serum half-life, resulting in improved
15 pharmacokinetic profiles.

Antibodies

Antibodies for use in the context of the present disclosure include whole antibodies and
20 functionally active fragments thereof, i.e. molecules that specifically bind to IL-13, IL-17A and/or IL-17F, also termed antigen-binding fragments. Features described herein with respect to antibodies also apply to antibody fragments unless context dictates otherwise.

Whole antibodies, also known as “immunoglobulins (Ig)” generally relate to intact or full-length antibodies i.e. comprising the elements of two heavy chains and two light chains, inter-connected by disulphide bonds, which assemble to define a characteristic Y-shaped three-dimensional structure. Classical natural whole antibodies are monospecific in that they bind one antigen type, and bivalent in that they have two independent antigen binding domains. The terms “intact antibody”, “full-length antibody” and “whole antibody” are used
25 interchangeably to refer to a monospecific bivalent antibody having a structure similar to a native antibody structure, including an Fc region as defined herein.
30

Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region (C_L). Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region (CH) constituted of
35 three constant domains CH₁, CH₂ and CH₃, or four constant domains CH₁, CH₂, CH₃ and CH₄, depending on the Ig class. The “class” of an Ig or antibody refers to the type of constant

region and includes IgA, IgD, IgE, IgG and IgM and several of them can be further divided into subclasses, e.g. IgG1, IgG2, IgG3, IgG4. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical
5 complement system.

The V_H and V_L regions of the antibody according to the present invention can be further subdivided into regions of hypervariability (or "hypervariable regions") determining the recognition of the antigen, termed complementarity determining regions (CDR), interspersed
10 with regions that are more structurally conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The CDRs and the FR together form a variable region. By convention, the CDRs in the heavy chain variable region of an antibody or antigen-binding fragment thereof are referred as CDR-H1, CDR-H2
15 and CDR-H3 and in the light chain variable regions as CDR-L1, CDR-L2 and CDR-L3. They are numbered sequentially in the direction from the N-terminus to the C-terminus of each chain.

CDRs are conventionally numbered according to a system devised by Kabat et al. This
20 system is set forth in Kabat et al., 1991, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al. (supra)"). This numbering system is used in the present specification except where otherwise indicated.

25 The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering, corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region, of the basic variable domain structure. The correct Kabat numbering of residues
30 may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering
35 system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus, unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26

to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1),
5 residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

In addition to the CDR loops, a fourth loop exists between CDR-2 (CDR-L2 or CDR-H2) and CDR-3 (CDR-L3 or CDR-H3) which is formed by framework 3 (FR3). The Kabat numbering
10 system defines framework 3 as positions 66-94 in a heavy chain and positions 57-88 in a light chain.

Based on the alignment of sequences of different members of the immunoglobulin family, numbering schemes have been proposed and are for example described in Kabat et al.,
15 1991, and Dondelinger et al., 2018, *Frontiers in Immunology*, Vol 9, article 2278.

The term "constant domain(s)", "constant region", as used herein are used interchangeably to refer to the domain(s) of an antibody which is outside the variable regions. The constant domains are identical in all antibodies of the same isotype but are different from one isotype
20 to another. Typically, the constant region of a heavy chain is formed, from N to C terminal, by CH₁-hinge -CH₂-CH₃-optionally CH₄, comprising three or four constant domains.

The constant domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular
25 the effector functions which may be required. For example, the constant domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes
30 and antibody effector functions are not required. It will be appreciated that sequence variants of these constant domains may also be used. For example, IgG4 molecules in which the serine at position 241 (numbered according to the Kabat numbering system) has been changed to proline as described in Angal et al. (Angal et al., 1993. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody as
35 observed during SDS-PAGE analysis *Mol Immunol* 30, 105-108) and termed IgG4P herein, may be used.

"Fc", "Fc fragment", "Fc-domain" and "Fc region" are used interchangeably to refer to the C-terminal region of an antibody comprising the constant region of an antibody excluding the first constant immunoglobulin domain. Thus, Fc refers to the last two constant domains, CH₂ and CH₃, of IgA, IgD, and IgG, or the last three constant domains of IgE and IgM, and the flexible hinge N-terminal to these domains. The human IgG1 heavy chain Fc region is defined herein to comprise residues C226 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In the context of human IgG1, the lower hinge refers to positions 226-236, the CH₂ domain refers to positions 237-340 and the CH₃ domain refers to positions 341-447 according to the EU index as in Kabat. The corresponding Fc region of other immunoglobulins can be identified by sequence alignments.

In the context of the present disclosure, when present, the constant region or Fc region may be natural, as defined above, or else may be modified in various ways, provided that it comprises a functional FcR binding domain, and preferably a functional FcRn binding domain. Preferably, the modified constant region or Fc region lead to improve functionalities and/or pharmacokinetics. The modifications may include deletion of certain portions of the Fc fragment. The modifications may further include various amino acid substitutions able to affect the biological properties of the antibody. Mutations for increasing FcRn binding and thus in vivo half-life may also be present. The modifications may further include modification in the glycosylation profile of the antibody. The natural Fc fragment is glycosylated in the CH₂ domain with the presence, on each of the two heavy chains, of an N-glycan bound to the asparagine residue at position 297 (Asn297). In the context of the present disclosure, the antibody may be glyco-modified, i.e. engineered to have a particular glycosylation profile, which, for example, leads to improved properties, e.g. improved effector function, or improved serum half-life.

The antibodies described herein are isolated. An "isolated" antibody is one which has been separated (e.g. by purification means) from a component of its natural environment.

The term "antibody" encompasses monovalent, i.e. antibodies comprising only one antigen binding domain (e.g. one-armed antibodies comprising a full-length heavy chain and a full-length light chain interconnected, also termed "half-antibody"), and multivalent antibodies, i.e. antibodies comprising more than one antigen binding domain.

The term "antibody" according to the invention also encompasses antigen-binding fragments of antibodies. Antigen-binding fragments of antibodies include single chain antibodies (e.g. scFv, and dsscFv), Fab, Fab', F(ab')₂, Fv, single domain antibodies or

nanobodies (e.g. V_H or V_L , or V_{HH} or V_{NAR}). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2011/117648, WO2005/003169, WO2005/003170 and WO2005/003171.

- 5 The 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).

The term "Fab fragment" as used herein refers to an antibody fragment comprising a light chain fragment comprising a V_L (variable light) domain and a constant domain of a light chain (CL), and a V_H (variable heavy) domain and a first constant domain (CH_1) of a heavy chain.

A typical "Fab' fragment" comprises a heavy and a light chain pair in which the heavy chain comprises a variable region V_H , a constant domain CH_1 and a natural or modified hinge region and the light chain comprises a variable region V_L and a constant domain CL. Dimers of a Fab' according to the present disclosure create a $F(ab')_2$ where, for example, dimerisation may be through the hinge.

The term "single domain antibody" as used herein refers to an antibody fragment consisting of a single monomeric variable antibody domain. Examples of single domain antibodies include V_H or V_L or V_{HH} or V_{NAR} .

The term "Fv" refers to two variable domains, for example co-operative variable domains, such as a cognate pair or affinity matured variable domains, i.e. a V_H and V_L pair.

25 "Single chain variable fragment" or "scFv" as employed herein refers to a single chain variable fragment, comprising or consisting of a heavy chain variable domain (V_H) and a light chain variable domain (V_L) which is stabilised by a peptide linker between the V_H and V_L variable domains. The V_H and V_L variable domains may be in any suitable orientation, for example the C-terminal of V_H may be linked to the N-terminal of V_L or the C-terminal of V_L may be linked to the N-terminal of V_H .

35 "Disulphide-stabilised single chain variable fragment" or "dsscFv" as employed herein refers to a single chain variable fragment which is stabilised by a peptide linker between the V_H and V_L variable domains and also includes an inter-domain disulphide bond between V_H and V_L . (see for example, Weatherill et al., Protein Engineering, Design & Selection, 25 (321-329), 2012, WO2007109254).

“Disulphide-stabilised variable fragment” or “dsFv” as employed herein refers to a single chain variable fragment which does not include a peptide linker between the V_H and V_L variable domains and is instead stabilised by an interdomain disulphide bond between V_H and V_L.

In one embodiment, the multi-specific antibody of the present invention is an antagonistic antibody. As used herein, the term “antagonistic antibody” describes an antibody that is capable of inhibiting or neutralising the biological signaling activity of one or more antigens, for example by blocking binding, or reducing binding of IL-13, IL-17A and/or IL-17F to their receptors.

Antibodies for use in the present invention may be, but are not limited to, monoclonal, humanised, fully human or chimeric antibodies.

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; WO92/02551; WO2004/051268 and International Patent Application number WO2004/106377.

Screening for antibodies can be performed using assays to measure binding to an antigen, and/or assays to measure the ability to block the binding of an antigen to one or more of its receptors. An example of a binding assay is an ELISA, for example, using a fusion protein of IL-13, which is immobilized on plates, and employing a conjugated secondary antibody to detect anti-IL-13 antibody bound to the IL-13. An example of a blocking assay is a flow cytometry based assay measuring the blocking of IL-13 ligand protein binding to an IL-13R. A fluorescently labelled secondary antibody is used to detect the amount of IL-13 ligand protein binding to the IL-13R.

- Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule (see, e.g. US 5,585,089; WO91/09967). It will be appreciated that it may only be necessary to transfer the specificity
- 5 determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived.
- 10 Chimeric antibodies are composed of elements derived from two different species such that the element retains the characteristics of the species from which it is derived. Generally a chimeric antibody will comprise a variable region from one species, for example a mouse, rat, rabbit or similar and constant region from another species such as a human.
- 15 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
- 20 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.

- Fully human antibodies are those antibodies in which the variable regions and the constant
- 25 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 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
- 30 replaced by their human counterparts eg. 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.

Multi-specific antibodies

- 35 The antibody of the present invention is a multi-specific antibody. "Multispecific or Multi-specific antibody" as employed herein refers to an antibody as described herein which has at

least two binding domains, i.e. two or more binding domains, for example two or three binding domains, wherein the at least two binding domains independently bind two different antigens or two different epitopes on the same antigen. Multi-specific antibodies are generally monovalent for each specificity (antigen). Multi-specific antibodies described
5 herein encompass monovalent and multivalent, e.g. bivalent, trivalent, tetravalent multi-specific antibodies.

A paratope is a region of an antibody which recognises and binds to an antigen. An antibody of the invention may be a multi-paratopic antibody. "Multi-paratopic antibody" as
10 employed herein refers to an antibody as described herein which comprises two or more distinct paratopes, which interact with different epitopes either from the same antigen or from two different antigens. Multi-paratopic antibodies described herein may be biparatopic, triparatopic, tetraparatopic.

"Antigen binding domain" as employed herein refers to a portion of the antibody, which comprises a part or the whole of one or more variable domains, for example a part or the whole of a pair of variable domains V_H and V_L , that interact specifically with the target antigen. A binding domain may comprise a single domain antibody. In one embodiment, each binding domain is monovalent. Preferably each binding domain comprises no more
20 than one V_H and one V_L .

"Specifically" as employed herein refers to a binding domain that only recognises the antigen to which it is specific, or a binding domain that has significantly higher binding affinity for the antigen to which it is specific compared to affinity for antigens to which it is non-specific.
25 Binding affinity may be measured by standard assays, for example surface plasmon resonance, such as BIAcore.

A variety of multi-specific antibody formats have been generated. Different classifications have been proposed, but multispecific IgG antibody formats generally include bispecific IgG, appended IgG, multispecific (e.g. bispecific) antibody fragments, multispecific (e.g. bispecific) fusion proteins, and multispecific (e.g. bispecific) antibody conjugates, as described for example in Spiess et al., Alternative molecular formats and therapeutic applications for bispecific antibodies. Mol Immunol. 67(2015):95-106.
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Techniques for making bispecific antibodies include, but are not limited to, CrossMab technology (Klein et al. Engineering therapeutic bispecific antibodies using CrossMab technology, Methods 154 (2019) 21-31), Knobs-in-holes engineering (e.g. WO1996027011,
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WO1998050431), DuoBody technology (e.g. WO2011131746), Azymetric technology (e.g. WO2012058768). Further technologies for making bispecific antibodies have been described for example in Godar et al., 2018, Therapeutic bispecific antibody formats: a patent applications review (1994-2017), Expert Opinion on Therapeutic Patents, 28:3, 251-276.

- 5 Bispecific antibodies include in particular CrossMab antibodies, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, Knobs-in-holes common LC, Knobs-in-holes assembly, Charge pair, Fab-arm exchange, SEEDbody, Triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body and orthogonal Fab.

- 10 Appended IgG classically comprise full-length IgG engineered by appending additional antigen-binding domain or antigen-binding fragment to the N- and/or C-terminus of the heavy and/or light chain of the IgG. Examples of such additional antigen-binding fragments include sdAb antibodies (e.g. V_H or V_L), Fv, scFv, dsscFv, Fab, scFav. Appended IgG antibody formats include in particular DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgC(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, 15 scFv4-Ig, Zbody and DVI-IgG (four-in-one), for example as described in Spiess et al., Alternative molecular formats and therapeutic applications for bispecific antibodies. Mol Immunol. 67(2015):95-106.

- 20 Multispecific antibody fragments include nanobody, nanobody-HAS, BiTEs, diabody, DART, TandAb, scDiabody, sc-Diabody-CH3, Diabody-CH3, Triple Body, Miniantibody; Minibody, Tri Bi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv3, scFv-KIH, Fab-scFv-Fc, Tetravalent HCAB, scDiabody-Fc, Diabody-Fc, Tandem scFv-Fc; and intrabody, as described, for example, Spiess et al., Alternative molecular formats and therapeutic applications for bispecific antibodies. Mol Immunol. 67(2015):95-106.
- 25 Multispecific fusion proteins include Dock and Lock, ImmTAC, HSAbody, scDiabody-HAS, and Tandem scFv-Toxin.

