

ANTI-TSLP ANTIBODIES AND USES THEREOF

CLAIM OF PRIORITY

This application claims priority to Chinese Application No. 202311318859.2, filed on October 12, 2023. The entire contents of the foregoing application are incorporated herein by
5 reference.

TECHNICAL FIELD

The present disclosure is related to the field of immunotherapy, more specifically, involves antibodies or antigen-binding fragments thereof that can specifically bind to TSLP.
10 The disclosure also relates to pharmaceutical compositions comprising the same, and the use thereof.

BACKGROUND

Autoimmune diseases are conditions arising from an abnormal immune response to a
15 normal body part. There are at least 80 types of autoimmune diseases. The cause of autoimmune disease is generally not well understood. Some autoimmune diseases such as lupus run in families, and some other autoimmune diseases may be triggered by infections or other environmental factors. Some common autoimmune diseases include e.g., celiac disease, type 1 diabetes mellitus, Graves' disease, inflammatory bowel disease, multiple sclerosis,
20 psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

Recent clinical and commercial success of therapeutic antibodies has created great interest in using antibodies to treat various immune-related disorders. There is a need to develop antibodies for use in various antibody-based therapeutics to treat immune disorders.

25 SUMMARY

The present disclosure is related to anti-TSLP antibodies or antigen-binding fragments thereof that can specifically bind to TSLP. The antibodies or antigen-binding fragments thereof can be used for treating diseases or disorders (e.g., cancer or immune disorders).

In one aspect, the disclosure is related to an antibody or antigen-binding fragment
30 thereof that binds to TSLP (thymic stromal lymphopoietin) comprising: a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2, and 3, in some embodiments, the VH CDR1 region comprises an amino acid sequence that is at least

80% identical to a selected VH CDR1 amino acid sequence, the VH CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR2 amino acid sequence, and the VH CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR3 amino acid sequence; and a light chain variable region (VL) comprising CDRs 1, 2, and 3, in some embodiments, the VL CDR1 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR1 amino acid sequence, the VL CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR2 amino acid sequence, and the VL CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR3 amino acid sequence, in some embodiments, the selected VH CDRs 1, 2, and 3 amino acid sequences and the selected VL CDRs, 1, 2, and 3 amino acid sequences are one of the following: (1) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 37, 38, 39, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 40, 41, 42, respectively; (2) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 86, 38, 39, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 40, 41, 42, respectively; (3) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 117, 118, 119, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 120, 121, 122, respectively.

In some embodiments, the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 37, 38, 39, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 40, 41, 42, respectively, in some embodiments, the VH CDR1 is determined according to AbM definition, in some embodiments, the VH CDR2, VH CDR3, and VL CDRs 1, 2, 3, are determined according to Kabat definition. In some embodiments, the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 86, 38, 39, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 40, 41, 42, respectively, according to Kabat definition. In some embodiments, the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 117, 118, 119, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 120, 121, 122, respectively, according to Chothia definition.

In one aspect, the disclosure is related to an antibody or antigen-binding fragment thereof that binds to TSLP comprising a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90% identical to a selected VH sequence, and a light

chain variable region (VL) comprising an amino acid sequence that is at least 90% identical to a selected VL sequence, in some embodiments, the selected VH sequence and the selected VL sequence are one of the following: (1) the selected VH sequence is SEQ ID NO: 43, and the selected VL sequence is SEQ ID NO: 44; and (2) the selected VH sequence is SEQ ID NO: 45, and the selected VL sequence is SEQ ID NO: 46. In some embodiments, the antibody or antigen-binding fragment specifically binds to human TSLP and/or monkey TSLP. In some embodiments, the antibody or antigen-binding fragment is a human or humanized antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding fragment is selected from a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, scFv, and nanobody.

In one aspect, the disclosure is related to an antibody or antigen-binding fragment thereof that binds to TSLP comprising a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 that are identical to VH CDR1, VH CDR2, and VH CDR3 of a selected VH sequence; and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 that are identical to VL CDR1, VL CDR2, and VL CDR3 of a selected VL sequence, in some embodiments, the selected VH sequence and the selected VL sequence are one of the following: (1) the selected VH sequence is SEQ ID NO: 43, and the selected VL sequence is SEQ ID NO: 44; and (2) the selected VH sequence is SEQ ID NO: 45, and the selected VL sequence is SEQ ID NO: 46.

In one aspect, the disclosure is related to an antibody or antigen-binding fragment thereof that cross-competes with the antibody or antigen-binding fragment thereof described herein.

In some embodiments, the antibody or antigen-binding fragment thereof is a bispecific or a multi-specific antibody or an antigen-binding fragment thereof.

In one aspect, the disclosure is related to a nucleic acid comprising a polynucleotide encoding a polypeptide comprising: (1) an immunoglobulin heavy chain or a fragment thereof comprising a VH comprising CDRs 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 37, 38, 39, respectively; SEQ ID NOs: 86, 38, 39, respectively; or SEQ ID NOs: 117, 118, 119, respectively; and in some embodiments, the VH, when paired with a light chain variable region (VL) comprising the amino acid sequence set forth in SEQ ID NO: 44 or 46, binds to TSLP; or (2) an immunoglobulin light chain or a fragment thereof comprising a VL comprising CDRs 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 40, 41, 42, respectively; or SEQ ID NOs: 120, 121, 122, respectively; and in some embodiments, the VL, when paired with a VH comprising the amino acid sequence set

forth in SEQ ID NO: 43 or 45, binds to TSLP. In some embodiments, the VH when paired with a VL specifically binds to human TSLP. In some embodiments, the immunoglobulin heavy chain or the fragment thereof is a human or humanized immunoglobulin heavy chain or a fragment thereof. In some embodiments, the nucleic acid encodes a single-chain variable
5 fragment (scFv), a bispecific or a multi-specific antibody or an antigen-binding fragment thereof. In some embodiments, the nucleic acid is cDNA.

In one aspect, the disclosure is related to a vector comprising one or more of the nucleic acids described herein, or a nucleic acid encoding the antibody or antigen-binding fragment thereof described herein.

10 In one aspect, the disclosure is related to a cell comprising the vector described herein. In some embodiments, the cell is a CHO cell. In one aspect, the disclosure is related to a cell comprising one or more of the nucleic acids described herein, or a nucleic acid encoding the antibody or antigen-binding fragment thereof described herein.

In one aspect, the disclosure is related to a method of producing an antibody or an
15 antigen-binding fragment thereof, or an antigen-binding protein construct, the method comprising (a) culturing the cell described herein under conditions sufficient for the cell to produce the antibody or the antigen-binding fragment thereof, or the antigen-binding protein construct; and (b) collecting the antibody or the antigen-binding fragment thereof, or the antigen-binding protein construct produced by the cell.

20 In one aspect, the disclosure is related to an antibody-drug conjugate comprising a therapeutic agent covalently bound to the antibody or antigen-binding fragment thereof described herein. In some embodiments, the therapeutic agent is a cytotoxic or cytostatic agent.

In one aspect, the disclosure is related to a pharmaceutical composition comprising a
25 pharmaceutically acceptable carrier and the antibody or antigen-binding fragment thereof, or the antibody-drug conjugate described herein.

In one aspect, the disclosure is related to a kit comprising the antibody or antigen-binding fragment thereof, or the antibody-drug conjugate described herein.

In one aspect, the disclosure is related to a method of treating a subject having cancer,
30 the method comprising administering a therapeutically effective amount of a composition comprising the antibody or antigen-binding fragment thereof, the antibody-drug conjugate, or the pharmaceutical composition described herein, to the subject.

In one aspect, the disclosure is related to a method of treating a subject having an immune disorder, the method comprising administering a therapeutically effective amount of

a composition comprising the antibody or antigen-binding fragment thereof, the antibody-drug conjugate, or the pharmaceutical composition described herein, to the subject. In some embodiments, the immune disorder is allergy, asthma, or atopic dermatitis. In some embodiments, the immune disorder is a type II or mixed allergic disease.

5 In some embodiments, the subject is a human or a non-human animal.

In one aspect, the present disclosure provides an isolated anti-TSLP antibody or an antigen-binding fragment thereof, comprising: HCDR1 set forth in SEQ ID NO: 37, HCDR2 set forth in SEQ ID NO: 38, HCDR3 set forth in SEQ ID NO: 39, LCDR1 set forth in SEQ ID NO: 40, LCDR2 set forth in SEQ ID NO: 41, and LCDR3 set forth in SEQ ID NO: 42.

10 In one aspect, the present disclosure relates to a polynucleotide encoding the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein).

In one aspect, the present disclosure relates to an expression vector, comprising the polynucleotide (e.g., any polypeptides described herein).

15 In one aspect, the present disclosure relates to a host cell integrated with the polynucleotide (e.g., any polypeptides described herein) or the expression vector thereof.

In one aspect, the present disclosure relates to an immunoconjugate, comprising the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein).

20 In one aspect, the present disclosure relates to a pharmaceutical composition, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein), or the immunoconjugate thereof (e.g., any immunoconjugates described herein), and an optional pharmaceutically acceptable excipient.

25 In one aspect, the present disclosure relates to a kit, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein), the immunoconjugate thereof (e.g., any immunoconjugates described herein), or the pharmaceutical composition (e.g., any pharmaceutical compositions described herein).