- 30 Multispecific antibody conjugates include IgG-IgG; Cov-X-Body; and scFv1 -PEG-scFv2. Additional multispecific antibody formats have been described for example in Brinkmann and Kontermann, The making of bispecific antibodies, mAbs, 9:2, 182-212 (2017), in particular in Figure 2, for example tandem scFv, triplebody, Fab-VHH, taFv-Fc, scFv₄-Ig, scFv₂-Fcab, scFv₄-IgG. Bibodies, tribodies and methods for producing the same are disclosed for example in WO99/37791.

- 35 The invention provides a multi-specific antibody which binds human IL-13, human IL-17A, and/or human IL-17F.

In one embodiment, the multi-specific antibody comprises an antigen binding site that binds to human IL-13, wherein the IL-13 binding site comprises a light chain variable region comprising the sequence given in SEQ ID NO:15 for CDR-L1, the sequence given in SEQ ID NO:16 for CDR-L2 and the sequence given in SEQ ID NO:17 for CDR-L3.

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In one embodiment, the multi-specific antibody comprises an antigen-binding site that binds to human IL-13, wherein the IL-13 binding site comprises a heavy chain variable region comprising the sequence given in SEQ ID NO:18 for CDR-H1, the sequence given in SEQ ID NO:19 for CDR-H2 and the sequence given in SEQ ID NO:20 for CDR-H3.

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In one embodiment, the IL-13 binding site comprises a light chain variable region comprising the sequence given in SEQ ID NO:27.

In one embodiment, the IL-13 binding site comprises a heavy chain variable region

15 comprising the sequence given in SEQ ID NO:28.

In one embodiment, the IL-13 binding site comprises a light chain variable region comprising the sequence given in SEQ ID NO:31.

20 In one embodiment, the IL-13 binding site comprises a heavy chain variable region comprising the sequence given in SEQ ID NO:32.

In one embodiment, the multi-specific antibody comprises an antigen binding site that binds to human IL-17A and human IL-17F comprising:

25 a light chain variable region comprising the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

In one embodiment, the multi-specific antibody comprises an antigen binding site that binds to human IL-17A and human IL-17F comprising:

30 a heavy chain variable region comprising the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6 for CDR-H3.

35 In one embodiment, the antigen binding site that binds to human IL-17A and human IL-17F comprises a light chain variable region comprising the sequence given in SEQ ID NO:7.

In one embodiment, the antigen binding site that binds to human IL-17A and human IL-17F comprises a heavy chain variable region comprising the sequence given in SEQ ID NO:9.

In one embodiment, the multi-specific antibody lacks an Fc-domain and half-life is provided
5 by an antigen binding site that binds to serum albumin.

In one embodiment, the multi-specific antibody comprises the sequence given in SEQ ID NO:57 or SEQ ID NO: 59.

10 In one embodiment, the multi-specific antibody comprises the sequence given in SEQ ID NO:61 or SEQ ID NO: 63.

In one embodiment, the multi-specific antibody comprises the sequence given in SEQ ID NO:59 and the sequence given in SEQ ID NO: 63.

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In one embodiment, the multi-specific antibody comprises or consists of:

a polypeptide chain of formula (I):

$V_H-CH_1-(CH_2)_s-(CH_3)_t-X-(V_1)_p$; and

a polypeptide chain of formula (II):

20 $V_L-C_L-Y-V_2$;

wherein:

V_H represents a heavy chain variable domain;

CH_1 represents domain 1 of a heavy chain constant region;

CH_2 represents domain 2 of a heavy chain constant region;

25 CH_3 represents domain 3 of a heavy chain constant region;

X represents a bond or linker;

V_1 represents a dsscFv, a dsFv, or a scFv;

V_L represents a light chain variable domain;

C_L represents a domain from a light chain constant region, such as Ckappa;

30 Y represents a bond or linker;

V_2 represents a dsscFv, a dsFv, or a scFv;

p represents 0 or 1;

s represents 0 or 1;

t represents 0 or 1;

35 wherein when p is 0, X is absent and when q is 0, Y is absent; and

wherein the polypeptide chain of formula (I) comprises a protein A binding domain; and wherein the polypeptide chain of formula (II) does not bind protein A.

In one embodiment when s is 0 and t is 0, the multi-specific antibody according to the present disclosure is provided as a dimer of a heavy and light chain of:
5 Formula (I) and (II) respectively, wherein the V_H -CH₁ portion together with the V_L -C_L portion form a functional Fab or Fab' fragment.

In one embodiment when s is 1 and t is 1, the multi-specific antibody according to the present disclosure is provided as a dimer of two heavy chains and two light chains of:
10 Formula (I) and (II) respectively, wherein the two heavy chains are connected by interchain interactions, notably at the level of CH₂-CH₃, and wherein the V_H -CH₁ portion of each heavy chain together with the V_L -C_L portion of each light chain, form a functional Fab or Fab' fragment. In such embodiment, the two V_H -CH₁. CH₂. CH₃ portions together with the two V_L -
15 C_L portions form a functional full-length antibody. In such embodiment, the full-length antibody may comprise a functional Fc region.

V_H represents a heavy chain variable domain. In one embodiment V_H is humanised. In one embodiment the V_H is fully human.
20

V_L represents a light chain variable domain. In one embodiment V_L is humanised. In one embodiment the V_L is fully human.

Generally, V_H and V_L together form an antigen binding domain. In one embodiment V_H and V_L form a cognate pair.
25

"Cognate pair" as employed herein refers to a pair of variable domains from a single antibody, which was generated *in vivo*, i.e. the naturally occurring pairing of the variable domains isolated from a host. A cognate pair is therefore a V_H and V_L pair. In one example,
30 the cognate pair bind the antigen co-operatively.

"Variable region" or "variable domain" as employed herein refers to the region in an antibody chain comprising the CDRs and a framework, in particular a suitable framework.

35 Variable regions for use in the present disclosure will generally be derived from an antibody, which may be generated by any method known in the art.

“Derived from” as employed herein refers to the fact that the sequence employed or a sequence highly similar to the sequence employed was obtained from the original genetic material, such as the light or heavy chain of an antibody.

- 5 “Highly similar” as employed herein is intended to refer to an amino acid sequence which over its full length is 95% similar or more, such as 96, 97, 98 or 99% similar.

Variable regions for use in the present invention, as described herein above for V_H and V_L may be from any suitable source and may be for example, fully human or humanised.

10

In one embodiment, the binding domain formed by V_H and V_L are specific to a first antigen.

In one embodiment, the binding domain of V_1 is specific to a second antigen.

In one embodiment, the binding domain of V_2 is specific to a third antigen.

- 15 In one embodiment, each one of V_H - V_L , V_1 , and V_2 , as present, separately binds its respective antigen.

In one embodiment, the CH_1 domain is a naturally occurring domain 1 from an antibody heavy chain or a derivative thereof. In one embodiment, the CH_2 domain is a naturally occurring domain 2 from an antibody heavy chain or a derivative thereof. In one

- 20 embodiment, the CH_3 domain is a naturally occurring domain 3 from an antibody heavy chain or a derivative thereof.

In one embodiment, the C_L fragment, in the light chain, is a constant kappa sequence or a derivative thereof. In one embodiment, the C_L fragment, in the light chain, is a constant

- 25 lambda sequence or a derivative thereof.

A derivative of a naturally occurring domain as employed herein is intended to refer to where at least one amino acid in a naturally occurring sequence have been replaced or deleted, for example to optimize the properties of the domain such as by eliminating undesirable

- 30 properties but wherein the characterizing feature(s) of the domain is/are retained. In one embodiment, a derivative of a naturally occurring domain comprises two, three, four, five, six, seven, eight, ten, eleven or twelve amino acid substitutions or deletions compared to a naturally occurring sequence.

- 35 In one embodiment, one or more natural or engineered inter chain (i.e. inter light and heavy chain) disulphide bonds are present in the functional Fab or Fab' fragment.

In one embodiment, a "natural" disulfide bond is present between a CH₁ and C_L in the polypeptide chains of Formula (I) and (II).

When the C_L domain is derived from either Kappa or Lambda, the natural position for a bond forming cysteine is 214 in human cKappa and cLambda (Kabat numbering 4th edition 1987).

The exact location of the disulfide bond forming cysteine in CH₁ depends on the particular domain actually employed. Thus, for example in human gamma-1 the natural position of the disulfide bond is located at position 233 (Kabat numbering 4th edition 1987). The position of the bond forming cysteine for other human isotypes such as gamma 2, 3, 4, IgM and IgD are known, for example position 127 for human IgM, IgE, IgG2, IgG3, IgG4 and 128 of the heavy chain of human IgD and IgA2B.

Optionally, there may be a disulfide bond between the V_H and V_L of the polypeptides of formula I and II.

In one embodiment, the multi-specific antibody according to the disclosure has a disulfide bond in a position equivalent or corresponding to that naturally occurring between CH₁ and C_L.

In one embodiment, a constant region comprising CH₁ and a constant region such as C_L has a disulfide bond which is in a non-naturally occurring position. This may be engineered into the molecule by introducing cysteine(s) into the amino acid chain at the position or positions required. This non-natural disulfide bond is in addition to or as an alternative to the natural disulfide bond present between CH₁ and C_L. The cysteine(s) in natural positions can be replaced by an amino acid such as serine which is incapable on forming a disulfide bridge.

Introduction of engineered cysteines can be performed using any method known in the art. These methods include, but are not limited to, PCR extension overlap mutagenesis, site-directed mutagenesis or cassette mutagenesis (see, generally, Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, 1989; Ausbel et al., Current Protocols in Molecular Biology, Greene Publishing & Wiley-Interscience, NY, 1993). Site-directed mutagenesis kits are commercially available, e.g. QuikChange® Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). Cassette mutagenesis can be performed based on Wells *et al.*, 1985, Gene, 34:315-323. Alternatively, mutants can be made by total gene synthesis by annealing, ligation and PCR amplification and cloning of overlapping oligonucleotides.

In one embodiment, a disulfide bond between CH₁ and C_L is completely absent, for example the interchain cysteines may be replaced by another amino acid, such as serine. Thus, in one embodiment there are no interchain disulphide bonds in the functional Fab fragment of the molecule. Disclosures such as WO2005/003170, incorporated herein by reference, describe how to provide Fab fragments without an inter chain disulphide bond.

Examples of antibody formats for use in the present invention include appended IgG and appended Fab, wherein a whole IgG or a Fab fragment, respectively, is engineered by appending at least one additional antigen-binding domain (e.g. one, two, three or four additional antigen-binding domains), for example a single domain antibody (such as V_H or V_L, or VHH), a scFv, a dsscFv, a dsFv to the N- and/or C-terminus of the light chain of said IgG or Fab, and optionally to the heavy chain of said IgG or Fab, for example as described in WO2009/040562, WO2010035012, WO2011/030107, WO2011/061492, WO2011/061246 and WO2011/086091 all incorporated herein by reference. An appended IgG comprising a full-length IgG engineered by appending a dsFv to the C-terminus of the light chain (and optionally to the heavy chain) of the IgG, was first disclosed in WO2015/197789, incorporated herein by reference.

A preferred antibody format for use in the present invention comprises a Fab linked to two scFvs or dsscFvs, each scFv or 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 International Patent Application Publication No WO2015/197772, which is hereby incorporated by reference in its entirety and particularly with respect to the discussion of antibody fragments.

V₁ represents a dsscFv, a dsFv, or a scFv.

V₂ represents a dsscFv, a dsFv, or a scFv.

In one embodiment, when V₁ and/or V₂ are a dsFv or a dsscFv, the disulfide bond between the variable domains V_H and V_L of V₁ and/or V₂ is between two of the residues listed below (unless the context indicates otherwise Kabat numbering is employed in the list below). Wherever reference is made to Kabat numbering the relevant reference is Kabat *et al.*, 1991 (5th edition, Bethesda, Md.), in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

In one embodiment the disulfide bond is in a position selected from the group comprising:

- V_H37 + V_L95C see for example Protein Science 6, 781-788 Zhu et al (1997);
 - V_H44 + V_L100 see for example; for example, Weatherill et al., Protein Engineering, Design & Selection, 25 (321-329), 2012);
 - 5 • V_H44 + V_L105 see for example J Biochem. 118, 825-831 Luo et al (1995);
 - V_H45 + V_L87 see for example Protein Science 6, 781-788 Zhu et al (1997);
 - V_H55 + V_L101 see for example FEBS Letters 377 135-139 Young et al (1995);
 - V_H100 + V_L50 see for example Biochemistry 29 1362-1367 Glockshuber et al (1990);
 - 10 • V_H100b + V_L49; see for example Biochemistry 29 1362-1367 Glockshuber et al (1990);
 - V_H98 + V_L 46; see for example Protein Science 6, 781-788 Zhu et al (1997);
 - V_H101 + V_L46; see for example Protein Science 6, 781-788 Zhu et al (1997);
 - V_H105 + V_L43 see for example; Proc. Natl. Acad. Sci. USA Vol. 90 pp.7538-7542 Brinkmann *et al* (1993); or Proteins 19, 35-47 Jung et al (1994),
 - 15 • V_H106 + V_L57 see for example FEBS Letters 377 135-139 Young *et al* (1995)
- and a position corresponding thereto in a variable region pair located in the molecule.

In one embodiment, the disulphide bond is formed between positions V_H44 and V_L100.

- 20 The amino acid pairs listed above are in the positions conducive to replacement by cysteines such that disulfide bonds can be formed. Cysteines can be engineered into these desired positions by known techniques. In one embodiment, therefore, an engineered cysteine according to the present disclosure refers to where the naturally occurring residue at a given amino acid position has been replaced with a cysteine residue.