30 In one aspect, the present disclosure relates to use of the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit in the preparation of a medicament for treating or preventing a disease or disorder (e.g., a cancer or autoimmune disease). Alternatively, the present disclosure relates to the anti-TSLP antibody or the antigen-binding fragment thereof, the

immunoconjugate thereof, the pharmaceutical composition, or the kit for use in the preparation of an inhibitor of TSLP. Alternatively, the present disclosure relates to a method of inhibiting TSLP, comprising administering to a subject in need thereof the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the
5 pharmaceutical composition, or the kit.

In one aspect, the present disclosure relates to use of the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit in the preparation of a medicament for treating or preventing a disease or disorder (e.g., a cancer or autoimmune disease). Alternatively, the present disclosure
10 relates to a method of treating or preventing a disease or disorder (e.g., a cancer or autoimmune disease), comprising administering to a subject in need thereof the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit. Alternatively, the present disclosure relates to the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof,
15 the pharmaceutical composition, or the kit for use in the treatment or prevention of a disease or disorder (e.g., a cancer or autoimmune disease). In some embodiments, the disease or disorder is type II and mixed allergic disease, e.g., asthma and/or atopic dermatitis.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention
20 belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification,
25 including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF THE DRAWINGS

30 FIGS. 1A-1B show the effects of IL-4, IL-13, and/or TSLP on the release of CCL-17 from PBMCs. FIG. 1A shows the induction of the release of CCL-17 from PBMCs by TSLP alone, IL-4 alone, IL-13 alone, and a mixture of the three (3MIX), respectively. FIG. 1B shows the inhibitory effect of immunoglobulin IgG1 (control), Dupilumab, Tezepelumab, and

the combination of Dupilumab and Tezepelumab on the release of CCL-17.

FIGS. 2A-2D show the effects of TSLP-mediated DC maturation on the releases of cytokines IL-5 and IL-13 mediated by naïve T cells. FIG. 2A shows a schematic diagram of TSLP-stimulated DCs driving naïve T cells to develop into Th2 cells, which release cytokines such as IL-4, IL-5, and IL-13. FIG. 2B shows that co-culturing DCs stimulated by TSLP with naïve T cells significantly enhanced the release of Th2 cytokines (e.g., IL-5/IL-13) compared to DCs stimulated with TSLP or CD4⁺ T cells only. FIGS. 2C-2D show that the inhibition levels of IL-5 release in each test group are comparable, while for IL-13 release, administration of anti-IL-4R α antibody alone or anti-TSLP antibody alone was almost unable to inhibit its release, and the combination use of anti-IL-4R α antibody and anti-TSLP antibody can significantly inhibit its release in a dose-dependent manner.

FIG. 3 shows the molecular structures of three bispecific antibodies.

FIGS. 4A-4B show the blocking function of each bispecific antibody prepared in Example 2 on IL-4R α /IL-4/IL-13 and TSLP/TSLPR pathways. FIG. 4A shows the blocking function of each bispecific antibody prepared in Example 2 on the IL-4R α /IL-4/IL-13 pathway. FIG. 4B shows the blocking function of each bispecific antibody prepared in Example 2 on the TSLP/TSLPR pathway.

FIGS. 5A-5B show the blocking activity of 11H7E11 chimeric antibody and scFv antibody against TSLP/TSLPR. FIG. 5A shows the results of detecting the blocking activity of 11H7E11 chimeric antibody against TSLP/TSLPR using CTLL2-mCD127-hTSLPR-stat5-Luc2 reporter gene cells. FIG. 5B shows the results of the inhibitory effects of 11H7E11 chimeric antibody and scFv antibody on TSLP induced secretion of the chemokine CCL-17 from human mDCs.

FIGS. 6A-6B show a schematic diagram of the structural form of an exemplary bispecific antibody (FIG. 6A) and the structures of peptide chain # 1 and peptide chain # 2 therein (FIG. 6B).

FIGS. 7A-7B show the CE-SDS results of the candidate molecule from Example 8 (FIG. 7A), and no significant increase in aggregates and fragmentation products observed for candidate molecule H6-5G-hz11H7-2-scFv (FIG. 7B).

FIGS. 8A-8B show the *in vitro* biological functional test results of the influence of bispecific antibodies with different lengths of linkers on the blocking functions. FIG. 8A shows the blocking function of each candidate bispecific antibody in Example 8 on the IL-4R α /IL-4/IL-13 pathway. FIG. 8B shows the blocking function of each candidate bispecific antibody in Example 8 on the TSLP/TSLPR pathway.

FIG. 10 shows the results of the CTLL2 reporter gene cell activity experiment of bispecific antibody binding to TSLP.

FIGS. 12A-12D show the therapeutic effect of bispecific antibodies on asthmatic mice. FIG. 12A shows the total number of lymphocytes after treatment. FIG. 12B shows the total number of eosinophils after treatment. FIG. 12C shows the total number of monocytes after treatment. FIG. 12D shows the total number of neutrophils after treatment.

FIG. 14 shows the changes in blood drug concentration at different time points after administering bispecific antibodies D5-5G-hz11H7-2-scFv and D5-11H7-YTE at a dose of 10 mg/kg to FcRn humanized mice.

FIG. 16 lists VH and VL sequences of antibodies discussed in the disclosure.

FIG. 18 lists additional sequences discussed in the disclosure.

25 **DETAILED DESCRIPTION**

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There is currently development of antibody drugs targeting multiple targets for various allergic diseases such as atopic dermatitis, asthma etc.

Unless otherwise defined, the technical and scientific terms used herein have the same meanings as commonly understood by the person of ordinary skill in the art.

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Definitions

The term “antibody” used herein refers to a protein or peptide that can specifically recognize and bind to antigen(s), covering various structures of natural and artificial antibodies, including but not limited to monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (e.g., bispecific antibodies), single chain antibodies, single domain antibodies, full-length antibodies, and antibody fragments that exhibit desired biological activity. In some embodiments, antibodies can be classified into 5 isotypes based on heavy chain categories, i.e., IgG, IgM, IgD, IgA, and IgE.

When referring to an antibody, the term “isolated” herein means that the antibody is essentially free of other cellular components that bind to it in the natural state. For example, an isolated antibody can be an antibody removed from the native or natural environment.

The term “bispecific” used herein refers to an antigen-binding construct (e.g., an antibody) comprising two antigen-binding portions (such as antigen-binding fragments) that each has specific binding specificity, for example, the first antigen-binding fragments and the second antigen-binding fragments bind specifically to epitopes on the first and second antigens, or bind specifically to different epitopes of the same antigen.

The term “antigen-binding fragment” of an antibody refers to a portion or a fragment of a full-length or whole antibody that has fewer amino acid residues than the whole antibody or the full-length antibody, but is capable of binding to an antigen or competing with a full-length antibody (i.e., the full-length antibody from which the antigen-binding fragment is derived) to bind to an antigen. Antigen-binding fragments can be prepared by recombinant DNA technology, or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding fragments include but are not limited to Fab, Fab', F(ab')₂, Fd, Fv, single chain Fv, diabody, single domain antibody (sdAb), and nanobody. For example, Fab fragments can be obtained by digesting full-length antibodies with papain. In addition, F(ab')₂ is generated via the digestion of a complete antibody by pepsin below disulfide bonds in a hinge region, which is a dimer of Fab' and a divalent antibody fragment. F(ab')₂ can be reduced under neutral conditions by breaking the disulfide bond in the hinge region, thereby converting F(ab')₂ dimer into Fab' monomers. A Fab' monomer is substantially a Fab fragment with the hinge

region. Fv fragment is consisted of the VL (light chain variable region) and VH (heavy chain variable region) domains in a single arm of an antibody. The two domains VL and VH of the Fv fragment can be encoded by independent genes, but recombination methods can also be used, linking the two domains by using synthetic linker peptides to produce the same as a single protein chain, where the VL and VH regions pair to form a single chain Fv (scFv) .

The term “scFv” herein comprises the VH and VL domains of an antibody present in a single polypeptide chain.

The term “CDR” (complementary determining region), also known as “hypervariable region (HVR)”, used herein refers to each region of the antibody variable domain that is highly variable in sequence and/or forms a structurally defined loop. Natural antibodies typically comprise three CDRs located in the heavy chain variable region (i.e., HCDR1 to HCDR3) and three CDRs located in the light chain variable region (LCDR1 to LCDR3). Multiple definitions well known in this field can be used to identify CDRs of heavy and light chains, e.g., Chothia based on the three-dimensional structure of antibodies and the topology of the CDR loops, Kabat based on antibody sequence variability (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health, 1987), AbM (University of Bath), Contact (University College London), International ImMunoGeneTics (IMGT) database (the international ImMunoGeneTics information system, <http://imgt.cines.fr>), and North CDR definition based on the affinity propagation clustering using a large number of crystal structures (North *et al.*, “A New Clustering of Antibody CDR Loop Conformations”, Journal of Molecular Biology, 406, 228-256, 2011).

Table 1. CDRs determined by using the different numbering schemes

CDR	Kabat	AbM	Chothia	Contact	IMGT
LCDR1	L24-L34	L24-L34	L26-L32	L30-L36	L27-L32
LCDR2	L50-L56	L50-L56	L50-L52	L46-L55	L50-L52
LCDR3	L89-L97	L89-L97	L91-L96	L89-L96	L89-L96
HCDR1*	H31-H35B	H26-H35B	H26-H32	H30-H35B	H26-H35B
HCDR1**	H31-H35	H26-H35	H26-H32	H30-H35	H26-H35
HCDR2	H50-H65	H50-H58	H53-H55	H47-H58	H51-H57
HCDR3	H95-H102	H95-H102	H96-H101	H93-H101	H93-H102

* Based on Kabat Numbering

** Based on Chothia/Martin Numbering

When referring to an antibody, the terms “variable region”, “V region”, or “variable

domain” can be used interchangeably to refer to a domain of the antibody heavy or light chains involved in the specific binding of the antibody to antigens, typically comprising an amino acid sequence arranged in the order of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 from the N-terminal to the C-terminal.

- 5 The term “chimeric antibody” refers to an antibody that comprises sequences derived from two different antibodies (usually from different species), for example, in which (a) a constant region or a portion thereof is modified, substituted, or exchanged such that an antigen-binding site is linked to a constant region having different or altered categories, effector functions, and/or species sources, or a completely different molecule providing a new biological function(e.g., an enzyme, a toxin, a hormone, a growth factor, and a drug) to the chimeric antibody, or the like; or (b) a variable region or a portion thereof is modified, substituted, or exchanged by a variable region with different or altered antigenic specificities.

- 15 The term “humanized antibody” used herein refers to an antibody that retains the antigen-specific reactivity of a non-human antibody (e.g., an alpaca monoclonal antibody, or a murine antibody) and has low immunogenicity when administered to human as a therapeutic drug. In some embodiments, it comprises CDRs derived from non-human animals, FRs derived from human, and optionally constant regions derived from human.

- 20 The term “epitope” used herein, also known as “antigenic determinant”, refers to a portion of an antigen that can be recognized and specifically bound by antibodies. An antigen can have multiple epitopes, which are typically formed by surface-exposed molecule groups, such as amino acids or sugar side chains.

 The term “flexible linker peptide”, “linker peptide” or “linker” herein refers to a short amino acid sequence consisting of amino acids, used for the linkage of peptide segments. A flexible linker peptide can comprise glycine (G), alanine (A), threonine (T) residues, etc.

- 25 The term “binding” or “specific binding” used herein means that the binding effect is selective for antigens and may be distinguished from undesired or non-specific interactions. For example, an antibody that specifically binds to a target antigen means that compared to the binding thereof to other non-target molecules, the antibody binds to the target antigen with higher affinity, stronger binding activity, easier binding, and/or longer binding duration.

- 30 “Affinity” or “binding affinity” refers to the inherent binding ability that reflects the interaction between members of a binding pair. For example, the affinity of molecule X for its partner Y can generally be represented by the equilibrium dissociation constant (K_D), which is a ratio of the dissociation rate constant (k_{dis} and k_{off}) to the association rate constant (k_a or k_{on}). Affinity can be measured by common methods known in the art. One specific

method for measuring affinity is the ForteBio kinetic binding assay.

The “percentage identity” of an amino acid sequence refers to the percentage of amino acid residues of a candidate sequence that are the same as those of a reference sequence in the total number of amino acid residues of the reference sequence, after
5 comparing the candidate sequence with the reference sequence and introducing gaps (if necessary) to achieve the maximum sequence identity percentage, without considering any conservative substitutions as part of sequence identity. Two or more sequences can be aligned to determine the percentage of sequence identity of amino acid sequences using tools known in the art, such as BLASTp, ClusterW2 (see Higgins DG *et al.*, Methods Enzymol 1996,
10 266:383-402; Larkin MA *et al.*, Bioinformatics 2007, 23:2947-2948), ALIGN or Megalign (DNASTAR) software, etc.

For peptide sequences, “conservative modification” includes the substitution, deletion, or insertion of amino acids in the peptide sequence with other amino acids of the same class (such as chemically or functionally similar amino acids), without substantially
15 altering the expected functional activity of the peptide sequence. For example, conservative substitution often causes a certain amino acid to be replaced with a similar amino acid. A list of conserved substitutions of functionally similar amino acids are known in the art. The following is a list of 8 groups of conservatively substituted amino acids: 1) alanine (A), glycine (G); 2) aspartic acid (D), glutamic acid (E); 3) asparagine (N), glutamine (Q); 4)
20 arginine (R), lysine (K); 5) isoleucine (I), leucine (L), methionine (M), valine (V); 6) phenylalanine (F), tyrosine (Y), tryptophan (W); 7) serine (S), threonine (T); and 8) cysteine (C), methionine (M).

The terms “subject”, “patient”, and “individual” are used herein interchangeably, comprising mammal or non-mammalian vertebrate (such as chicken, emu, fish), wherein the
25 mammal comprises, but is not limited to, domesticated animal (such as cow, sheep, cat, dog, pig, and horse), primate (such as human, non-human primate such as monkey), rabbit, and rodent (such as mouse, rat, guinea pig, hamster), preferably human.

The term “treat”, “treating”, or “treatment” herein refers to alleviating or relieving a certain disease or condition, slowing the rate of onset or progression of a certain disease or
30 condition, reducing the risk of developing a certain disease or condition, or delaying the progression of a symptom related to a certain disease or condition, reducing or terminating a symptom related to a certain disease or condition, producing a complete or partial reversal of a certain disease or condition, curing a certain disease or condition, or a combination of the above. The desired therapeutic effects herein include, but are not limited to, preventing the

occurrence or recurrence of a disease, alleviating a condition, diminishing any direct or indirect pathological consequences of a disease, slowing the rate of disease progression, improving or mitigating a disease state, and relieving or improving prognosis.

As used herein, the term “optional” refers to the presence or absence of the object modified thereby, for example, “a pharmaceutical composition comprising an optional pharmaceutically acceptable excipient” means that the pharmaceutical composition may or may not comprise the pharmaceutically acceptable excipient.

The term “therapeutically effective amount” or “effective dosage” refers to a dose or concentration that is effective in preventing or improving symptoms related to a disease or condition and/or alleviating the severity of a disease or condition at a desired dose and for a desired period of time. The therapeutically effective amount of the preparation, antibody or antigen-binding fragment thereof, bispecific antibody or composition of the present disclosure may vary depending on various factors such as disease status, age, gender and weight of the individual, and the ability of the antibody or antigen-binding portion to elicit the desired response in the individual. The therapeutically effective amount can also be considered as a preparation, antibody or antigen-binding fragment thereof, bispecific antibody or composition exhibiting therapeutic beneficial effects significantly greater than any toxic or harmful effects caused thereby. The term “effective amount” refers to an amount of active ingredient or medicament that is sufficient to provide clinical benefits to the subject, including but not limit to, improving, relieving or alleviating a disease, condition or related symptoms thereof, delaying or stopping disease progression.

As used herein, the term “pharmaceutically acceptable” or “clinically acceptable” refers to the mentioned carrier, vehicle, diluent, excipient, and/or salt that are generally chemically and/or physically compatible with other ingredients in the formulation and physiologically compatible with the subject.

The terms “X” and “Xaa” herein are equivalent and refer to an unspecified amino acid, the range covered by which is specified through definitions in relevant expressions. In order to distinguish multiple “X” in a amino acid sequence, multiple X that appear successively are numbered (e.g., written as X_n) and the ranges covered thereby are defined respectively.

Unless otherwise specified, the terms “comprise, comprises and comprising” or their equivalents (e.g., contain, contains, containing, include, includes, including) herein are open-ended expressions and should be understood as “include but not limited to”, which means that in addition to the listed elements, components and steps, other unspecified elements,

components and steps may also be covered.

Unless otherwise specified, all the numbers used herein in the specification to express amounts of ingredients, measured values, or conditions should be understood as being modified in all cases by the term “about”, the meaning of which should be considered as being within an acceptable range of error for a corresponding value. When being connected to a percentage, the term “about” can represent such as $\pm 1\%$, preferably $\pm 0.5\%$, more preferably 0.1% .

Unless otherwise indicated, the singular term herein encompasses the corresponding plural references, and *vice versa*. Likewise, unless otherwise indicated clearly in the context, the word “or” herein is intended to include “and”.

The term “pharmaceutical composition” herein means that various active ingredients comprised therein can be administered simultaneously, separately, or at regular or irregular intervals, and the various active ingredients can be mixed together or exist separately and individually (e.g., in the form of their respective pharmaceutical compositions).

For the purpose of describing and disclosing, all patents, patent applications and other established publications are expressly incorporated herein by reference. These publications are provided solely for their disclosure prior to the filing date of this application. All statements regarding the dates of these documents or the representation of the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the dates of these documents or the contents of these documents.

A more detailed description of the technical solution in the present disclosure will be made through exemplary embodiments below, but the protection scope of the present disclosure is not limited thereto.

Anti-TSLP antibodies or antigen-binding fragments thereof

The present disclosure provides an isolated anti-TSLP antibody or an antigen-binding fragment thereof that can specifically bind to TSLP to inhibit the maturation of dendritic cells (DCs) and the production of chemokine CCL-17 induced by TSLP, thereby blocking TSLP activity and reducing inflammation. The anti-TSLP antibody according to the present disclosure can be a murine antibody, a chimeric antibody, a humanized antibody, a fully humanized antibody, or can be a monoclonal antibody, a polyclonal antibody, a monospecific antibody, or a multi-specific antibody (such as a bispecific antibody), as long as the antibody can specifically recognize and bind to TSLP.