- 25 Introduction of engineered cysteines can be performed using any method known in the art. These methods include, but are not limited to, PCR extension overlap mutagenesis, site-directed mutagenesis or cassette mutagenesis (see, generally, Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, 1989; Ausbel et al., Current Protocols in Molecular Biology, Greene Publishing & Wiley-Interscience, NY, 1993). Site-directed mutagenesis kits are commercially available, e.g. QuikChange® Site-Directed Mutagenesis kit (Stratagen, La Jolla, CA). Cassette mutagenesis can be performed based on Wells *et al.*, 1985, Gene, 34:315-323.
- 30 Alternatively, mutants can be made by total gene synthesis by annealing, ligation and PCR amplification and cloning of overlapping oligonucleotides.
- 35

Accordingly, in one embodiment when V_1 and/or V_2 are a dsFv or a dsscFv, the variable domains V_H and V_L of V_1 and/or the variable domains V_H and V_L of V_2 , may be linked by a disulfide bond between two cysteine residues, wherein the position of the pair of cysteine residues is selected from the group consisting of: V_H37 and V_L95 , V_H44 and V_L100 , V_H44 and V_L105 , V_H45 and V_L87 , V_H100 and V_L50 , V_H100b and V_L49 , V_H98 and V_L46 , V_H101 and V_L46 , V_H105 and V_L43 and V_H106 and V_L57 .

In one embodiment when V_1 and/or V_2 are a dsFv or a dsscFv, the variable domains V_H and V_L of V_1 and/or the variable domains V_H and V_L of V_2 may be linked by a disulfide bond between two cysteine residues, one in V_H and one in V_L , which are outside of the CDRs wherein the position of the pair of cysteine residues is selected from the group consisting of V_H37 and V_L95 , V_H44 and V_L100 , V_H44 and V_L105 , V_H45 and V_L87 , V_H100 and V_L50 , V_H98 and V_L46 , V_H105 and V_L43 and V_H106 and V_L57 .

In one embodiment when V_1 is a dsFv or a dsscFv, the variable domains V_H and V_L of V_1 are linked by a disulphide bond between two engineered cysteine residues, one at position V_H44 and the other at V_L100 . In one embodiment when V_2 is a dsFv or a dsscFv, the variable domains V_H and V_L of V_2 are linked by a disulphide bond between two engineered cysteine residues, one at position V_H44 and the other at V_L100 .

In one embodiment when V_1 is a dsscFv, a dsFv, or a scFv, the V_H domain of V_1 is attached to X.

In one embodiment when V_1 is a dsscFv, a dsFv, or a scFv, the V_L domain of V_1 is attached to X.

In one embodiment when V_2 is a dsscFv, a dsFv, or a scFv, the V_H domain of V_2 is attached to Y.

In one embodiment when V_2 is a dsscFv, a dsFv, or a scFv, the V_L domain of V_2 is attached to Y.

The skilled person will appreciate that when V_1 and/or V_2 represents a dsFv, the multi-specific antibody will comprise a third polypeptide encoding the corresponding free V_H or V_L domain which is not attached to X or Y. When V_1 and V_2 are a dsFv then the "free variable domain" (i.e. the domain linked to via a disulphide bond to the remainder of the polypeptide) will be common to both chains. Thus, whilst the actual variable domain fused or linked via X

or Y to the polypeptide may be different in each polypeptide chain, the free variable domains paired therewith will generally be identical to each other.

In some embodiments, p is 1. In some embodiments, p is 0.

5 In some embodiments, s is 1. In some embodiments, s is 0.

In some embodiments, t is 1. In some embodiments, t is 0.

In some embodiments, s is 1 and t is 1. In some embodiments, s is 0 and t is 0.

10 In one embodiment, p is 1, q is 1, r is 0, s is 0 and t is 0, and V_1 and V_2 both represent a dsscFv. Thus, in one aspect, there is provided a multi-specific antibody which binds human IL-13, human IL-17A and/or human IL-17F comprising or consisting of:

a) a polypeptide chain of formula (Ia):

$V_H-CH_1-X-V_1$; and

b) a polypeptide chain of formula (IIa):

15 $V_L-C_L-Y-V_2$;

wherein:

V_H represents a heavy chain variable domain;

CH_1 represents domain 1 of a heavy chain constant region;

X represents a bond or linker;

20 Y represents a bond or linker;

V_1 represents a scFv, a dsscFv, or a dsFv;

V_L represents a light chain variable domain;

C_L represents a domain from a light chain constant region, such as Ckappa;

V_2 represents a scFv, a dsscFv or a dsFv;

25 wherein at least one of V_1 or V_2 is a dsscFv or a dsFv;

wherein the polypeptide chain of formula (Ia) comprises a protein A binding domain; and

wherein the polypeptide chain of formula (IIa) does not bind protein A.

30 In such embodiment, V_2 does not bind protein A, i.e. the scFv, dsscFv or dsFv of V_2 does not comprise a protein A binding domain. In one embodiment, V_2 , i.e. the scFv, dsscFv or dsFv of V_2 , comprises a VH1 domain. In another embodiment, V_2 , i.e. the scFv, dsscFv or dsFv of V_2 , comprises a VH3 domain which does not bind protein A. In one embodiment, V_2 , i.e. the scFv, dsscFv or dsFv of V_2 , comprises a VH2 domain. In one embodiment, V_2 , i.e. the scFv, dsscFv or dsFv of V_2 , comprises a VH4 domain. In one embodiment, V_2 , i.e. the scFv, dsscFv or dsFv of V_2 , comprises a VH5 domain. In one embodiment, V_2 , i.e. the scFv,

35 dsscFv or dsFv of V_2 , comprises a VH5 domain. In one embodiment, V_2 , i.e. the scFv,

dsscFv or dsFv of V_2 , comprises a VH6 domain. In one embodiment, the polypeptide chain of formula (Ia) comprises only one protein A binding domain present in V_H or V_1 . In one embodiment, the polypeptide chain of formula (Ia) comprises only one protein A binding domain present in V_1 . In another embodiment, the polypeptide chain of formula (Ia)
 5 comprises two protein A binding domains present in V_H and V_1 respectively.

In another embodiment, p is 0, q is 1, r is 0, s is 1, t is 1, and V_2 is a dsscFv. Thus, in one aspect, there is provided a multi-specific antibody which binds human IL-13, human IL-17A and/or human IL-17F, and comprising or consisting of:

10 a) a polypeptide chain of formula (Ib):

V_H -CH₁-CH₂-CH₃; and

b) a polypeptide chain of formula (IIb):

V_L -C_L-Y- V_2 ;

wherein:

15 V_H represents a heavy chain variable domain;

CH₁ represents domain 1 of a heavy chain constant region;

CH₂ represents domain 2 of a heavy chain constant region;

CH₃ represents domain 3 of a heavy chain constant region;

Y represents a bond or linker;

20 V_L represents a light chain variable domain;

C_L represents a domain from a light chain constant region, such as Ckappa;

V_2 represents a dsscFv;

wherein the polypeptide chain of formula (Ib) comprises a protein A binding domain; and
 wherein the polypeptide chain of formula (IIb) does not bind protein A.

25

In such embodiment, V_2 does not bind protein A, i.e. the dsscFv of V_2 does not comprise a protein A binding domain. In one embodiment, V_2 , i.e. the dsscFv of V_2 , comprises a VH1 domain. In another embodiment, V_2 , i.e. the dsscFv of V_2 , comprises a VH3 domain which does not bind protein A. In one embodiment, the polypeptide chain of formula (Ib) comprises
 30 only one protein A binding domain present in V_H or CH₂-CH₃. In another embodiment, the polypeptide chain of formula (Ib) comprises two protein A binding domains present in V_H and CH₂-CH₃ respectively.

In another embodiment, p is 0, q is 1, r is 0, s is 1, t is 1, and V₂ is a dsFv. Thus, in one aspect, there is provided a multi-specific antibody which binds human IL-13, human IL-17A and/or human IL-17F comprising or consisting of:

a) a polypeptide chain of formula (Ic):

5 V_H-CH₁-CH₂-CH₃; and

b) a polypeptide chain of formula (IIc):

V_L-C_L-Y-V₂;

wherein:

V_H represents a heavy chain variable domain;

10 CH₁ represents domain 1 of a heavy chain constant region;

CH₂ represents domain 2 of a heavy chain constant region;

CH₃ represents domain 3 of a heavy chain constant region;

Y represents a bond or linker;

V_L represents a light chain variable domain;

15 C_L represents a domain from a light chain constant region, such as C_κ;

V₂ represents a dsFv;

wherein the polypeptide chain of formula (Ic) comprises a protein A binding domain; and wherein the polypeptide chain of formula (IIc) does not bind protein A.

20 In such embodiment, V₂, i.e. the dsFv of V₂, does not bind protein A. In one embodiment, the polypeptide chain of formula (Ic) comprises only one protein A binding domain present in V_H or CH₂-CH₃. In another embodiment, the polypeptide chain of formula (Ic) comprises two protein A binding domains present in V_H and CH₂-CH₃ respectively.

25 In one embodiment of the multi-specific antibody of the invention,

V_L and V_H comprise an antigen binding site that binds to human IL-17A and/or human IL-17F,

V₁ comprises an antigen binding site that binds to human serum albumin, and

V₂ comprises an antigen binding site that binds to human IL-13.

30

In one embodiment, V_L comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3; V_H comprises the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6 for CDR-H3.

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In one embodiment, V_1 comprises a light chain variable region comprising the sequence given in SEQ ID NO:39 for CDR-L1, the sequence given in SEQ ID NO:40 for CDR-L2 and the sequence given in SEQ ID NO:41 for CDR-L3; and a heavy chain variable region comprising the sequence given in SEQ ID NO:42 for CDR-H1, the sequence given in SEQ ID NO:43 for CDR-H2 and the sequence given in SEQ ID NO:44 for CDR-H3.

In one embodiment, V_2 comprises a light chain variable region comprising the sequence given in SEQ ID NO:15 for CDR-L1, the sequence given in SEQ ID NO:16 for CDR-L2 and the sequence given in SEQ ID NO:17 for CDR-L3; and a heavy chain variable region comprising the sequence given in SEQ ID NO:18 for CDR-H1, the sequence given in SEQ ID NO:19 for CDR-H2 and the sequence given in SEQ ID NO:20 for CDR-H3;

In one embodiment, V_L comprises the sequence given in SEQ ID NO:7 and V_H comprises the sequence given in SEQ ID NO:9.

In one embodiment, V_1 comprises a light chain variable region comprising the sequence given in SEQ ID NO:45 and a heavy chain variable region comprising the sequence given in SEQ ID NO:46.

In one embodiment, V_1 comprises a light chain variable region comprising the sequence given in SEQ ID NO:49 and a heavy chain variable region comprising the sequence given in SEQ ID NO:50.

In one embodiment, the light chain variable region and heavy chain variable region of V_1 are connected by a linker, said linker comprising the sequence given in SEQ ID NO:68.

In one embodiment, V_1 is a scFv comprising the sequence given in SEQ ID NO:53 or a dsscFv comprising the sequence given in SEQ ID NO:55.

In one embodiment, V_2 comprises a light chain variable region comprising the sequence given in SEQ ID NO:27 and a heavy chain variable region comprising the sequence given in SEQ ID NO:28.

In one embodiment, V_2 comprises a light chain variable region comprising the sequence given in SEQ ID NO:31 and a heavy chain variable region comprising the sequence given in SEQ ID NO:32.

In one embodiment, the light chain variable region and heavy chain variable region of V₂ are connected by a linker, said linker comprising the sequence given in SEQ ID NO:66.

In one embodiment, V₂ is a scFv comprising the sequence given in SEQ ID NO:35 or a
5 dsscFv comprising the sequence given in SEQ ID NO:37.

In one embodiment, X is a linker comprising the sequence given in SEQ ID NO:67.

In one embodiment, Y is a linker comprising the sequence given in SEQ ID NO:65.
10

In one embodiment, the polypeptide chain of formula (Ia) comprises the sequence given in SEQ ID NO:57 or SEQ ID NO: 59.

In one embodiment, the polypeptide chain of formula (IIa) comprises the sequence given in
15 SEQ ID NO:61 or SEQ ID NO: 63.

In one embodiment, the polypeptide chain of formula (Ia) comprises the sequence given in SEQ ID NO:59 and the polypeptide chain of formula (IIa) comprises the sequence given in SEQ ID NO: 63.
20

It will be appreciated that one or more amino acid substitutions, additions and/or deletions may be made to the sequences provided by the present invention without significantly altering the ability of the antibody to bind to an antigen and neutralise its biological activity. The effect of any amino acid substitutions, additions and/or deletions can be readily tested
25 by one skilled in the art, for example by using the methods described herein, particularly those illustrated in the Examples, to determine antigen binding and inhibition of biological activity.

Accordingly, the present invention provides a multi-specific antibody comprising CDRs as
30 defined by the sequences given in SEQ ID NOs:1, 2, 3, 4, 5, 6, 15, 16, 17, 18, 19, 20, 39, 40, 41, 42, 43 and 44, in which one or more amino acids in one or more of the CDRs has been substituted with another amino acid, for example a similar amino acid as defined herein below.

35 "Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is

of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- 5 - lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk,
- 10 A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New
- 15 York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.*, 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.*, 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.*, 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

- 20 In one embodiment, the CDRs of the multi-specific antibody comprise sequences which have at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequences given in SEQ ID NOs:1, 2, 3, 4, 5, 6, 15, 16, 17, 18, 19, 20, 39, 40, 41, 42, 43 and 44.

- In one embodiment, V_L comprises a sequence which has at least 70%, 80%, 90%, 95% or
- 25 98% identity or similarity to the sequence given in SEQ ID NO:7 and V_H comprises a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:9.

- In one embodiment, V₁ comprises a light chain variable region comprising a sequence which
- 30 has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:45 and/or a heavy chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:46.

- In one embodiment, V₁ comprises a light chain variable region comprising a sequence which
- 35 has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:49 and/or a heavy chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:50.

In one embodiment, the light chain variable region and heavy chain variable region of V_1 are connected by a linker, said linker comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:68.

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In one embodiment, V_1 is a scFv comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:53 or a dsScFv comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:55.

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In one embodiment, V_2 comprises a light chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:27 and/or a heavy chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:28.

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In one embodiment, V_2 comprises a light chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:31 and/or a heavy chain variable region comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:32.

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In one embodiment, the light chain variable region and heavy chain variable region of V_2 are connected by a linker, said linker comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:66.