In some embodiments, the present disclosure provides an isolated anti-TSLP antibody or an antigen-binding fragment thereof, comprising: HCDR1 set forth in SEQ ID NO: 37, HCDR2 set forth in SEQ ID NO: 38, HCDR3 set forth in SEQ ID NO: 39; and/or LCDR1 set forth in SEQ ID NO: 40, LCDR2 set forth in SEQ ID NO: 41, and LCDR3 set forth in SEQ ID NO: 42.

In the present disclosure, in the isolated anti-TSLP antibody or the antigen-binding fragment thereof, the heavy chain variable region CDR1 (HCDR1) is defined by the AbM numbering system, the heavy chain variable region CDR2 and CDR3 (HCDR2 and HCDR3) and light chain variable region CDRs (LCDRs) are defined by the Kabat numbering system. Variable regions defined using other numbering systems are also within the protection scope of this disclosure.

In some preferred embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure comprises: a VH having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or higher identity to the amino acid sequence set forth in SEQ ID NO: 43 or 45; and a VL having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or higher identity to the amino acid sequence set forth in SEQ ID NO: 44 or 46.

In some particular embodiments, compared with the amino acid sequence set forth in SEQ ID NO: 43 or 45, the different amino acids in the amino acid sequence with at least 80% identity thereto mainly exist (or all exist) in the FR region (framework region). In some particular embodiments, compared with the amino acid sequence set forth in SEQ ID NO: 44 or 46, the different amino acids in the amino acid sequences with at least 80% identity thereto mainly exist (or all exist) in the FR region (framework region).

In some preferred embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure comprises: a VH set forth in SEQ ID NO: 43 or 45; and a VL set forth in SEQ ID NO: 44 or 46. In some preferred embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure comprises: HCDRs comprised in a VH set forth in SEQ ID NO: 43 or 45; and LCDRs comprised in a VL set forth in SEQ ID NO: 44 or 46.

In some embodiments, the isolated anti-TSLP antibody or the antigen-binding

fragment thereof according to the present disclosure comprises:

(1) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43, and a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44; or

(2) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 45, and a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 46;

wherein, compared with the amino acid sequence set forth in any one of SEQ ID NOs: 43, 44, 45, and 46, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto all exist in the FR region.

In some embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure comprises:

(1) VH set forth in SEQ ID NO: 43, and VL set forth in SEQ ID NO: 44; or

(2) VH set forth in SEQ ID NO: 45, and VL set forth in SEQ ID NO: 46.

In some embodiments, the anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure is an anti-TSLP scFv. In some particular embodiments, the scFv comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein each variable region or a portion thereof is derived from the same antibody or different antibodies, preferably from the same antibody. A disulfide bond may be or may be not comprised between VH and VL of the scFv. In some embodiments, the anti-TSLP scFv comprises a linker. In some embodiments, the scFv molecule can be constructed in the order of VH-linker-VL or VL-linker-VH. In some embodiments, the linker has low immunogenicity, preferably using flexible peptides (such as 5-25, 10-20 amino acids in length, including such as glycine, or glycine and serine) as the linker (such as G_m linker, GS linker, etc.).

In some particular embodiments, the linker comprises an amino acid sequence of (GGGGS)_n, i.e., (G4S)_n, where n is an integer from 1 to 5, preferably n is 2, 3, or 4, and most preferably n is 4. In some examples, an amino acid sequence of the linker is GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 63).

In some embodiments, the anti-TSLP scFv according to the present disclosure comprises: (1) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43 or 45; (2) a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44 or 46; and (3) a (G4S)_n linker for linking the VH to VL, n is an integer from 1 to 5, preferably n is 2, 3, or 4.

In some preferred embodiments, the anti-TSLP scFv according to the present disclosure comprises:

(1) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43, a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44, and a (G4S)_n linker for linking the VH to VL, n is an integer from 1 to 5, preferably n is 2, 3, or 4; or

(2) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 45, a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 46, and a (G4S)_n linker for linking the VH to VL, n is an integer from 1 to 5, preferably n is 2, 3, or 4;

wherein, compared with the amino acid sequence set forth in any one of SEQ ID NOs: 43, 44, 45, and 46, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto all exist in the FR region.

In some preferred embodiments, the anti-TSLP scFv according to the present disclosure comprises:

(1) VH set forth in SEQ ID NO: 43, VL set forth in SEQ ID NO: 44, and the (G4S)_n linker for linking the VH to VL, with n being 2, 3, or 4; or

(2) VH set forth in SEQ ID NO: 45, VL set forth in SEQ ID NO: 46, and the (G4S)_n linker for linking the VH to VL, with n being 2, 3, or 4.

In some preferred embodiments, the anti-TSLP scFv according to the present disclosure comprises:

(1) VH set forth in SEQ ID NO: 43, VL set forth in SEQ ID NO: 44, and a linker set

forth in SEQ ID NO: 63; or

(2) VH set forth in SEQ ID NO: 45, VL set forth in SEQ ID NO: 46, and a linker set forth in SEQ ID NO: 63.

In some alternative embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure further comprises a heavy chain constant region and a light chain constant region. In further preferred embodiments, the heavy chain constant region is selected from the constant regions of human IgG1, IgG2, IgG3, and IgG4, or the variants thereof; and the light chain constant region is selected from the constant regions of human κ and λ chains, or the variants thereof. Exemplary variants include IgG1, IgG2, or IgG4 heavy chain constant region variants that undergo site directed modification and amino acid substitution to the heavy chain constant regions, such as the AAA mutation, DLE mutation (Shields *et al.*, 2002; Lazar *et al.*, 2006), YTE mutation, and LS mutation (Ghetie *et al.*, 1997; Zalevsky *et al.*, 2010) known in the art.

In some embodiments, the heavy chain constant region (CH) is of the IgG1 LALA subtype. In some embodiments, the heavy chain constant region comprises an amino acid sequence set forth in SEQ ID NO: 47 or an amino acid sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or higher identity thereto. In some embodiments, the heavy chain constant region comprises an amino acid sequence set forth in SEQ ID NO: 82 or SEQ ID NO: 83.

In some embodiments, the light chain constant region is a human κ chain constant region. In some embodiments, the light chain constant region comprises an amino acid sequence set forth in SEQ ID NO: 48 or an amino acid sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or higher identity thereto.

In some embodiments, the isolated anti-TSLP antibody comprises a heavy chain and a light chain, wherein

the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43 or 45, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and

the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44 or 46, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48.

In some embodiments, the isolated anti-TSLP antibody comprises a heavy chain and a light chain, wherein

(1) the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and

the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48; or

(2) the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 45, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and

the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 46, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48;

wherein, compared with the amino acid sequence set forth in any one of SEQ ID NOs: 43, 44, 45, and 46, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto all exist in the FR region.

In some embodiments, the isolated anti-TSLP antibody comprises a heavy chain and a light chain, wherein

(1) the heavy chain comprises a VH set forth in SEQ ID NO: 43, and a CH set forth in

SEQ ID NO: 47; and

the light chain comprises a VL set forth in SEQ ID NO: 44, and a CL set forth in SEQ ID NO: 48; or

(2) the heavy chain comprises a VH set forth in SEQ ID NO: 45, and a CH set forth in
5 SEQ ID NO: 47; and

the light chain comprises a VL set forth in SEQ ID NO: 46, and a CL set forth in SEQ ID NO: 48.

In some embodiments, the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure is of IgG1, IgG2, IgG3, or IgG4 type.

10 In some embodiments, the antigen-binding fragment of the isolated anti-TSLP antibody according to the present disclosure is selected from a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, and nanobody.

Various variants of the isolated anti-TSLP antibody or the antigen-binding fragment
15 thereof described herein retain the ability of specifically binding to the antigen TSLP.

In some embodiments, the K_D value of the isolated anti-TSLP antibody or the antigen-binding fragment thereof according to the present disclosure binding to human TSLP is less than 1×10^{-9} M, preferably less than 8×10^{-10} M, for example less than 5×10^{-10} M, and exhibits good blocking activities against TSLP/TSLPR.

20 The disclosure provides several antibodies and antigen-binding fragments thereof that specifically bind to TSLP.

The antibodies and antigen-binding fragments described herein are capable of binding to TSLP. The disclosure provides e.g., anti-TSLP antibodies 11H7E11, and the antibodies derived therefrom.

25 The CDR sequences for 11H7E11, and 11H7E11 derived antibodies (e.g., chimeric antibodies or humanized antibodies) include CDRs of the heavy chain variable domain, SEQ ID NOs: 37, 38, 39, respectively, and CDRs of the light chain variable domain, SEQ ID NOs: 40, 41, 42, respectively, wherein the VH CDR1 is determined according to AbM definition, wherein the VH CDR2, VH CDR3, and VL CDRs 1, 2, 3, are determined according to Kabat
30 definition. The CDRs can also be defined by Kabat or Chothia system. Under the Kabat numbering, the CDR sequences of the heavy chain variable domain are set forth in SEQ ID NOs: 86, 38, 39, respectively, and CDR sequences of the light chain variable domain are set forth in SEQ ID NOs: 40, 41, 42, respectively. Under the Chothia numbering, the CDR sequences of the heavy chain variable domain are set forth in SEQ ID NOs: 117, 118, 119,

respectively, and CDR sequences of the light chain variable domain are set forth in SEQ ID NOs: 120, 121, 122, respectively.