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In one embodiment, V_2 is a scFv comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:35 or a dsScFv comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:37.

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In one embodiment, X is a linker comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:67.

In one embodiment, Y is a linker comprising a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:65.

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In one embodiment, the polypeptide chain of formula (Ia) comprises a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:57 or SEQ ID NO: 59.

- 5 In one embodiment, the polypeptide chain of formula (IIa) comprises a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:61 or SEQ ID NO: 63.

- 10 In one embodiment, the polypeptide chain of formula (Ia) comprises a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:59 and the polypeptide chain of formula (IIa) comprises a sequence which has at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 63.

Epitope

- 15 An epitope is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In
- 20 certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.
- 25 One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference antibody of the invention, the reference antibody is allowed to bind to a protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the protein or peptide is
- 30 assessed. If the test antibody is able to bind to the protein or peptide following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the reference antibody. On the other hand, if the test antibody is not able to bind to protein or peptide following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference
- 35 antibody of the invention.

To determine if an antibody competes for binding with a reference antibody, the above-described binding methodology is performed in two orientations. In a first orientation, the reference antibody is allowed to bind to a protein/peptide under saturating conditions followed by assessment of binding of the test antibody to the protein/peptide molecule. In a
5 second orientation, the test antibody is allowed to bind to the protein/peptide under saturating conditions followed by assessment of binding of the reference antibody to the protein/peptide. If, in both orientations, only the first (saturating) antibody is capable of binding to the protein/peptide, then it is concluded that the test antibody and the reference antibody compete for binding to the protein/peptide. As will be appreciated by the skilled
10 person, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks)
15 binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50%, 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res, 1990:50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of
20 the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (e.g., peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due
25 to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

30 Antibodies may compete for binding to IL-17A or IL-17F with, or bind to the same epitope as, a multi-specific antibody which comprises a CDR-L1/CDR-L2/CDR-L3/CDR-H1/CDR-H2/CDR-H3 sequence combination of SEQ ID NOs: 1/2/3/4/5/6.

Antibodies may compete for binding to IL-13 with, or bind to the same epitope as, a multi-specific antibody which comprises a CDR-L1/CDR-L2/CDR-L3/CDR-H1/CDR-H2/CDR-H3
35 sequence combination of SEQ ID NOs: 15/16/17/18/19/20.

Antibodies may compete for binding to serum albumin with, or bind to the same epitope as, a multi-specific antibody which comprises a CDR-L1/CDR-L2/CDR-L3/CDR-H1/CDR-H2/CDR-H3 sequence combination of SEQ ID NOs: 39/40/41/42/43/44.

5 Effector molecules

If desired, a multi-specific antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982, Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195, WO 89/01476 and WO 03031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP 0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (e.g. kills) cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (e.g. methotrexate, 6-mercaptapurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g. mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g. daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g. dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (e.g. vincristine and vinblastine).

10

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y , Lu^{177} , Bismuth ^{213} , Californium ^{252} , Iridium ^{192} and Tungsten ^{188} /Rhenium ^{188} ; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

15 Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumour necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g. angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

25

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I , ^{131}I , ^{111}In and ^{99}Tc .

35

In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO 05/117984.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the

product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO 98/25971). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

Suitably PEG molecules may be covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment or diFab which is PEGylated, *i.e.* has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.* according to the method disclosed in EP 0948544 or EP 1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

In one embodiment the multi-specific antibody is not attached an effector molecule.

20 Polynucleotides / Vectors / Host cells

The present invention also provides an isolated polynucleotide encoding a polypeptide chain of an IL-13/IL-17AF multi-specific antibody molecule.

25 A variant polynucleotide may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40, up to 50, up to 75 or more nucleic acid substitutions and/or deletions from the sequences given in the sequence listing. Generally, a variant has 1-20, 1-50, 1-75 or 1-100 substitutions and/or deletions.

30 Suitable variants may be at least about 70% homologous to a polynucleotide of any one of nucleic acid sequences disclosed herein, typically at least about 80 or 90% and more suitably at least about 95%, 97% or 99% homologous thereto. Variants may retain at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity. Variants typically retain about 60% - about 99% identity, about 80% - about 99% identity, about 90% - about 99% identity or about 95% - about 99% identity. Homology and identity at these levels is generally present at least with respect to the coding regions of the polynucleotides. Methods of measuring homology are well known in the art and it will be understood by those of skill in

the art that in the present context, homology is calculated on the basis of nucleic acid identity. Such homology may exist over a region of at least about 15, at least about 30, for instance at least about 40, 60, 100, 200 or more contiguous nucleotides (depending on the length). Such homology may exist over the entire length of the unmodified polynucleotide sequence.

The homologue may differ from a sequence in the relevant polynucleotide by less than about 3, 5, 10, 15, 20 or more mutations (each of which may be a substitution, deletion or insertion). For example, the homologue may differ by 3-50 mutations, often 3-20 mutations. These mutations may be measured over a region of at least 30, for instance at least about 40, 60 or 100 or more contiguous nucleotides of the homologue.

The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an IL-13/IL-17AF multi-specific antibody. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the IL-13/IL-17AF multi-specific antibody. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO cells.

The present invention also provides a process for the production of an IL-13/IL-17AF multi-specific antibody, comprising culturing a host cell containing a vector under conditions suitable for expression of protein from DNA encoding the IL-13/IL-17AF multi-specific antibody, and isolating the IL-13/IL-17AF multi-specific antibody.

Production of multispecific antibodies

There are a number of approaches for generating multi-specific, notably bi-specific antibodies. Morrison *et al* (Coloma and Morrison 1997, Nat Biotechnol. 15, 159-163) describes the fusion of single chain variable fragments (scFv) to whole antibodies, e.g. IgG.

Schoonjans *et al.*, 2000, Journal of Immunology, 165, 7050-7057, describes the fusion of scFv to antibody Fab fragments. WO2015/197772 describes the fusion of disulphide stabilised scFv (dsscFv) to Fab fragments.

5 Standard approaches described in the prior art comprise the expression in a host cell of at least two polypeptides, each one coding for a heavy chain (HC) or a light chain (LC) of a whole antibody or antigen binding fragment thereof e.g. a Fab, to which an additional antigen binding fragment of an antibody can be fused to the N- and/or C- terminal position of the heavy chain and/or the light chain. When trying to recombinantly produce such multi-specific antibodies by expressing two (one light chain and one heavy chain to form an
10 appended Fab) or four polypeptides (two light chains and two heavy chains to form an appended IgG), it usually requires expressing the light chain in excess over the heavy chain, in order to ensure the proper folding of the heavy chain upon assembly with its corresponding light chain. In particular, the CH₁ (domain 1 of the heavy chain constant
15 region) is prevented from folding on itself by BIP proteins, which can be displaced by a corresponding LC; therefore, the correct folding of the CH₁/HC is dependent on the availability of its corresponding LC (Lee et al., 1999, Molecular Biology of the Cell, Vol. 10, 2209–2219).

20 We have observed that those methods of expressing multi-specific antibodies may result in the production of the light chain in excess over the heavy chain, which remains in the host cell harvest, and that the excess of light chain tends to form dimeric complexes (or “LC dimers”) which are present as a by-product of the production process with the desired multi-specific antibody, notably monomeric, and thus need to be purified away.

25 Importantly, the technical problem associated with the formation of dimers of light chains, when fused on N- and/or C-terminal to additional antigen binding fragment(s), has not been identified so far, and the commonly used analytical methods have not allowed the detection and quantification of those appended LC dimers amongst the heterogenous products of the
30 production process. This may result in a significant bias when estimating the amount of the products using standard analytical methods.

Thus, there is a need to improve multi-specific antibodies and methods of production thereof, which allow the isolation and removal of the appended LC dimers easily and efficiently at the
35 earliest steps of the production process, and thus improve the yield of the protein of interest for use in therapy, which is the multi-specific antibody, in particular in its monomeric form.

The multi-specific antibodies of the present invention have been engineered to provide improved multi-specific antibodies with equivalent functionality and stability, whilst increasing the yield of “multi-specific antibody” material, notably monomeric, obtained after purification, notably after a one-step purification comprising a protein A affinity chromatography.

5

Advantageously, the multi-specific antibodies of the present disclosure can be more efficiently purified with a purification method which is improved over the methods commonly used in the prior art, notably in that the improved method comprises fewer steps, which is cost and time effective at the industrial scale. In particular, the multi-specific antibodies of the present disclosure maximise the quantity of proteins of interest (i.e, the correct multi-specific antibody format) obtained after a one-step purification method comprising a protein A affinity chromatography, whereby the purification of the multi-specific antibodies of interest and the removal of the appended LC dimers occur concurrently. Advantageously, the methods of production and purification of the multi-specific antibodies of the present disclosure do not require an additional purification step to capture the free, unbound light chains in excess, notably the appended LC dimers.

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Protein A

Protein A is a 42 kDa surface protein originally found in the cell wall of the bacteria *Staphylococcus aureus*. Protein A has been widely used to detect, quantify and purify immunoglobulins. Protein A has been reported to bind the Fab portion derived from the VH3 family antibodies, and the Fc gamma region in the constant region portion of IgG (between the CH₂ and CH₃ domains). The crystal structure of the complex formed by protein A and the Fab has been described for example in Graille et al., 2000, PNAS, 97(10): 5399–5404. In the context of the present disclosure, protein A encompasses natural protein A and any variant or derivative thereof, to the extent that the protein A variant or derivative maintains its ability to bind VH3 domains and/or Fc gamma domains.

25

The polypeptide chain of formula (I) of the present invention comprises a protein A binding domain. In one embodiment, the polypeptide chain of formula (I) comprises one, two or three protein A binding domains.

30

“Protein A binding domain” as employed herein is intended to refer to a binding domain which specifically binds to protein A. A Protein A binding domain may refer to a VH3 domain or a portion of a VH3 domain which binds protein A, i.e. which comprises a protein A binding interface. The portion of a VH3 domain which binds protein A does not comprise the CDRs

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of the VH3 domain, i.e. the protein A binding interface of the VH3 does not involve the CDRs; consequently, it will be understood that a protein A binding domain does not compete with an antigen binding domain as disclosed in the present application.

- 5 In one embodiment, the polypeptide chain of formula (I) comprises a protein A binding domain which is present in V_H and/or CH₂-CH₃ and/or V₁. In one embodiment, the polypeptide chain of formula (I) comprises one, two or three protein A binding domains, which is/are present in V_H and/or CH₂-CH₃ and/or V₁. In one embodiment, the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V_H or
- 10 V₁. In one embodiment, s is 0, t is 0 and the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V_H or V₁. In one embodiment, the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V_H. In one embodiment, s is 0, t is 0, p is 0, and the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V_H. In one embodiment,
- 15 the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V₁. In one embodiment, s is 0, t is 0, p is 1, and the polypeptide chain of formula (I) comprises only one protein A binding domain which is present in V₁.

- In one embodiment, the polypeptide chain of formula (I) comprises two protein A binding
- 20 domains. In one embodiment, the polypeptide chain of formula (I) comprises two protein A binding domains which are present in V_H and CH₂-CH₃ respectively. In another embodiment, the polypeptide chain of formula (I) comprises two protein A binding domains which are present in V_H and V₁ respectively. In another embodiment, the polypeptide chain of formula (I) comprises two protein A binding domains which are present in CH₂-CH₃ and V₁
- 25 respectively.

In one embodiment, the polypeptide chain of formula (I) comprises three protein A binding domains, each one being present in V_H, CH₂-CH₃ and V₁.

- 30 Natural protein A can interact in particular with the Fc gamma region, in the constant region portion of IgG. More particularly, protein A can interact with a binding domain between the CH₂ and the CH₃. In one embodiment when s is 1, t is 1, both CH₂ and CH₃ are naturally occurring domains of the IgG class.

- 35 In some embodiments, the protein A binding domain(s) comprise(s) or consist(s) of a VH3 domain or variant thereof which binds protein A. In some embodiments, the protein A binding domain(s) comprise(s) or consist(s) of a naturally occurring VH3 domain. In some

embodiments, a variant of a VH3 domain which binds protein A is a variant of a naturally occurring VH3 domain, said naturally occurring VH3 domain being unable to bind protein A.

The polypeptide chain of formula (II) of the present disclosure does not bind protein A. In
5 one embodiment, the binding domain of V₂ does not bind protein A.

In some embodiments, V₂ comprises or consists of a VH1 and/or a VH2 and/or a VH4 and/or a VH5 and/or a VH6 and does not comprise a VH3 domain. In some embodiments, V₂ comprises or consists of a VH3 domain or variant thereof which does not bind protein A. In
10 some embodiments, V₂ comprises or consists of a naturally occurring VH3 domain being unable to bind protein A. In some embodiments, a variant of a VH3 domain which does not bind protein A is a variant of a naturally occurring VH3, said naturally occurring VH3 domain being able to bind protein A.

15 Human VH3 germline genes and VH3 domains (or frameworks) have been well characterized. Many of the naturally occurring VH3 domains have the capacity to bind protein A but certain naturally occurring VH3 domains do not have the capacity to bind protein A (see Roben et al., 1995, J Immunol.;154(12):6437-6445).

20 A VH3 domain for use in the present disclosure can be obtained by several methods. In one embodiment, a VH3 domain for use in the present disclosure is a naturally occurring VH3 domain, selected for its ability or inability to bind protein A, depending on its position within the polypeptide (I) and/or (II) of the disclosure. For example, a panel of antibodies may be generated against an antigen of interest by immunisation of a non-human animal, then
25 humanised, and the humanised antibodies may be screened and selected based on their ability or inability to bind protein A via the humanised VH3 domain, for example against a protein A affinity column. Alternatively, display technologies (e.g. phage display, yeast display, ribosome display, bacterial display, mammalian cell surface display, mRNA display, DNA display) may be used to screen antibody libraries and select antibodies comprising a
30 VH3 domain which binds, notably via a protein A binding interface which does not involve the CDRs, or does not bind protein A.