Furthermore, in some embodiments, the antibodies or antigen-binding fragments thereof described herein can also contain one, two, or three heavy chain variable region CDRs selected from the group of SEQ ID NOs: 37, 38, 39; SEQ ID NOs: 86, 38, 39; and SEQ ID NOs: 117, 118, 119; and/or one, two, or three light chain variable region CDRs selected from the group of SEQ ID NOs: 40, 41, 42; and SEQ ID NOs: 120, 121, 122.

In some embodiments, the anti-TSLP antibodies can have a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2, 3, wherein the CDR1 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VH CDR1 amino acid sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VH CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected VH CDR3 amino acid sequence, and a light chain variable region (VL) comprising CDRs 1, 2, 3, wherein the CDR1 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected VL CDR1 amino acid sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected VL CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected VL CDR3 amino acid sequence. The selected VH CDRs 1, 2, 3 amino acid sequences and the selected VL CDRs, 1, 2, 3 amino acid sequences are shown in **FIGS. 15A-15C**.

In some embodiments, the anti-TSLP antibody or an antigen-binding fragment described herein can contain a heavy chain variable domain containing one, two, or three of the CDR1 with zero, one or two amino acid insertions, deletions, or substitutions; CDR2 with zero, one or two amino acid insertions, deletions, or substitutions; CDR3 with zero, one or two amino acid insertions, deletions, or substitutions, and the VH CDRs 1, 2, and 3 are shown in **FIGS. 15A-15C**. In some embodiments, the antibody or an antigen-binding fragment described herein can contain a light chain variable domain containing one, two, or three of the CDR1 with zero, one or two amino acid insertions, deletions, or substitutions; CDR2 with zero, one or two amino acid insertions, deletions, or substitutions; CDR3 with zero, one or two amino acid insertions, deletions, or substitutions, and the VL CDRs 1, 2, 3 are shown in **FIGS. 15A-15C**.

The insertions, deletions, and substitutions can be within the CDR sequence, or at one or both terminal ends of the CDR sequence.

The disclosure also provides antibodies or antigen-binding fragments thereof that bind to TSLP. The antibodies or antigen-binding fragments thereof contain a heavy chain variable region (VH) comprising or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VH sequence, and a light chain variable region (VL) comprising or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a selected VL sequence. In some embodiments, the selected VH sequence is SEQ ID NO: 43, and the selected VL sequence is SEQ ID NO: 44. In some embodiments, the selected VH sequence is SEQ ID NO: 45, and the selected VL sequence is SEQ ID NO: 46.

In some embodiments, the antibody or antigen-binding fragment thereof can have 3 VH CDRs that are identical to the CDRs of any VH sequences as described herein. In some embodiments, the antibody or antigen-binding fragment thereof can have 3 VL CDRs that are identical to the CDRs of any VL sequences as described herein.

The disclosure also provides nucleic acid comprising a polynucleotide encoding a polypeptide comprising an immunoglobulin heavy chain or an immunoglobulin heavy chain. The immunoglobulin heavy chain or immunoglobulin light chain comprises CDRs as shown in **FIGS. 15A-15C**, or have sequences as shown in **FIG. 16**. When the polypeptides are paired with corresponding polypeptide (e.g., a corresponding heavy chain variable region or a corresponding light chain variable region), the paired polypeptides bind to TSLP (e.g., human TSLP or monkey TSLP).

The anti-TSLP antibodies and antigen-binding fragments can also be antibody variants (including derivatives and conjugates) of antibodies or antibody fragments and multi-specific (e.g., bispecific) antibodies or antibody fragments. Additional antibodies provided herein are polyclonal, monoclonal, multi-specific (multimeric, e.g., bispecific), human antibodies, chimeric antibodies (e.g., human-mouse chimera), single-chain antibodies, intracellularly-made antibodies (i.e., intrabodies), and antigen-binding fragments thereof. The antibodies or antigen-binding fragments thereof can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass. In some embodiments, the antibody or antigen-binding fragment thereof is an IgG antibody or antigen-binding fragment thereof.

Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired affinity and specificity of the full-length antibody. Thus, a fragment of an

antibody that binds to TSLP will retain an ability to bind to TSLP. An Fv fragment is an antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs or a subset thereof confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) can have the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

Antigen-binding protein constructs

The present disclosure provides antibodies and the antigen-binding fragments thereof that target TSLP. The anti-TSLP antibodies and the antigen-binding fragments thereof can have various forms.

In general, wildtype antibodies (also called immunoglobulins) can be made up of two classes of polypeptide chains, light chains and heavy chains. A non-limiting antibody of the present disclosure can be an intact, four immunoglobulin chain antibody comprising two heavy chains and two light chains. The heavy chain of the antibody can be of any isotype including IgM, IgG, IgE, IgA, or IgD or sub-isotype including IgG1, IgG2, IgG2a, IgG2b, IgG3, IgG4, IgE1, IgE2, etc. The light chain can be a kappa light chain or a lambda light chain. An antibody can comprise two identical copies of a light chain and/or two identical copies of a heavy chain. The heavy chains, which each contain one variable domain (or variable region, VH) and multiple constant domains (or constant regions), bind to one another via disulfide bonding within their constant domains to form the “stem” of the antibody. The light chains, which each contain one variable domain (or variable region, VL) and one constant domain (or constant region), each bind to one heavy chain via disulfide binding. The variable region of each light chain is aligned with the variable region of the heavy chain to which it is bound. The variable regions of both the light chains and heavy chains contain three hypervariable regions sandwiched between more conserved framework regions (FR).

These hypervariable regions, known as the complementary determining regions (CDRs), form loops that comprise the principal antigen-binding surface of the antibody. The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the

other chain, contribute to the formation of the antigen-binding region.

In some embodiments, the antibody is an intact immunoglobulin molecule (e.g., IgG1, IgG2a, IgG2b, IgG3, IgM, IgD, IgE, IgA). The IgG subclasses (IgG1, IgG2, IgG3, and IgG4) are highly conserved, differ in their constant region, particularly in their hinges and upper
 5 CH2 domains. The sequences and differences of the IgG subclasses are known in the art, and are described, e.g., in Vidarsson, et al, "IgG subclasses and allotypes: from structure to effector functions." *Frontiers in immunology* 5 (2014); Irani, et al. "Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases." *Molecular immunology* 67.2 (2015): 171-182; Shakib, Farouk,
 10 ed. *The human IgG subclasses: molecular analysis of structure, function and regulation*. Elsevier, 2016; each of which is incorporated herein by reference in its entirety.

The antibody can also be an immunoglobulin molecule that is derived from any species (e.g., human, rodent, mouse, rat, camelid). Antibodies disclosed herein also include, but are not limited to, polyclonal, monoclonal, monospecific, polyspecific antibodies, and
 15 chimeric antibodies that include an immunoglobulin binding domain fused to another polypeptide. The antigen-binding domain or antigen-binding fragment is a portion of an antibody that retains specific binding activity of the intact antibody, i.e., any portion of an antibody that is capable of specific binding to an epitope on the intact antibody's target molecule. It includes, e.g., Fab, Fab', F(ab')₂, and variants of these fragments. Thus, in some
 20 embodiments, an antibody or an antigen-binding fragment thereof can be, e.g., a scFv, a Fv, a Fd, a dAb, a bispecific antibody, a bispecific scFv, a diabody, a linear antibody, a single-chain antibody molecule, a multi-specific antibody formed from antibody fragments, and any polypeptide that includes a binding domain which is, or is homologous to, an antibody binding domain. Non-limiting examples of antigen-binding domains include, e.g., the heavy
 25 chain and/or light chain CDRs of an intact antibody, the heavy and/or light chain variable regions of an intact antibody, full length heavy or light chains of an intact antibody, or an individual CDR from either the heavy chain or the light chain of an intact antibody.

In some embodiments, the antigen-binding fragment can form a part of a chimeric antigen receptor (CAR). In some embodiments, the chimeric antigen receptor are fusions of
 30 single-chain variable fragments (scFv) as described herein, fused to CD3-zeta transmembrane- and endodomain. In some embodiments, the chimeric antigen receptor also comprises intracellular signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS). In some embodiments, the chimeric antigen receptor comprises multiple signaling domains, e.g., CD3z-CD28-41BB or CD3z-CD28-OX40, to increase

potency. Thus, in one aspect, the disclosure further provides cells (e.g., T cells) that express the chimeric antigen receptors as described herein.

In some embodiments, the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) can bind to two different
5 antigens or two different epitopes.

In some embodiments, the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) can comprises one, two, or three heavy chain variable region CDRs selected from **FIGS. 15A-15C**. In some
10 embodiments, the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) can comprises one, two, or three light chain variable region CDRs selected from **FIGS. 15A-15C**.

In some embodiments, the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) described herein can be conjugated to a therapeutic agent. The antibody-drug conjugate comprising the antibody or
15 antigen-binding fragment thereof can covalently or non-covalently bind to a therapeutic agent. In some embodiments, the therapeutic agent is a cytotoxic or cytostatic agent (e.g., monomethyl auristatin E, monomethyl auristatin F, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin, maytansinoids such as DM-1 and DM-4,
20 dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, epirubicin, and cyclophosphamide and analogs).