Alternatively, a VH3 domain for use in the present disclosure is a variant of a naturally occurring VH3. In one embodiment, a VH3 variant comprises a sequence of a naturally
35 occurring VH3 able to bind protein A, and further comprising at least one amino acid mutation, which abolishes its ability to bind protein A. In one embodiment, a VH3 variant which binds protein A comprises a sequence of a naturally occurring VH3 unable to bind

protein A, and further comprises at least one amino acid mutation. In such embodiment, the mutation(s) is/are responsible for the VH3 domain to gain the ability to bind protein A, i.e. the mutation(s) contribute(s) to the generation of a protein A binding domain which was not naturally present.

5

In one embodiment, a VH3 variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid mutations. In one embodiment, a VH3 variant comprises a mutation at the position 15, 17, 19, 57, 59, 64, 65, 66, 68, 70, 81 or 82 on the VH3, numbering according to Kabat and as described for example in Graille et al., 2000, PNAS, 97(10): 5399–5404). The mutation may be a substitution, a deletion, or an insertion. In one embodiment, the VH3 variant comprises a substitution at the position 15, 17, 19, 57, 59, 64, 65, 66, 68, 70, 81 or 82 on the VH3, numbering according to Kabat.

15

Naturally occurring VH1, VH2, VH4, VH5 and VH6 do not bind protein A. In one embodiment, a V_H domain which does not bind protein A is a VH1. In one embodiment, a V_H domain which does not bind protein A is a VH2. In one embodiment, a V_H domain which does not bind protein A is a VH4. In one embodiment, a V_H domain which does not bind protein A is a VH5. In one embodiment, a V_H domain which does not bind protein A is a VH6.

20

Pharmaceutical Compositions, Dosages and Dosage Regimes

A multi-specific antibody of the invention may be provided in a pharmaceutical composition. The pharmaceutical composition will normally be sterile and will typically include a pharmaceutically acceptable carrier and/or adjuvant. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically acceptable adjuvant and/or carrier.

30

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. The carrier may be suitable for parenteral, e.g. intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Alternatively, the carrier may be suitable for non-parenteral administration, such as a topical, epidermal or mucosal route of administration. The carrier may be suitable for oral administration. Depending on the route of administration, the

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modulator may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compositions of the invention may include one or more pharmaceutically acceptable salts. A "*pharmaceutically acceptable salt*" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include acid addition salts and base addition salts.

Pharmaceutically acceptable carriers comprise aqueous carriers or diluents. Examples of suitable aqueous carriers that may be employed in the pharmaceutical 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 desirable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

Therapeutic 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.

Pharmaceutical compositions of the invention may comprise additional active ingredients.

Also within the scope of the present invention are kits comprising antibodies or modulatory agents of the invention and instructions for use. The kit may further contain one or more additional reagents, such as an additional therapeutic or prophylactic agent as discussed above.

The modulators and/or antibodies of the invention or formulations or compositions thereof may be administered for prophylactic and/or therapeutic treatments.

In therapeutic applications, compounds are administered to a subject already suffering from a disorder or condition as described above, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an increase in frequency or

duration of symptom-free periods. An amount adequate to accomplish this is defined as a "*therapeutically effective amount*".

In prophylactic applications, formulations are administered to a subject at risk of a disorder or condition as described above, in an amount sufficient to prevent or reduce the subsequent effects of the condition or one or more of its symptoms. An amount adequate to accomplish this is defined as a "*prophylactically effective amount*". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject.

A subject for administration may be a human or non-human animal. The term "*non-human animal*" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. Administration to humans is typical.

An antibody/modulator or pharmaceutical composition of the 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. Examples of routes of administration for compounds or pharmaceutical compositions of the invention include intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal 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. Alternatively, antibody/modulatory agent or pharmaceutical composition of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration. The antibody/modulatory agent or pharmaceutical composition of the invention may be for oral administration.

A suitable dosage of an antibody/modulatory agent or pharmaceutical composition 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 that 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 compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, 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 may be, for example, in the range of from about 0.01µg/kg to about 1000mg/kg body weight, typically from about 0.1µg/kg to about 100mg/kg body weight, of the patient to be treated. For example, a suitable dosage may be from about 1µg/kg to about 10mg/kg body weight per day or from about 10 µg/kg to about 5 mg/kg body weight per day.

10

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single dose may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Dosage unit form as
15 used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

20 Administration may be in single or multiple doses. Multiple doses may be administered via the same or different routes and to the same or different locations. Alternatively, doses can be via a sustained release formulation, in which case less frequent administration is required. Dosage and frequency may vary depending on the half-life of the antagonist in the patient and the duration of treatment desired.

25

As mentioned above, modulators/antibodies or pharmaceutical compositions of the invention may be co-administered with one or other more other therapeutic agents.

Combined administration of two or more agents may be achieved in a number of different
30 ways. Both may be administered together in a single composition, or they may be administered in separate compositions as part of a combined therapy. For example, the one may be administered before, after or concurrently with the other.

Therapeutic indications

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Antibodies of present invention may be used in treating, preventing or ameliorating any condition that is associated with IL-13 and/or IL-17A and/or IL-17F activity; for example, any

condition which results in whole or in part from signalling through an IL-13, IL-17A and/or IL-17F receptor.

Such diseases include primary and metastatic cancers, including carcinomas of breast,
 5 colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver,
 gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and
 urothelium), female genital tract (including cervix, uterus, and ovaries as well as
 choriocarcinoma and gestational trophoblastic disease), male genital tract (including
 10 prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the
 thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas,
 sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma),
 tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas,
 glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and
 meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias,
 15 and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas), rheumatoid arthritis,
 osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis,
 reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, ulcerative colitis,
 inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, allergic
 diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant
 20 rejection, acute or chronic immune disease associated with organ transplantation,
 sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease,
 Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis,
 Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis,
 uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases,
 25 parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis,
 Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary
 cirrhosis, hemolytic anemia, malignancies, heart failure, Addison's disease, sporadic,
 polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome,
 adult (acute) respiratory distress syndrome, alopecia, alopecia areata, arthropathy, Reiter's
 30 disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis,
 chlamydia, yersinia and salmonella associated arthropathy, atheromatous
 disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris,
 pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia,
 Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious
 35 anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis,
 giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, acquired
 immunodeficiency related diseases, hepatitis B, hepatitis C, common varied

immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue

5 disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjogren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung

10 disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism,

15 acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple

20 sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjorgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease,

25 hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcoholinduced liver injury, choleosatatis, idiosyncratic liver disease, drug-induced hepatitis, non-alcoholic steatohepatitis, allergy, group B streptococci (GBS) infection, mental disorders, depression,

30 schizophrenia, Th2 Type and Th1 Type mediated diseases, acute and chronic pain, different forms of pain, cancers, lung cancer, breast cancer, stomach cancer, bladder cancer, colon cancer, pancreatic cancer, ovarian cancer, prostate cancer, rectal cancer, hematopoietic malignancies, leukemia, lymphoma, Abetalipoproteinemia, acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute

35 myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcoholinduced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis (including seasonal

allergic rhinitis), non-allergic rhinitis, allograft rejection, alpha-1-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis,

5 arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar

10 disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, CreutzfeldtJakob disease, culture

15 negative sepsis, cystic fibrosis, cytokine therapy associated disorders, dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic atherosclerotic disease, diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine

20 receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hemophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative

25 sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallervorden-Spatz disease, Hashimoto's thyroiditis, hay fever, heart transplant rejection, hemochromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle arrhythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitivity reactions,

30 hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular

35 atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphedema, malaria, malignant lymphoma, malignant histiocytosis, malignant melanoma, meningitis,

meningococchemia, metabolic/idiopathic, migraine headache, mitochondrial multi. system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine-Thomas Shi-Drager and Machado-Joseph), mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, 5 neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic muscular atrophies, neutropenic fever, nonhodgkins lymphoma, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, okt3 therapy, orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, 10 pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, 15 monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiomyopathy syndrome, preeclampsia, progressive supranuclear palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, senile 20 chorea, senile dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, subacute sclerosing panencephalitis, syncope, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory 25 response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital 30 encephalitis/aseptic meningitis, vitall-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, atopic eczema, atopic dermatitis, autoimmune 35 dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis,

bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (cis) with risk for multiple sclerosis, childhood onset psychiatric disorder, dacryocystitis, dermatomyositis, diabetic retinopathy, disk herniation, disk prolaps, drug
 5 induced immune hemolytic anemia, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barre syndrome (GBS), Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease,
 10 inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratoconjunctivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B
 15 hepatitis, optic neuritis, osteolysis, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery, disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), primary Parkinsonism, prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent
 20 neuromyelitis optica, restenosis, rheumatic heart disease, sapho (synovitis, acne, pustulosis, hyperostosis, and osteitis), secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, sneddon-wilkinson dermatosis, spondylitis ankylosans, Stevens-Johnson syndrome (SJS), temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor necrosis
 25 factor receptor, type 1 allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, or wound healing, aspirin sensitive asthma, atopic asthma, chronic hand eczema, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic
 30 myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angiodema, helminth infections, onchocercal dermatitis, Eosinophil-Associated Gastrointestinal Disorders, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, nasal micropolyposis and polyposis, food allergy, aspirin intolerance, and obstructive sleep apnoea, chronic asthma, Crohn's
 35 disease and endomyocardial fibrosis, cancer (e.g., glioblastoma (such as glioblastoma multiforme), non-Hodgkin's lymphoma (NHL)), fibrosis, inflammatory bowel disease,

pulmonary fibrosis (including idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis secondary to sclerosis), COPD, and hepatic fibrosis.

Multi-specific antibodies of the present invention may be especially useful for treating or preventing atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis. Thus, in one embodiment, a multi-specific antibody or pharmaceutical composition of the present invention is provided for use in a method of treatment of the human or animal body by therapy. In one embodiment, the multi-specific antibody or pharmaceutical composition is provided for use in a method of treating atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis. In one embodiment, the invention provides a method of treating or preventing atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis, comprising administering a therapeutically effective amount of a multi-specific antibody or pharmaceutical composition to a patient in need thereof.

The following Examples illustrate the invention.

EXAMPLES

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EXAMPLE 1. Generation and selection of therapeutic anti-IL-13 antibody CA650

Rats were immunised with either purified human IL-13 (Peprotech) or rat fibroblasts expressing human IL-13 (expressing approx 1 ug/ml in culture supernatant), or in some cases, a combination of the two. Following 3 to 6 shots, animals were sacrificed and PBMC, spleen, bone marrow and lymph nodes harvested. Sera was monitored for binding to human IL-13 in ELISA and also for the ability to neutralise hIL-13 in the HEK-293 IL-13R-STAT-6 reporter cell assay (HEK-Blue assay, Invivogen).

B cell cultures were set up and supernatants were first screened for their ability to bind hIL-13 in a bead-based assay in an Applied Biosystems FMAT assay. This was a homogeneous assay using biotinylated human IL-13 coated onto streptavidin beads and a goat anti-rat Fc-Cy5 conjugate as a reveal agent. Positives from this assay were then progressed into a HEK-293 IL-13R-STAT-6 reporter cell assay (HEK-Blue assay, Invivogen) to identify neutralisers. Neutralising supernatants were then profiled in the Biacore to estimate off-rate and also to characterise the mode of action of neutralisation. Neutralisation was categorised as either bin 1 or bin 2. Bin 1 represents an antibody that binds to human IL-13 and prevents

binding of IL-13R α 1 and as a result also blocks IL-4R from binding. Bin 1 antibodies may also prevent binding of IL-13 to IL-13R α 2. Bin 2 represents an antibody that binds hIL-13 in such a way that allows binding to IL-13R α 1 but prevents recruitment of IL-4R into the complex. We were selecting antibodies that operated via bin 1.

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Approx. 7500 IL-13-specific positives were identified in the primary FMAT screen from a total of 27 x 100-plate SLAM experiments. 800 wells demonstrated neutralisation in the HEK-blue assay. 170 wells had desirable Biacore profiles, i.e. bin 1 antibodies with off-rates $< 5 \times 10^{-4} \text{ s}^{-1}$. Variable region cloning from these 170 wells was attempted and 160 successfully yielded fluorescent foci. 100 wells generated heavy and light chain variable region gene pairs following reverse transcription (RT)-PCR. These V-region genes were cloned as mouse IgG1 full-length antibodies and re-expressed in a HEK-293 transient expression system. Sequence analysis revealed that there were 27 unique families of anti-human IL-13 antibody. These recombinant antibodies were then retested for their ability to block recombinant hIL-13 (*E.coli*-derived and mammalian-derived), recombinant variant hIL-13 (R130Q) (*E.coli*-derived), natural wild type and variant hIL-13 (human donor-derived) and cynomolgus IL-13 (mammalian-derived) in the cell-based assay. Recombinant antibodies were also tested for their ability to bind variant human IL-13 (R130Q) and cynomolgus IL-13 in the Biacore. Following this characterisation, antibody families were selected to fulfil our criteria, i.e. sub-100 pM antibody with minimal drop-off in potency and affinity for all human and cynomolgus IL-13 preparations.

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Based on neutralisation potency, affinity and donor content in humanised grafts (see below), humanised CA650 was selected for further progression.

EXAMPLE 2. Antibody CA650 Humanisation

Antibody 650 was humanised by grafting the CDRs from the rat V-region onto human germline antibody V-region frameworks. In order to recover the activity of the antibody, a number of framework residues from the rat V-region were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair *et al.* (1991) (Humanised antibodies. WO91/09967). Alignments of the rat antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figure 1, together with the designed humanised sequences. (Figure 1(A) light chain graft 650 and Figure 1(B) heavy chain graft 650). The CDRs grafted from the donor to the acceptor

sequence are as defined by Kabat (Kabat *et al.*, 1987), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair *et al.*, 1991 Humanised antibodies. WO91/09967).