In some embodiments, the multi-specific antibody is a bispecific antibody. Bispecific antibodies can be made by engineering the interface between a pair of antibody molecules to
25 maximize the percentage of heterodimers that are recovered from recombinant cell culture. For example, the interface can contain at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chain(s) are
30 created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers. This method is described, e.g., in WO 96/27011, which is incorporated by reference in its entirety.

Any of the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) described herein may be conjugated to a stabilizing molecule (e.g., a molecule that increases the half-life of the antibody or antigen-binding fragment thereof in a subject or in solution). Non-limiting examples of stabilizing molecules include: a polymer (e.g., a polyethylene glycol) or a protein (e.g., serum albumin, such as human serum albumin). The conjugation of a stabilizing molecule can increase the half-life or extend the biological activity of an antibody or an antigen-binding fragment *in vitro* (e.g., in tissue culture or when stored as a pharmaceutical composition) or *in vivo* (e.g., in a human).

The antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies) can also have various forms. Many different formats of antigen-binding constructs are known in the art, and are described e.g., in Suurs, et al. "A review of bispecific antibodies and antibody constructs in oncology and clinical challenges," Pharmacology & therapeutics (2019), which is incorporated herein by reference in the entirety.

In some embodiments, the antigen-binding protein construct is a BiTe, a (scFv)₂, a nanobody, a nanobody-HSA, a DART, a TandAb, a scDiabody, a scDiabody-CH₃, scFv-CH₂-CL-scFv, a HSAbody, scDiabody-HAS, or a tandem-scFv. In some embodiments, the antigen-binding protein construct is a VHH-scAb, a VHH-Fab, a Dual scFab, a F(ab')₂, a diabody, a crossMab, a DAF (two-in-one), a DAF (four-in-one), a DutaMab, a DT-IgG, a knobs-in-holes common light chain, a knobs-in-holes assembly, a charge pair, a Fab-arm exchange, a SEEDbody, a LUZ-Y, a Fcab, a κλ-body, an orthogonal Fab, a DVD-IgG, a IgG(H)-scFv, a scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zyboby, DVI-IgG, Diabody-CH₃, a triple body, a miniantibody, a minibody, a TriBi minibody, scFv-CH₃ KIH, Fab-scFv, a F(ab')₂-scFv₂, a scFv-KIH, a Fab-scFv-Fc, a tetravalent HCAb, a scDiabody-Fc, a Diabody-Fc, a tandem scFv-Fc, an Intrabody, a dock and lock, a ImmTAC, an IgG-IgG conjugate, a Cov-X-Body, or a scFv1-PEG-scFv₂.

In some embodiments, the antigen-binding protein construct can be a TrioMab. In a TrioMab, the two heavy chains are from different species, wherein different sequences restrict the heavy-light chain pairing.

In some embodiments, the antigen-binding protein construct has two different heavy chains and one common light chain. Heterodimerization of heavy chains can be based on the knob-in-holes or some other heavy chain pairing technique.

In some embodiments, CrossMAb technique can be used produce bispecific antibodies. CrossMAb technique can be used enforce correct light chain association in bispecific heterodimeric IgG antibodies, this technique allows the generation of various bispecific antibody formats, including bi-(1+1), tri-(2+1) and tetra-(2+2) valent bispecific antibodies, as well as non-Fc tandem antigen-binding fragment (Fab)-based antibodies. These formats can be derived from any existing antibody pair using domain crossover, without the need for the identification of common light chains, post-translational processing/in vitro chemical assembly or the introduction of a set of mutations enforcing correct light chain association. The method is described in Klein et al., "The use of CrossMAb technology for the generation of bi-and multi-specific antibodies." MAbs. Vol. 8. No. 6. Taylor & Francis, 2016, which is incorporated by reference in its entirety. In some embodiments, the CH1 in the heavy chain and the CL domain in the light chain are swapped.

The antigen-binding protein construct can be a Duobody. The Fab-exchange mechanism naturally occurring in IgG4 antibodies is mimicked in a controlled matter in IgG1 antibodies, a mechanism called controlled Fab exchange. This format can ensure specific pairing between the heavy-light chains.

In Dual-variable-domain antibody (DVD-Ig), additional VH and variable light chain (VL) domain are added to each N-terminus for bispecific targeting. This format resembles the IgG-scFv, but the added binding domains are bound individually to their respective N-termini instead of a scFv to each heavy chain N-terminus.

In scFv-IgG, the two scFv are connected to the C-terminus of the heavy chain (CH3). The scFv-IgG format has two different bivalent binding sites and is consequently also called tetravalent. There are no heavy-chain and light-chain pairing problem in the scFv-IgG.

In some embodiments, the antigen-binding protein construct can be a IgG-IgG format. Two intact IgG antibodies are conjugated by chemically linking the C-terminals of the heavy chains.

The antigen-binding protein construct can also have a Fab-scFv-Fc format. In Fab-scFv-Fc format, a light chain, heavy chain and a third chain containing the Fc region and the scFv are assembled. It can ensure efficient manufacturing and purification.

In some embodiments, antigen-binding protein construct can be a TF. Three Fab fragments are linked by disulfide bridges. Two fragments target the tumor associated antigen (TAA) and one fragment targets a hapten. The TF format does not have an Fc region.

ADAPTIR has two scFvs bound to each side of an Fc region. It abandons the intact IgG as a basis for its construct, but conserves the Fc region to extend the half-life and

facilitate purification.

Bispecific T cell Engager ("BiTE") consists of two scFvs, VLA VHA and VHB VLB on one peptide chain. It has only binding domains, no Fc region.

In BiTE-Fc, an Fc region is fused to the BiTE construct. The addition of Fc region
5 enhances half-life leading to longer effective concentrations, avoiding continuous IV.

Dual affinity retargeting (DART) has two peptide chains connecting the opposite fragments, thus VLA with VHB and VLB with VHA, and a sulfur bond at their C-termini fusing them together. In DART, the sulfur bond can improve stability over BiTEs.

In DART-Fc, an Fc region is attached to the DART structure. It can be generated by
10 assembling three chains, two via a disulfide bond, as with the DART. One chain contains half of the Fc region which will dimerize with the third chain, only expressing the Fc region. The addition of Fc region enhances half-life leading to longer effective concentrations, avoiding continuous IV.

In tetravalent DART, four peptide chains are assembled. Basically, two DART
15 molecules are created with half an Fc region and will dimerize. This format has bivalent binding to both targets, thus it is a tetravalent molecule.

Tandem diabody (TandAb) comprises two diabodies. Each diabody consists of an VHA and VLB fragment and a VHA and VLB fragment that are covalently associated. The two diabodies are linked with a peptide chain. It can improve stability over the diabody
20 consisting of two scFvs. It has two bivalent binding sites.

The ScFv-scFv-toxin includes toxin and two scFv with a stabilizing linker. It can be used for specific delivery of payload.

In modular scFv-scFv-scFv, one scFv directed against the TAA is tagged with a short recognizable peptide is assembled to a bsAb consisting of two scFvs, one directed against
25 CD3 and one against the recognizable peptide.

In ImmTAC, a stabilized and soluble T cell receptor is fused to a scFv recognizing CD3. By using a TCR, the ImmTAC is suitable to target processed, e.g. intracellular, proteins.

Tri-specific nanobody has two single variable domains (nanobodies) with an additional module for half-life extension. The extra module is added to enhance half-life.

30 In Trispecific Killer Engager (TriKE), two scFvs are connected via polypeptide linkers incorporating human IL-15. The linker to IL-15 is added to increase survival and proliferation of NKs.

In some embodiments, the antibodies or the antigen-binding fragments thereof (e.g., the anti-TSLP antibody), or the related antibody drug conjugates (ADC), have a light chain

constant region that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 48, and a heavy chain constant region that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47.

- 5 In some embodiments, antibodies or the antigen-binding fragments thereof (e.g., the anti-TSLP antibody) is an scFv. The scFv can be in the VH-linker-VL or VL-linker-VH format. In some embodiments, the linker described herein is a flexible linker, e.g., a GS linker. In some embodiments, the linker described herein is a flexible linker, e.g., a GS linker. In some embodiments, the GS linker includes on or more repeats (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 10 repeats) of GS, SG, GGGGS (SEQ ID NO: 127). In some embodiments, the GS linker includes an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 63. Details of flexible linkers can be found, e.g., Chen, X., et al. "Fusion protein linkers: property, design and functionality." *Advanced Drug Delivery Reviews* 65.10 (2013): 1357-1369, which is incorporated herein by reference in its entirety.
- 15 In some embodiments, the antigen-binding protein construct described herein is a multi-specific antibody (e.g., a bispecific antibody).

Polynucleotides, vectors and host cells

- 20 The present disclosure relates to a polynucleotide encoding the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein), an expression vector comprising the polynucleotide, and a host cell integrated with the polynucleotide or the expression vector thereof.

- 25 In some embodiments, the expression vector may be any expression vector capable of expressing the antibody or antigen-binding portion thereof as described herein, including but not limited to naked plasmids, phagemids, yeast plasmids, adenoviruses, adeno-associated viruses, herpesviruses (such as herpes simplex viruses), retroviruses (such as lentiviruses), poxviruses, papillomavirus, papovaviruses (such as SV40), rhabdoviruses, or baculoviruses. For facilitating production and purification, the expression vector may also comprise a secretory signal peptide, an expression tag, etc.