- 5 Genes encoding initial V-region sequences were designed and constructed by an automated synthesis approach by Entelechon GmbH, and modified to generate the grafted versions gL8 and gH9 by oligonucleotide directed mutagenesis. The gL8 sequence was sub-cloned into the UCB Celltech human light chain expression vector pVhCK, which contains DNA encoding the human C-Kappa constant region (Km3 allotype). The gH9 sequence was sub-
10 cloned into pVhg1Fab, which contains DNA encoding human heavy chain gamma-1 CH₁ constant region.

- Human V-region IGKV1-39 plus JK2 J-region (International Immunogenetics Information System® IMGT, <http://www.imgt.org>) was chosen as the acceptor for antibody 650 light
15 chain CDRs. The light chain framework residues in graft gL8 are all from the human germline gene, with the exception of residues 58 and 71 (numbering according to Kabat), where the donor residues Isoleucine (I58) and Tyrosine (Y71) were retained, respectively. Retention of residues I58 and Y71 was essential for full potency of the humanised antibody.

- 20 Human V-region IGHV1-69 plus JH4 J-region (IMGT, <http://www.imgt.org>) was chosen as the acceptor for the heavy chain CDRs of antibody 650. The heavy chain framework residues in grafts gH9 are all from the human germline gene, with the exception of residues 67, 69 and 71 (numbering according to Kabat), where the donor residues Alanine (A67), Phenylalanine (F69) and Valine (V71) were retained, respectively. Retention of residues
25 A67, F69 and V71 was essential for full potency of the humanised antibody. The Glutamine residue at position 1 of the human framework was replaced with Glutamic acid (E1) to afford the expression and purification of a homogeneous product: the conversion of Glutamine to pyroGlutamate at the N-terminus of antibodies and antibody fragments is widely reported. The final selected variable graft sequences gL8 and gH9 are shown in Figure 1(A) and
30 Figure 1(B) respectively.

The amino acid and DNA sequences encoding the CDRs, heavy and light variable regions, scFv and dsscFV formats of antibody 650 are shown in Figure 2.

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EXAMPLE 3. Generation of anti-IL-17AF antibody 496.g3

The production of the antibody CA028_00496.g3 (also referred to herein as antibody 496.g3) against human IL-17A and human IL-17F has been previously described in WO2012/095662. The antibody binds human IL-17A, IL-17F and IL-17A/F heterodimer with pM affinity. The amino acid and DNA sequences encoding the CDRs, heavy and light variable regions and light chain and heavy chain of the Fab format of antibody 496.g3 are shown in Figure 2. The 496.g3 (IL-17A/F binding) Fab constant regions comprised the human C-kappa constant region (K1m3 allotype) and the human gamma-1 CH₁ constant region and hinge (G1m17 allotype).

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EXAMPLE 4. Generation of anti-human albumin antibody 645

The production of the anti-human albumin antibody 645 has been previously described in WO2013/068571. The amino acid and DNA sequences encoding the CDRs, heavy and light variable regions, scFv and dsscFV formats of antibody 645 are shown in Figure 2.

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EXAMPLE 5. Multi-specific antibody IL-13/IL-17AF – construction of transient plasmids and expression in cells.

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Multi-specific antibodies were designed with the anti-IL-17AF V-region (496.g3) fixed in the Fab position; the anti-albumin V-region (645gL4gH5) and IL-13 (1539gL8gH9) were reformatted into disulfide-linked scFv in the HL orientation (dsHL) and linked to the C-termini of the respective heavy and light chain constant regions of the Fab via a 11 -amino acid glycine-serine rich linkers. (Figure 7). The amino acid and DNA sequences encoding the full-length heavy chain and light chain of the multi-specific antibody are shown in Figure 2.

25

The light chain and heavy chain genes were independently cloned into mammalian expression vectors for transient expression under the control of a hCMV promoter. Equal ratios of both plasmids were transfected into the CHO-S XE cell line (UCB) using the commercial ExpiCHO expifectamine transient expression kit (Thermo Scientific). The cultures were incubated in Corning roller bottles with vented caps at 37°C, 8.0% CO₂, 190 rpm. After 18-22 h, the cultures were fed with the appropriate volumes of CHO enhancer and feeds for the HiTiter method as provided by the manufacturer. Cultures were reincubated at 32°C, 8.0% CO₂, 190 rpm for an additional 10 to 12 days. The supernatant was harvested by centrifugation at 4000 rpm for 1 h at 4°C prior to filter-sterilization through a 0.45 µm followed by a 0.2 µm filter. Expression titres were quantified by Protein G HPLC

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using a 1 ml GE HiTrap Protein G column (GE Healthcare) and Fab standards produced in-house. The expression titre is shown in Table 1.

Table 1: Expression titres from transient expression in CHO S –XE cell line

Antibody	Concentration. (mg/L)
IL-17AF-IL-13 multi-specific antibody	160

5

EXAMPLE 6. IL-13/IL-17AF multi-specific antibody - mammalian cell line development.

To demonstrate stable expression of IL-13/IL-17AF multi-specific antibody a stably
 10 expressing mammalian cell line was created. A CHO cell line was transfected with a vector containing 496.g3 Fab, 1539gH9gL8 dsscFv HL (LC, INS0025609), 645gH5gL4 dsscFv HL (HC, INS0025306) and a selectable marker. The cell lines were cloned and evaluated for fit to a suitable manufacturing process. To assess the quality and quantity of the protein and to ensure the optimal cell line was selected, the cell line was evaluated in a small scale model
 15 of a manufacturing fed-batch bioreactor. A CHO cell line was selected expressing IL-13/IL-17AF multi-specific antibody at greater than 1.8 g/L and greater than 75 % monomer.

EXAMPLE 7. Purification process for IL-13/IL-17AF multi-specific antibody.

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The multi-specific antibody protein was purified by native protein A capture step followed by a preparative size exclusion polishing step. Clarified supernatants from standard transient CHO expression were loaded onto a MabSelect (GE Healthcare) column giving a 5 min contact time and washed with binding buffer (20mM Hepes pH7.4 + 150mM NaCl). Bound
 25 material was eluted with a 0.1M sodium citrate pH3.1 step elution and neutralised with 2M Tris/HCl pH8.5 and quantified by absorbance at 280nm.

Size exclusion chromatography (SE-UPLC) was used to determine the purity status of the eluted product. The antibody (~2 µg) was loaded on to a BEH200, 200 Å, 1.7 µm, 4.6 mm
 30 ID x 300 mm column (Waters ACQUITY) and developed with an isocratic gradient of 0.2 M phosphate pH 7 at 0.35 mL/min. Continuous detection was by absorbance at 280 nm and multi-channel fluorescence (FLR) detector (Waters). The eluted multi-specific antibody was found to be 72 % monomer.

The neutralised samples were concentrated using Amicon Ultra-15 concentrator (10kDa molecular weight cut off membrane) and centrifugation at 4000xg in a swing out rotor. Concentrated samples were applied to a XK16/60 Superdex200 column (GE Healthcare) equilibrated in PBS, pH7.4 and developed with an isocratic gradient of PBS, pH7.4 at 1ml/min. Fractions were collected and analysed by size exclusion chromatography on a BEH200, 200Å, 1.7 µm, 4.6 mm ID x 300 mm column (Aquity) and developed with an isocratic gradient of 0.2 M phosphate pH 7 at 0.35 mL/min, with detection by absorbance at 280nm and multi-channel fluorescence (FLR) detector (Waters). Selected monomer fractions were pooled, 0.22 µm sterile filtered and final samples were assayed for concentration by A280 Scanning on DropSense96 (Trinean). Endotoxin level was less than 1.0EU/mg as assessed by Charles River's EndoSafe® Portable Test System with Limulus Amebocyte Lysate (LAL) test cartridges.

Monomer status of the final multi-specific antibody was determined by size exclusion chromatography on a BEH200, 200Å, 1.7 µm, 4.6 mm ID x 300 mm column (Aquity) and developed with an isocratic gradient of 0.2 M phosphate pH 7 at 0.35 mL/min, with detection by absorbance at 280nm and multi-channel fluorescence (FLR) detector (Waters). The final multi-specific antibody was found to be >99 % monomeric as shown in Figure 3(A).

For analysis by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) samples were prepared by adding 4 x Novex NuPAGE LDS sample buffer (Life Technologies) and either 10X NuPAGE sample reducing agent (Life Technologies) or 100 mM N-ethylmaleimide (Sigma-Aldrich) to ~ 5µg purified protein, and were heated to 100°C for 3 min. The samples were loaded onto a 10 well Novex 4-20% Tris-glycine 1.0 mm SDS-polyacrylamide gel (Life Technologies) and separated at a constant voltage of 225 V for 40 min in Tris-glycine SDS running buffer (Life Technologies). Novex Mark12 wide-range protein standards (Life Technologies) were used as standards. The gel was stained with Coomassie Quick Stain (Generon) and destained in distilled water.

On non-reducing SDS-PAGE the multi-specific antibody, theoretical molecular weight (MW) of ~100 kDa, migrated to ~120 kDa. When the multi-specific antibody protein was reduced, both chains migrated at a mobility rate approaching their respective theoretical MWs, heavy chain (HC) ~52 kDa and light chain (LC) ~51 kDa. Additional bands on the non-reduced gel at ~45 - 50 kDa are 'free' LC and HC missing the disulphide bond in the Fab portion of the molecule, they do not migrate to the same position as the LC and HC in lane 2 as they are not fully reduced. (Figure 3(B)).

EXAMPLE 8. Antigen Binding of IL-13/IL-17AF multi-specific antibody molecule.**(i) Antigen binding affinity**

The binding kinetics of human and cynomolgus IL-13, IL-17A, AF, F and albumin were assessed by surface plasmon resonance (Biacore T200).

A goat anti-human IgG, F(ab')₂ fragment specific antibody (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip via amine coupling chemistry to a level of approximately 5000RU. Each analysis cycle consisted of capture of the IL-13/IL-17AF/albumin multi-specific antibody molecule to the anti F(ab')₂ surface, injection of analyte (at 25°C at a flow rate of 30µl per minute) followed by surface regeneration. Human and cynomolgus analytes were injected at 2-fold serial dilutions in HBS-EP+ running buffer (GE Healthcare) at concentrations of 10nM to 0.3125nM for IL-13, 5nM to 0.156nM for IL-17A, AF, F and 100nM to 3.125nM for albumin. Antigens were prepared in-house, with the exception of human IL-13 (R&D Systems), cyno IL-13 (Sinobiologicals) and human serum albumin (Jackson ImmunoResearch). Buffer blank injections were included to subtract instrument noise and drift.

Kinetic parameters were determined using a 1:1 binding model using Biacore T200 Evaluation software (version 3.0) and summarised in Tables 2(1) and 2(2). Where dissociation rates (k_d) were measured as less than 1.0×10^{-5} they were fixed to 1.0×10^{-5} (the limit of detection of the Biacore T200 instrument as defined by the manufacturer GE Healthcare) to calculate affinity (K_D).

The results demonstrated that the multi-specific antibody bound effectively to human and cynomolgus IL-17A, IL-17AF, IL-17F, IL-13 and albumin.

Table 2(1): Kinetic Constants of the IL-13/IL-17AF multi-specific antibody binding to human antigens.

Analyte	k_a (1/Ms)	k_d (1/s)	K_D (pM)	n=
Human IL-17A	2.26E+06	1.57E-05	9	7
Human IL-17AF	3.70E+06	4.54E-05	15	4

Human IL-17F	1.67E+06	1.98E-04	128	7
Human IL-13	1.46E+06	3.84E-05	26	3
Human albumin	6.65E+04	1.54E-04	2508	3

Results represent the mean from the indicated number of determinations.

Table 2(2): Kinetic Constants of the IL-13/IL-17AF multi-specific antibody binding to cynomolgus antigens.

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Analyte	k_a (1/Ms)	k_d (1/s)	K_D (pM)	n=
Cyno IL-17A	1.65E+06	1.24E-05	7	4
Cyno IL-17AF	2.25E+06	2.87E-05	14	4
Cyno IL-17F	1.14E+06	2.82E-04	283	4
Cyno IL-13	9.48E+05	1.48E-04	156	3
Cyno albumin	8.18E+04	2.04E-04	2473	3

Results represent the mean from the indicated number of determinations.

(ii) Simultaneous antigen binding

- 10 Surface plasmon resonance was used to demonstrate that IL-17A, IL-13 and albumin can bind simultaneously to IL-13/IL-17AF multi-specific antibody using either human or cynomolgus forms of the proteins.

15 The method format for assessing simultaneous binding of analytes to IL-13/IL-17AF multi-specific antibody was to capture the antibody sample to an immobilized anti-human IgG F(ab')₂ fragment specific antibody. Human or cynomolgus IL-13, IL-17A, and albumin were then injected singly or in a mixed solution of all three analytes (at 30µl/min for 300s) over the captured IL-13/IL-17AF multi-specific antibody (final concentrations of 30nM IL-13, 15nM IL-17A, 150nM albumin).

20

At the end of each cycle the surface was regenerated at a flowrate of 10µl/min using a 60s injection of 50mM HCl followed by a 30s injection of 5mM NaOH and a final 60s injection of 50mM HCl.

- 25 The binding response of each antigen when injected singly was determined, and the sum of the individual responses was compared to the binding response when a mixture of all three antigens was injected.

The mean binding response of a mixture of human IL-17A, IL-13 and albumin to IL-13/IL-17AF multi-specific antibody was 100% of the sum of the individual binding response (summarised in Table 3), indicating that IL-13/IL-17AF multi-specific antibody was able to simultaneously and independently bind each antigen.

5

Table 3: Simultaneous Binding of IL-13/IL-17AF multi-specific antibody to Human IL-17A, IL-13 and Albumin

Analyte	Binding, n=1 (RU)	Binding, n=2 (RU)	Binding, n=3 (RU)	Mean
IL-13	12	19	19	
IL-17A	33	53	51	
Albumin	36	60	59	
IL-13 + IL-17A + Albumin	81	131	129	
Sum of Individual Binding Responses	81	131	129	
Binding of Mixture as a Percentage of Individual Binding Responses (%)	100	100	100	100

The mean binding response of a mixture of cynomolgus IL-17A, IL-13 and albumin to IL-13/IL-17AF multi-specific antibody was 97% of the sum of the individual binding response (summarised in Table 4), indicating that IL-13/IL-17AF multi-specific antibody was able to simultaneously and independently bind each antigen.