- 30 It is known by the skilled in the art that due to codon degeneracy, each antibody or peptide amino acid sequence can be encoded by multiple nucleic acid sequences. The nucleic acid sequence encoding the antibody or the fragment thereof according to the present disclosure can be synthesized using methods well known in the art, such as *de novo* solid-phase DNA synthesis or PCR amplification. Given that specific amino acid sequences have

been described in this disclosure, those skilled in the art can easily modify one or more codons of their respective encoding sequences to prepare many different nucleic acids without altering the amino acid sequences of each antibody or the antigen-binding fragment thereof, or a bispecific antibody according to the present disclosure.

5 The skilled in the art can construct nucleic acids encoding the antibodies or the antigen-binding fragments thereof, or bispecific antibodies of the present disclosure into suitable vectors by using conventional means known in the art, to be introduced into host cells for the expression of the target proteins. The vector components may include but are not limited to signal sequences, replication origins, one or more marker genes, enhancer
10 elements, promoters, and transcription termination sequences. In the vector, the nucleic acid encoding the target protein is operatively linked to the promoter.

 In some embodiments, the host cell is a prokaryotic cell. In other embodiments, the host cell is a eukaryotic cell. In some embodiments, the host cell is selected from yeast cells, mammalian cells, or any other cells suitable for preparing antigen-binding constructs. In
15 some examples, the mammalian cells include Chinese hamster ovary (CHO) cells, CHO-S cells, 293 cells, and monkey kidney cells.

 The above expression vectors can be introduced into suitable host cells using any conventional means known in the art, such as protoplast fusion, calcium phosphate precipitation, electroporation, virus transfection, gene gun, liposome transfection, or other
20 conventional techniques, but not limited thereto.

 Under the conditions suitable for the expression of the target protein, the above host cells are cultured under conventional conditions, and then the antibodies described herein are recovered from the host cells or the culture medium of the host cells through conventional protein separation and purification means (such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography (such as Protein
25 A column affinity chromatography), size exclusion chromatography, etc.).

 The disclosure also provides a nucleic acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to
30 any nucleotide sequence as described herein, and an amino acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any amino acid sequence as described herein.

 The disclosure also provides a nucleic acid sequence that has a homology of at least

1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any nucleotide sequence as described herein, and an amino acid sequence that has a homology of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any amino acid sequence as described herein.

In some embodiments, the disclosure relates to nucleotide sequences encoding any peptides that are described herein, or any amino acid sequences that are encoded by any nucleotide sequences as described herein. In some embodiments, the nucleic acid sequence is less than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300, 350, 400, 500, or 600 nucleotides. In some embodiments, the amino acid sequence is less than 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, or 400 amino acid residues.

In some embodiments, the amino acid sequence (i) comprises an amino acid sequence; or (ii) consists of an amino acid sequence, wherein the amino acid sequence is any one of the sequences as described herein.

In some embodiments, the nucleic acid sequence (i) comprises a nucleic acid sequence; or (ii) consists of a nucleic acid sequence, wherein the nucleic acid sequence is any one of the sequences as described herein.

The percentage of sequence homology (e.g., amino acid sequence homology or nucleic acid homology) can also be determined. How to determine percentage of sequence homology is known in the art. In some embodiments, amino acid residues conserved with similar physicochemical properties (percent homology), e.g. leucine and isoleucine, can be used to measure sequence similarity. Families of amino acid residues having similar physicochemical properties have been defined in the art. These families include e.g., amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). The homology percentage, in many cases, is higher than the identity percentage.

The disclosure provides one or more nucleic acid encoding any of the polypeptides as described herein. In some embodiments, the nucleic acid (e.g., cDNA) includes a polynucleotide encoding a polypeptide of a heavy chain as described herein. In some

embodiments, the nucleic acid includes a polynucleotide encoding a polypeptide of a light chain as described herein. In some embodiments, the nucleic acid includes a polynucleotide encoding a scFv polypeptide as described herein.

5 **Immunoconjugates**

The present disclosure relates to an immunoconjugate, comprising the anti-TSLP antibody or the antigen-binding fragment thereof (e.g., any anti-TSLP antibodies or the antigen-binding fragments thereof described herein).

In some embodiments, the present disclosure provides an antibody-drug conjugate
10 (ADC) comprising the aforementioned anti-TSLP antibody or the antigen-binding fragment thereof, conjugated with an inflammatory therapeutic agent. In some embodiments, the inflammatory therapeutic agent may be an asthma treatment drug. In some embodiments, the anti-TSLP antibody or the antigen-binding fragment thereof is directly conjugated with a therapeutic agent. In some embodiments, the anti-TSLP antibody or the antigen-binding
15 fragment thereof, is conjugated with a therapeutic agent through a linker. In the ADC, the linker for conjugating antibodies with therapeutic agents can be a cleavable linker, such as a peptide-based linker, disulfide bond, or hydrazone linker, or can be a non-cleavable linker.

Pharmaceutical compositions and routes of administration

20 Also provided herein are pharmaceutical compositions that contain at least one (e.g., one, two, three, or four) of the antigen-binding protein constructs, antibodies (e.g., bispecific antibodies), antigen-binding fragments, or antibody-drug conjugates described herein. Two or more (e.g., two, three, or four) of any of the antigen-binding protein constructs, antibodies, antigen-binding fragments, or antibody-drug conjugates described herein can be present in a
25 pharmaceutical composition in any combination. The pharmaceutical compositions may be formulated in any manner known in the art.

The present disclosure relates to a pharmaceutical composition, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof, or the immunoconjugate thereof, and an optional pharmaceutically acceptable excipient. The pharmaceutical composition herein
30 comprises a therapeutically effective amount of the above each ingredient.

In some embodiments, the pharmaceutical composition further comprises additional inflammatory therapeutic agent(s), for example, conventional asthma treatment drug(s), such as glucocorticoid anti-inflammatory drugs, etc.

The excipients described herein can be any pharmaceutically acceptable excipient,

such as but not limited to solvents, propellants, solubilizers, cosolvents, emulsifiers, colorants, disintegrants, fillers, lubricants, wetting agents, osmotic pressure regulators, stabilizers, glidants, flavoring agents, preservatives, suspending agents, antioxidants, penetration enhancers, pH regulators, surfactants, diluents, etc. For other available

5 pharmaceutically acceptable pharmaceutical excipients, they can be found, for example, in “Handbook of Pharmaceutical Excipients” (4th edition), R.C. Rowe *et al.*, translated by Junmin Zheng, 2005, Chemical Industry Press.

In some embodiments, the pharmaceutical composition thereof may be in a form of a sterile aqueous solution, a microemulsion, a liposome, or a powder. In some embodiments,
10 the pharmaceutical composition thereof may be in a form of a unit dose, facilitating administration to patients at a desired dose.

The dosage range for administration of the pharmaceutical composition according to the present disclosure can be determined by clinical physicians based on the administration method (including administration time, administration interval, administration route),
15 patient’s age, weight, gender or pathological condition, diet, excretion rate, sensitivity to the drug and the like according to experience.

Pharmaceutical compositions are formulated to be compatible with their intended route of administration (e.g., intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal). The compositions can include a sterile diluent (e.g., sterile
20 water or saline), a fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvents, antibacterial or antifungal agents, such as benzyl alcohol or methyl parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like, antioxidants, such as ascorbic acid or sodium bisulfite, chelating agents, such as ethylenediaminetetraacetic acid, buffers, such as acetates, citrates, or phosphates, and isotonic agents, such as sugars (e.g.,
25 dextrose), polyalcohols (e.g., mannitol or sorbitol), or salts (e.g., sodium chloride), or any combination thereof. Liposomal suspensions can also be used as pharmaceutically acceptable carriers (see, e.g., U.S. Patent No. 4,522,811). Preparations of the compositions can be formulated and enclosed in ampules, disposable syringes, or multiple dose vials. Where required (as in, for example, injectable formulations), proper fluidity can be maintained by,
30 for example, the use of a coating, such as lecithin, or a surfactant. Absorption of the antibody or antigen-binding fragment thereof can be prolonged by including an agent that delays absorption (e.g., aluminum monostearate and gelatin). Alternatively, controlled release can be achieved by implants and microencapsulated delivery systems, which can include biodegradable, biocompatible polymers (e.g., ethylene vinyl acetate, polyanhydrides,

polyglycolic acid, collagen, polyorthoesters, and polylactic acid; Alza Corporation and Nova Pharmaceutical, Inc.).

Compositions containing one or more of any of the antigen-binding protein constructs, antibodies, antigen-binding fragments, antibody-drug conjugates described herein
5 can be formulated for parenteral (e.g., intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal) administration in dosage unit form (i.e., physically discrete units containing a predetermined quantity of active compound for ease of administration and uniformity of dosage).

Toxicity and therapeutic efficacy of compositions can be determined by standard
10 pharmaceutical procedures in cell cultures or experimental animals (e.g., monkeys). One can determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population): the therapeutic index being the ratio of LD₅₀:ED₅₀. Agents that exhibit high therapeutic indices are preferred. Where an agent exhibits an undesirable side effect, care should be taken to minimize potential damage (i.e.,
15 reduce unwanted side effects). Toxicity and therapeutic efficacy can be determined by other standard pharmaceutical procedures.