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Table 4: Simultaneous Binding of IL-13/IL-17AF multi-specific antibody to Cynomolgus IL-17A, IL-13 and Albumin

15

Analyte	Binding, n=1 (RU)	Binding, n=2 (RU)	Binding, n=3 (RU)	Mean
IL-13	31	30	30	
IL-17A	57	56	60	
Albumin	63	61	60	
IL-13 + IL-17A + Albumin	146	141	145	
Sum of Individual Binding Responses	151	146	150	

Analyte	Binding, n=1 (RU)	Binding, n=2 (RU)	Binding, n=3 (RU)	Mean
Binding of Mixture as a Percentage of Individual Binding Responses (%)	97	96	97	97

EXAMPLE 9. Neutralisation of IL-13 by IL-13/IL-17AF multi-specific antibody.

- 5 The activity of the IL-13/IL-17AF multi-specific antibody to neutralise IL-13 was assessed using HEK 293 Human IL4/IL-13 SEAP reporter cell line assay. SEAP secretion was measured upon activation of the STAT6 pathway to assess IL-13 responses.

- HEK 293 Human IL4/IL-13 SEAP reporter cells (# hkb-il413) were obtained from Invivogen, San Diego. Culturing, freezing and maintenance of the cell line followed manufacturers protocol.

Recombinant human IL-13 was obtained from R&D Systems, Minneapolis, MN. (# 213-ILB)

- 15 Cells were plated such that cells were approximately 80% confluent upon stimulation in 96 flat bottom wells.

Cells were treated in duplicate with antibodies pre-incubated with 250 pg/mL IL-13 for 30mins at 37°C, 5% CO₂, 100% humidity, before adding to the cells for 24hrs.

- 20 After 24 hours 20µL of supernatant was pipetted from the cell stimulation and added to 180 µL of Quanti-blue #rep-qbs (Invivogen, San Diego), in accordance with manufacturers instructions.

- 25 Assay was developed until there was a visible colour change gradient and absorbance was read using a spectrophotometer at 620nm. IC₅₀s were calculated by non-linear regression using Graphpad prism (San Diego, CA). Figure 4 shows a representative graph of % inhibition of STAT6 signalling by IL-13/IL-17AF multi-specific antibody.

- 30 **Table 5: IC₅₀ value for inhibition of STAT6 signalling by IL-13/IL-17AF multi-specific antibody.**

pM	+/- SEM	ng/ml	+/- SEM	n=
4.118	1.319	0.4118	0.1319	3

EXAMPLE 10. Neutralizing effect of IL-13/IL-17AF multi-specific antibody on IL-6 responses by human dermal fibroblasts to human and cynomolgus IL-17A and IL-17F

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The purpose of this study was to determine the neutralizing capacity of IL-13/IL-17AF multi-specific antibody against human and cynomolgus IL-17A and IL-17F in a human primary cell system. A pro-inflammatory response is induced when IL-17 is present in combination with other cytokines such as TNF- α . This synergistic effect was therefore harnessed to produce an IL-6 release assay from primary normal neonate human dermal fibroblasts (nHDF) stimulated with IL-17 and TNF- α .

The ability of IL-13/IL-17AF multi-specific antibody to inhibit IL-17 induced IL-6 release from nHDFs was measured in this assay. Specifically, nHDFs were stimulated with human or cynomolgus IL-17A (50pM) or IL-17F (25,000pM) in combination with TNF- α (25pM) in the presence of a titration of IL-13/IL-17AF multi-specific antibody (concentration range 5000pM to 0.25pM for IL-17A study; 500,000pM to 25pM for IL-17F study). The resultant IL-6 response was then measured using homogeneous time-resolved FRET (HTRF).

nHDF cells (Sigma #106-05n) were cultured in complete media (DMEM + 10% FCS + 2 mM L-glutamine) and maintained in a tissue culture flask using standard techniques. Cells were harvested from the tissue culture flask using TrypLE (Invitrogen #12605036). The TrypLE was neutralised using complete medium (45ml) and the cells were centrifuged at 300 x g for 3 minutes. The cells were re-suspended in complete media (3-5ml), counted and adjusted to a concentration of 3.125×10^4 cells/mL before addition to the 384-well assay plate (Corning #3701) at 40 μ l / well. The cells were incubated for three hours at 37°C / 5% CO₂, to adhere to the plate. IL-13/IL-17AF multi-specific antibody was serially diluted in complete media in a 384-well dilution plate (Greiner #781281) to a final concentration range of 5000pM to 0.25pM for assessment against IL-17A, and 500,000pM to 25pM for assessment against IL-17F. Mixtures of TNF- α and IL-17 cytokine were prepared in complete media to final concentrations of TNF- α 25pM with either human or cynomolgus IL-17A 50pM or IL-17F 25,000pM. 30 μ l / well of these solutions were then added to a 384-well reagent plate (Greiner #781281). 10 μ l from the IL-13/IL-17AF multi-specific antibody serial dilution plate was then transferred to the reagent plate containing 30 μ l of the diluted cytokines. IL-13/IL-

17AF multi-specific antibody was then incubated with the cytokine mixture for one hour at 37°C / 5% CO₂. After the incubation, 10µl was transferred from the reagent plate to the assay plate containing cells. The assay plate was then incubated for 18 hours ± 2 hours at 37°C / 5% CO₂. After the incubation was complete, the Europium cryptate and Alexa 665 antibodies from the Cisbio IL-6 HTRF kit (Cisbio #62IL6PEB) were diluted in reconstitution buffer and mixed 1:1; as per the kit insert. Subsequently, 10µl / well of this antibody mixture was added to a white low volume 384-well HTRF plate (Greiner #784075). Supernatant from the assay plate was then transferred to the HTRF plate at 10µl / well. The HTRF plate was then incubated at room temperature for 2 hours with gentle shaking. The HTRF plates were then read on a Synergy Neo 2 plate reader as per the manufacturer's instructions, measuring the fluorescence at reads of 330/620nm and 330/665nm. The ratio values were then calculated using the following equation (330/665nm divided by 330/620nm) X 10,000 and used to determine the relative percentage inhibition as compared to the control wells, using Microsoft Excel. 4PL curve fitting and calculation of IC₅₀ values was performed using GraphPad Prism 7.0.

Results shown are the mean (+/- SEM) of three independent experiments. The IC₅₀ value of IL-13/IL-17AF multi-specific antibody was calculated as 42pM for human IL-17A and 49pM for cynomolgus IL-17A. The IC₅₀ value of IL-13/IL-17AF multi-specific antibody was calculated as 28,030pM for human IL-17F and 34,320pM for cynomolgus IL-17F. (Figure 5).

Table 6: Summary of potency, efficacy and hill slope values for IL-13/IL-17AF multi-specific antibody.

Values were calculated from three independent experiments.

Assay	IC ₅₀ (pM)	E _{max} (%)	Slope
Human IL-17A	42	100	2
Cynomolgus IL-17A	49	100	2.4
Human IL-17F	28030	100	3.2
Cynomolgus IL-17F	34320	100	2.6

EXAMPLE 11. Simultaneous neutralisation of IL-13, IL-17A and IL-17F by IL-13/IL-17AF multi-specific antibody in an NHEK CXCL1 release bioassay

The purpose of this assay was to assess the ability of IL-13/IL-17AF multi-specific antibody to simultaneously neutralise IL-13, IL-17A and IL-17F in a primary cell system. When NHEK are treated with IL-13 or IL-17A or IL-17F individually, they induce the secretion of CXCL1, a chemokine which is responsible for recruiting cells to sites of inflammation. In the context of atopic dermatitis, there is evidence that CXCL1 has a role in sensitising neurons such that they have a lower excitation threshold. This observation may be pertinent to the itch that patients experience (Yang T.B. and Kim B.S. 2019; "Pruritus in allergy and immunology" J Allergy Clin Immunol 144(2): 353-360).

- 10 NHEK (PromoCell, Heidelberg), were stored, cultured and used as per the manufacturers protocol. Cells were plated such that they were 100 % confluent upon stimulation in 48 flat bottom well plates. Cells were pre-incubated with increasing concentrations of anti-IL-13, anti-IL-17A, anti-IL-17F, or IL-13/IL-17AF multi-specific antibody respectively for 30 minutes before treatment with 100 ng/mL IL-13 and 100 ng/mL IL-17A, 1 µg/mL IL-17F for 72 hours.
- 15 After the elapsed time period, 50 µl cell-free supernatant was collected for quantification of CXCL1 concentration via ELISA as per the manufacturers protocol (R&D Systems). IC₅₀ of each experimental group was calculated by non-linear regression using Graphpad prism (San Diego, CA).
- 20 The results demonstrated that the IL-13/IL-17AF multi-specific antibody simultaneously neutralised IL-13, IL-17A and IL-17F activity. (Figure 6). When normalising for the number of binding sites available in the assay, IL-13/IL-17AF multi-specific antibody (81.3%) was more effective than anti-IL-17A (52.7 %), anti-IL-17F (0.7 %) or anti-IL-13 (48.8%) at inhibiting CXCL1 release. Increasing the concentration of anti-IL-17A, anti-IL-17F or anti-IL-
- 25 13 alone did not improve the maximal inhibition achieved. The results highlight the benefit of simultaneously inhibiting IL-13, IL-17A and IL-17F compared to a single cytokine neutralisation.

30 **EXAMPLE 12. Comparison of multi-specific IL-13/IL-17 antibody with IL-13/IL-17 antibodies of the prior art.**

Introduction

- 35 An example of an IL-13/IL-17AF multi-specific antibody according to the present invention is shown in Figure 7. It comprises a Fab domain with dual specificity for IL-17A and IL-17F,

linked to 2 scFv domains, one specific for IL-13 and the other for albumin. The anti-albumin domain confers the multi-specific antibody with an extended half-life.

Bi-specific antibodies which bind to IL-13 and IL-17 have previously been described by Abbvie (WO2013/102042A2) and Genentech (WO2015/127405A2). However, little is disclosed about how they bind and to what degree they bind to IL-13, IL-17A and IL-17F. In order to compare these properties, we made the antibodies described in the prior art and investigated their binding behaviours for the following characteristics:

- Affinity for IL-13
- Interaction of molecule with IL-13 and IL-13R α 1
- Affinity for IL-17A
- Affinity for IL-17F

Generation of comparator antibodies

BITS7201A (Genentech) was constructed using the sequences described in Example 6 of WO2015/127405A2.

DVD2166 and DVD2174 (Abbvie) were constructed using the sequences described in WO2013/102042A2, Table 6 and Table 7. These molecules were selected based on the reported activity of the individual arms of the bi-specifics against IL-13 and IL-17 being near-equivalent or equivalent to their respective parent antibody (WO2013/102042A2 Example 4, page 83 para 0195).

DNA constructs were transfected into CHO-SXE cells using the high titre protocol of the ExpiCHO transfection system (ThermoFisher Scientific). Upon harvest, cell cultures were centrifuged for at least an hour at 4 000 RPM and the supernatants were clarified by filtration using 0.22 μ M Stericup filter units.

DVD2166 + DVD2174 purification

DVD-IgG proteins were purified by applying clarified supernatants over a 10 ml MabSelect Sure column and washed with PBS, pH 7.4 for 3 column volumes (CVs). Proteins were eluted off the column with 0.1 M NaCitrate pH 3.6 step elution and neutralised with 2 M Tris-HCl, pH 8.5. Monomeric proteins were isolated by application onto a HiLoad 16x60 Superdex 200 pg column (Sigma) equilibrated with PBS, pH 7.4. Fractions containing monomeric proteins were pooled, sterile filtered and stored at 4°C.

BITS7201A purification

Parental knob and hole proteins were purified by applying clarified supernatants over a 10 mL MabSelect Sure column and washed with 3 CVs PBS, pH 7.4. Proteins were eluted off
5 the column with 0.1 M NaCitrate pH 3.6. In order to neutralise and stabilise the protein, the samples were diluted 1:1 with 1 M arginine/succinate buffer, pH 8.7. The parental antibodies were then applied onto a HiLoad 26x60 Superdex 200 pg column (Sigma) equilibrated with 0.15 M sodium acetate, 0.5 M Arginine buffer, pH 8.5. The bispecific material was subsequently generated by mixing parental antibodies at a 1:1 ratio in the presence of 5 mM
10 cysteamine and incubating overnight at room temperature. A second preparative gel filtration step was performed to remove any high molecular weight species from the exchanged bispecific material by applying to a HiLoad 26x60 Superdex 200 pg column equilibrated with PBS, pH 7.4. Fractions containing monomeric bispecific proteins were pooled, sterile filtered and stored at 4°C.

15

Comparison of binding characteristics

The binding kinetics of human IL-13, IL-17A, and IL-17F binding to IL-13/IL-17AF multi-specific antibody, termed "UCBXXXX", and prior art antibodies were assessed by surface
20 plasmon resonance (Biacore T200) and directly compared within a single experiment. In addition, surface plasmon resonance was used to assess whether the binding of IL-13/IL-17AF multi-specific antibody UCBXXXX or comparator molecules to IL-13 resulted in blocking of the IL-13 interaction with IL-13 receptors.

25 A goat anti-human IgG, F(ab')₂ fragment specific antibody (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip via amine coupling chemistry to a level of approximately 5000RU. For affinity assessment, each analysis cycle consisted of capture of IL-13/IL-17AF multi-specific antibody or comparator bispecific molecule to the anti F(ab')₂ surface (50 to 100RU), injection of analyte (at 25°C at a flow rate of 30µl per minute for 180s), after which
30 dissociation was monitored for 1200 s for IL-13 and IL-17A, and 600s for IL-17F. At the end of each cycle the surface was regenerated at a flowrate of 10 µl/min using a 60s injection of 50mM HCl followed by a 30s injection of 5mM NaOH and a final 60s injection of 50mM HCl. Analytes were injected at 2-fold serial dilutions in HBS-EP+ running buffer (GE Healthcare) at concentrations of 10nM to 0.3125nM for IL-13 and 5nM to 0.156nM for IL-17A and IL-17F.
35 IL-17A and IL-17F were prepared in-house whereas human IL-13 was sourced from R&D Systems. Buffer blank injections were included to subtract instrument noise and drift.

Kinetic parameters were determined using a 1:1 binding model using Biacore T200 Evaluation software (version 3.0). The results are summarised in Table 7.

Table 7: Comparison of kinetic constants for binding to IL-13, IL-17A and IL-17F.

Molecule	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (pM)
UCBXXXX*	IL-13	3.61E+06	1.11E-04	25.8
BITS7210A*		2.05E+06	5.69E-05	27.5
DVD2166		8.16E+05	1.50E-05	18.35
DVD2174		6.50E+05	3.66E-05	56.34
UCBXXXX*	IL-17A	3.20E+06	3.45E-05	8.2
BITS7210A*		1.27E+07	1.06E-03	84.4
DVD2166		5.59E+05	7.68E-05	137.4
DVD2174		4.76E+05	1.28E-04	268.4
UCBXXXX*	IL-17F	2.66E+06	2.19E-04	82.1
BITS7210A*		7.71E+06	5.74E-04	74.4
DVD2166		←←←←←←←←←← No Binding →→→→→→→→→→		
DVD2174		←←←←←←←←←← No Binding →→→→→→→→→→		
*Results represent the mean from two technical replicates within the same experiment				

5

To assess IL-13R α 1 receptor blocking, each antibody molecule was captured to the goat anti-human IgG, F(ab')₂ surface (to approximately 50 to 100RU), followed by injection of IL-13 (25nM for 180s at 10 μ l/min) and injection of IL-13R α 1 (R&D Systems, 100nM for 300s at 10 μ l/min). Blank injections of both IL-13 and IL-13R α 1 were included to subtract any drift or background responses. As summarised in Table 8, UCBXXXX was able to block the interaction of IL-13 with IL-13R α 1, whereas BITS7210A, DVD2166 and DVD 2174 molecules were not.

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Table 8: IL-13R α 1 receptor blocking by IL-13/IL-17AF multi-specific antibody and comparator molecules.

15

Molecule	Capture (RU)	IL-13 Binding (RU)	IL-13R α 1 Binding (RU)	IL-13R α 1 Blocker
UCBXXXX	65.4	7.3	0.2	Yes
BITS7210A	86	8.6	15.7	No
DVD2166	115.2	11.7	13.8	No
DVD2174	115.3	11.3	16.2	No

A further experiment was conducted to assess the ability of IL-13/IL-17AF multi-specific antibody to block binding to IL-13R α 1 and IL-13R α 2. In this experiment approximately 260RU of UCBXXXX was captured to an immobilised mouse anti human CH1 antibody (UCB in-house), followed by injection of IL-13 (25nM for 180s at 10 μ l/min) then either IL-13R α 1 or IL-13R α 2 (R&D Systems, 100nM for 300s at 10 μ l/min). Blank injections of IL-13, IL-13R α 1 and IL-13R α 2 were included to subtract any drift or background responses. The results demonstrated that UCBXXXX was able to block the interaction of IL-13 with both IL-13R α 1 and IL-13R α 2. (Table 9).

Table 9: IL-13R α 1 and IL-13R α 2 receptor blocking by IL-13/IL-17AF multi-specific antibody.

Capture (RU)	IL-13 Binding (RU)	IL-13R α 1 Binding (RU)	IL-13R α 2 Binding (RU)
264.4	27.0	0.8	0.3

Discussion

The comparator study demonstrated that IL-13/IL-17AF multi-specific antibody UCBXXXX, bispecific antibody BITS7210A, and dual-variable-domain antibodies DVD2166 and DVD2174 were able to bind with high affinity to IL-13. However, the characteristics of the binding interactions were very different. UCBXXXX was able to block the interaction of IL-13 with IL-13-R α 1, whereas BITS7210A, DVD2166 and DVD2174 were not.

The comparator study further demonstrated that multi-specific antibody UCBXXXX, bispecific antibody BITS7210A, and dual-variable-domain antibodies DVD2166 and DVD2174 were able to bind to IL-17. Again however, the characteristics of the binding interactions were very different. The binding affinity of UCBXXXX for IL-17A was significantly higher than that of BITS7210A and the DVD antibodies. UCBXXXX and BITS7210A bound with similar affinity to IL-17F whereas the DVD antibodies were not able to bind to IL-17F at all.

The interaction between IL-13 and IL-13R α 1, in the presence of these antibodies, is important in the context of reducing potential immunogenicity. Evidence suggests that immunogenicity, particularly the generation of anti-drug-antibodies (ADA), should be closely considered when investigating potential new bispecific antibody therapeutics. A major driver for ADA generation is the engagement and subsequent internalisation of the therapeutic antibody with target antigens expressed on the cell surface (Schellekens, H., 2002; Clin

Ther. 24(11):1720-40). Internalised therapeutic antibody / target antigen complexes undergo trafficking through various intracellular compartments, and can either be recycled back to the cell surface or undergo degradation (St Pierre et al, 2011); which can lead to peptide presentation by antigen presenting molecules and ADA generation.

5

In bispecific antibody BITS7201A, the IL-13 F(ab) portion of the molecule is the same as the anti-IL-13 antibody, lebrikizumab (see example 6 of WO2015/127405A2). It is believed that lebrikizumab binds to IL-13 at a site which allows IL-13 to bind to its receptors IL-13R α 1 and IL-13R α 2, but blocks the interaction with IL-4R α receptor (Popovic et al., 2017; J Mol Biol.

10 429(2):208-19). This particular type of interaction may enable internalisation of the antibody / target antigen / receptor complex through IL-13R α 2 and thus increase the potential for an immunogenic response. In a phase I clinical trial, BITS7201A was associated with a high incidence of anti-drug antibodies (ADAs) and was withdrawn from clinical development.

15 Similarly, the dual-variable-domain antibodies DVD2166 and DVD2174 were not able to block the interaction of IL-13 with IL-13-R α 1.

To mitigate the potential immunogenicity risk, UCBXXXX has been specifically designed so that, on engagement with its respective target antigens IL-13, IL-17A and IL-17F, they are
20 prevented from interacting with receptors on cells, thus decreasing the chance of internalisation, degradation and potential for ADA generation. The incidence of ADA with UCBXXXX in humans will only be known for certain once clinical data are available.

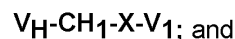
The data generated above indicates that IL-13/IL-17AF multi-specific antibody UCBXXXX
25 has the correct properties to become an effective IL-13/IL-17 antibody therapeutic, with improved efficacy and lower risk of immunogenicity.

30

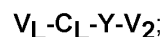
CLAIMS

1. A multi-specific antibody which binds human IL-13, human IL-17A and/or human IL-17F, comprising an IL-13 binding site which comprises:
a light chain variable region comprising the sequence given in SEQ ID NO:15 for CDR-L1, the sequence given in SEQ ID NO:16 for CDR-L2 and the sequence given in SEQ ID NO:17 for CDR-L3, and
a heavy chain variable region comprising the sequence given in SEQ ID NO:18 for CDR-H1, the sequence given in SEQ ID NO:19 for CDR-H2 and the sequence given in SEQ ID NO:20 for CDR-H3.
2. A multi-specific antibody according to claim 1, wherein the IL-13 binding site comprises a light chain variable region comprising the sequence given in SEQ ID NO:27 and a heavy chain variable region comprising the sequence given in SEQ ID NO:28.
3. A multi-specific antibody according to claim 1, wherein the IL-13 binding site comprises a light chain variable region comprising the sequence given in SEQ ID NO:31 and a heavy chain variable region comprising the sequence given in SEQ ID NO:32.
4. A multi-specific antibody according to any of claims 1 to 3, comprising an antigen binding site that binds to human IL-17A and human IL-17F which comprises:
a light chain variable region comprising the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3, and
a heavy chain variable region comprising the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6 for CDR-H3.
5. A multi-specific antibody according to claim 4, wherein the antigen binding site that binds to human IL-17A and human IL-17F comprises a light chain variable region comprising the sequence given in SEQ ID NO:7 and a heavy chain variable region comprising the sequence given in SEQ ID NO:9.
6. A multi-specific antibody according to any preceding claim which binds human IL-13, human IL-17A and/or human IL-17F, comprising:

a) a polypeptide chain of formula (Ia):



b) a polypeptide chain of formula (IIa):



wherein:

V_H represents a heavy chain variable domain;

CH_1 represents domain 1 of a heavy chain constant region;

X represents a bond or linker;

Y represents a bond or linker;

V_1 represents a scFv, a dsscFv, or a dsFv;

V_L represents a light chain variable domain;

C_L represents a domain from a light chain constant region, such as Ckappa;

V_2 represents a scFv, a dsscFv or a dsFv;

wherein the polypeptide chain of formula (Ia) comprises a protein A binding domain;

and

wherein the polypeptide chain of formula (IIa) does not bind protein A.

7. A multi-specific antibody according to claim 6, wherein:

V_L and V_H comprise an antigen binding site that binds to human IL-17A and human IL-17F,

V_2 comprises an antigen binding site that binds to human IL-13, and

V_1 comprises an antigen binding site that binds to human serum albumin;

wherein

V_L comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3, and

V_H comprises the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6 for CDR-H3;

wherein

V_1 comprises a light chain variable region comprising the sequence given in SEQ ID NO:39 for CDR-L1, the sequence given in SEQ ID NO:40 for CDR-L2 and the sequence given in SEQ ID NO:41 for CDR-L3; and a heavy chain variable region comprising the sequence given in SEQ ID NO:42 for CDR-H1, the sequence

given in SEQ ID NO:43 for CDR-H2 and the sequence given in SEQ ID NO:44 for CDR-H3; and

wherein

V₂ comprises a light chain variable region comprising the sequence given in SEQ ID NO:15 for CDR-L1, the sequence given in SEQ ID NO:16 for CDR-L2 and the sequence given in SEQ ID NO:17 for CDR-L3, and a heavy chain variable region comprising the sequence given in SEQ ID NO:18 for CDR-H1, the sequence given in SEQ ID NO:19 for CDR-H2 and the sequence given in SEQ ID NO:20 for CDR-H3.

8. A multi-specific antibody according to claim 6 or claim 7, wherein V_L comprises the sequence given in SEQ ID NO:7 and V_H comprises the sequence given in SEQ ID NO:9.
9. A multi-specific antibody according to any of claims 6 to 8, wherein V₂ comprises a light chain variable region comprising the sequence given in SEQ ID NO:27 and a heavy chain variable region comprising the sequence given in SEQ ID NO:28.
10. A multi-specific antibody according to any of claims 6 to 8, wherein V₂ comprises a light chain variable region comprising the sequence given in SEQ ID NO:31 and a heavy chain variable region comprising the sequence given in SEQ ID NO:32.
11. A multi-specific antibody according to any of claims 6 to 10, wherein V₁ comprises a light chain variable region comprising the sequence given in SEQ ID NO:45 and a heavy chain variable region comprising the sequence given in SEQ ID NO:46.
12. A multi-specific antibody according to any of claims 6 to 10, wherein V₁ comprises a light chain variable region comprising the sequence given in SEQ ID NO:49 and a heavy chain variable region comprising the sequence given in SEQ ID NO:50.
13. A multi-specific antibody according to any of claims 6 to 12, wherein the light chain variable region and heavy chain variable region of V₂ are connected by a linker, said linker comprising the sequence given in SEQ ID NO:66.
14. A multi-specific antibody according to claim 13, wherein V₂ is a scFv comprising the sequence given in SEQ ID NO:35 or a dsscFv comprising the sequence given in SEQ ID NO:37.

15. A multi-specific antibody according to any of claims 6 to 14, wherein the light chain variable region and heavy chain variable region of V_1 are connected by a linker, said linker comprising the sequence given in SEQ ID NO:68.
16. A multi-specific antibody according to claim 15, wherein V_1 is a scFv comprising the sequence given in SEQ ID NO:53 or a dsscFv comprising the sequence given in SEQ ID NO:55.
17. A multi-specific antibody according to any of claims 6 to 16, wherein Y is a linker comprising the sequence given in SEQ ID NO:65.
18. A multi-specific antibody according to any of claims 6 to 17, wherein X is a linker comprising the sequence given in SEQ ID NO:67.
19. A multi-specific antibody according to any preceding claim comprising the sequence given in SEQ ID NO:57 or SEQ ID NO: 59.
20. A multi-specific antibody according to any preceding claim, comprising the sequence given in SEQ ID NO:61 or SEQ ID NO: 63.
21. A multi-specific antibody according to any preceding claim comprising the sequence given in SEQ ID NO:59 and the sequence given in SEQ ID NO: 63.
22. An isolated polynucleotide encoding a multi-specific antibody as defined in any one of claims 1 to 21.
23. An expression vector carrying the polynucleotide of claim 22.
24. A host cell comprising the vector as defined in claim 23.
25. A method of producing the multi-specific antibody as defined in any one of claims 1-21, comprising culturing the host cell of claim 24 under conditions permitting production of the antibody, and recovering the antibody produced.
26. The method of claim 25 comprising a protein A purification step.

27. A pharmaceutical composition comprising an antibody as defined in any one of claims 1 to 21 and a pharmaceutically acceptable adjuvant and/or carrier.
28. An antibody as defined in any one of claims 1-21 or a pharmaceutical composition as defined in claim 27 for use in a method of treatment of the human or animal body by therapy.
29. The antibody or pharmaceutical composition according to claim 28, for use in the treatment or prevention of atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis.
30. A method of treating or preventing atopic dermatitis, chronic hand eczema, nasal micropolyposis or polyposis, food allergy, or eosinophilic esophagitis, comprising administering a therapeutically effective amount of a multi-specific antibody according to claims 1-21 or a pharmaceutical composition according to claim 27 to a patient in need thereof.