Exemplary doses include milligram or microgram amounts of any of the antigen-binding protein constructs, antibodies or antigen-binding fragments, or antibody-drug conjugates described herein per kilogram of the subject's weight (e.g., about 1 µg/kg to about
20 500 mg/kg; about 100 µg/kg to about 500 mg/kg; about 100 µg/kg to about 50 mg/kg; about 10 µg/kg to about 5 mg/kg; about 10 µg/kg to about 0.5 mg/kg; or about 0.1 mg/kg to about 0.5 mg/kg).

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. The disclosure also provides methods of
25 manufacturing the antibodies or antigen-binding fragments thereof, or antibody-drug conjugates for various uses as described herein.

In some embodiments, the pharmaceutical composition described herein comprises additional therapeutic agent(s), such as inflammatory therapeutic agent(s), for example, conventional asthma treatment drug(s), such as glucocorticoid anti-inflammatory drugs, etc.

30

Kits

The present disclosure relates to a kit, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, or the pharmaceutical composition thereof. The kit herein comprises a therapeutically effective amount of the above

each ingredient.

In some embodiments, the kit may further comprise an instruction for use. In some embodiments, the kit may further comprise reagent(s) for the diagnosis of a patient. In some embodiments, the kit may further comprise a device for administering to a patient, such as a syringe, etc.

In some embodiments, the kit may further comprise pharmaceutical excipients for assisting in administering the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, or the pharmaceutical composition thereof, to a patient, such as sterile water or normal saline.

Methods of making antibodies, antigen-binding fragments and antigen-binding protein constructs

An isolated fragment of human protein can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation.

Polyclonal antibodies can be raised in animals by multiple injections (e.g., subcutaneous or intraperitoneal injections) of an antigenic peptide or protein. In some embodiments, the antigenic peptide or protein is injected with at least one adjuvant. In some embodiments, the antigenic peptide or protein can be conjugated to an agent that is immunogenic in the species to be immunized. Animals can be injected with the antigenic peptide or protein more than one time (e.g., twice, three times, or four times).

The full-length polypeptide or protein can be used or, alternatively, antigenic peptide fragments thereof can be used as immunogens. The antigenic peptide of a protein comprises at least 8 (e.g., at least 10, 15, 20, or 30) amino acid residues of the amino acid sequence of the protein and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject (e.g., human or transgenic animal expressing at least one human immunoglobulin locus). An appropriate immunogenic preparation can contain, for example, a recombinantly-expressed or a chemically-synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a polypeptide, or an antigenic peptide thereof (e.g., part of the protein) as an immunogen. The antibody titer in the immunized subject can be monitored over time by

standard techniques, such as with an enzyme-linked immunosorbent assay (ELISA) using the immobilized polypeptide or peptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A or protein G chromatography to obtain the IgG fraction. At an appropriate time
5 after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler et al. (*Nature* 256:495-497, 1975), the human B cell hybridoma technique (Kozbor et al., *Immunol. Today* 4:72, 1983), the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer*
10 *Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985), or trioma techniques. The technology for producing hybridomas is well known (see, generally, *Current Protocols in Immunology*, 1994, Coligan et al. (Eds.), John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide or epitope of interest, e.g., using a
15 standard ELISA assay.

Variants of the antibodies or antigen-binding fragments described herein can be prepared by introducing appropriate nucleotide changes into the DNA encoding a human, humanized, or chimeric antibody, or antigen-binding fragment thereof described herein, or by peptide synthesis. Such variants include, for example, deletions, insertions, or substitutions of
20 residues within the amino acids sequences that make-up the antigen-binding site of the antibody or an antigen-binding domain. In a population of such variants, some antibodies or antigen-binding fragments will have increased affinity for the target protein. Any combination of deletions, insertions, and/or combinations can be made to arrive at an antibody or antigen-binding fragment thereof that has increased binding affinity for the target.
25 The amino acid changes introduced into the antibody or antigen-binding fragment can also alter or introduce new post-translational modifications into the antibody or antigen-binding fragment, such as changing (e.g., increasing or decreasing) the number of glycosylation sites, changing the type of glycosylation site (e.g., changing the amino acid sequence such that a different sugar is attached by enzymes present in a cell), or introducing new glycosylation
30 sites.

Antibodies disclosed herein can be derived from any species of animal, including mammals. Non-limiting examples of native antibodies include antibodies derived from humans, primates, e.g., monkeys and apes, cows, pigs, horses, sheep, camelids (e.g., camels

and llamas), chicken, goats, and rodents (e.g., rats, mice, hamsters and rabbits), including transgenic rodents genetically engineered to produce human antibodies.

Phage display (panning) can be used to optimize antibody sequences with desired binding affinities. In this technique, a gene encoding single chain Fv (comprising VH or VL) can be inserted into a phage coat protein gene, causing the phage to "display" the scFv on its outside while containing the gene for the protein on its inside, resulting in a connection between genotype and phenotype. These displaying phages can then be screened against target antigens, in order to detect interaction between the displayed antigen-binding sites and the target antigen. Thus, large libraries of proteins can be screened and amplified in a process called in vitro selection, and antibodies sequences with desired binding affinities can be obtained.

Human and humanized antibodies include antibodies having variable and constant regions derived from (or having the same amino acid sequence as those derived from) human germline immunoglobulin sequences. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation *in vivo*), for example in the CDRs.

In some embodiments, a covalent modification can be made to the antibodies, the antigen-binding fragments thereof, or the antigen-binding protein constructs (e.g., bispecific antibodies). These covalent modifications can be made by chemical or enzymatic synthesis, or by enzymatic or chemical cleavage. Other types of covalent modifications of the antibody or antibody fragment are introduced into the molecule by reacting targeted amino acid residues of the antibody or fragment with an organic derivatization agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues; or position 314 in Kabat numbering); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300,

due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. In some embodiments, to reduce glycan heterogeneity, the Fc region of the antibody can be further engineered to replace the Asparagine at position 297 with Alanine (N297A).

5 In some embodiments, to facilitate production efficiency by avoiding Fab-arm exchange, the Fc region of the antibodies was further engineered to replace the serine at position 228 (EU numbering) of IgG4 with proline (S228P). A detailed description regarding S228 mutation is described, e.g., in Silva et al. "The S228P mutation prevents in vivo and in vitro IgG4 Fab-arm exchange as demonstrated using a combination of novel quantitative
10 immunoassays and physiological matrix preparation." *Journal of Biological Chemistry* 290.9 (2015): 5462-5469, which is incorporated by reference in its entirety.

 In some embodiments, the methods described here are designed to make a bispecific antibody. Bispecific antibodies can be made by engineering the interface between a pair of antibody molecules to maximize the percentage of heterodimers that are recovered from
15 recombinant cell culture. For example, the interface can contain at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large
20 amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers. This method is described, e.g., in WO 96/27011, which is incorporated by reference in its entirety.

 In some embodiments, knob-into-hole (KIH) technology can be used, which involves
25 engineering CH3 domains to create either a "knob" or a "hole" in each heavy chain to promote heterodimerization. The KIH technique is described e.g., in Xu, Yiren, et al. "Production of bispecific antibodies in 'knobs-into-holes' using a cell-free expression system." *MAbs*. Vol. 7. No. 1. Taylor & Francis, 2015, which is incorporated by reference in its entirety. In some embodiments, one heavy chain has a T366W, and/or S354C (knob)
30 substitution (EU numbering), and the other heavy chain has an Y349C, T366S, L368A, and/or Y407V (hole) substitution (EU numbering). In some embodiments, one heavy chain has one or more of the following substitutions Y349C and T366W (EU numbering). The other heavy chain can have one or more the following substitutions E356C, T366S, L368A,

and Y407V (EU numbering). Furthermore, a substitution (-ppcpScp-->-ppcpPcp-) can also be introduced at the hinge regions of both substituted IgG.

Furthermore, an anion-exchange chromatography can be used to purify bispecific antibodies. Anion-exchange chromatography is a process that separates substances based on their charges using an ion-exchange resin containing positively charged groups, such as diethyl-aminoethyl groups (DEAE). In solution, the resin is coated with positively charged counter-ions (cations). Anion exchange resins will bind to negatively charged molecules, displacing the counter-ion. Anion exchange chromatography can be used to purify proteins based on their isoelectric point (pI). The isoelectric point is defined as the pH at which a protein has no net charge. When the $\text{pH} > \text{pI}$, a protein has a net negative charge and when the $\text{pH} < \text{pI}$, a protein has a net positive charge. Thus, in some embodiments, different amino acid substitution can be introduced into two heavy chains, so that the pI for the homodimer comprising two Arm A and the pI for the homodimer comprising two Arm B is different. The pI for the bispecific antibody having Arm A and Arm B will be somewhere between the two pIs of the homodimers. Thus, the two homodimers and the bispecific antibody can be released at different pH conditions. The present disclosure shows that a few amino acid residue substitutions can be introduced to the heavy chains to adjust pI.

Treatment methods

The present disclosure relates to use of the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit in the preparation of an inhibitor of TSLP.

The present disclosure relates to use of the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit in the preparation of a medicament for treating or preventing a type II or mixed allergic disease. Alternatively, the present disclosure relates to a method of treating or preventing a type II or mixed allergic disease, comprising administering to a subject in need thereof the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit. Alternatively, the present disclosure relates to the anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit for use in the treatment or prevention of a type II or mixed allergic disease.

In some embodiments, the type II and mixed allergic diseases are asthma, atopic dermatitis and the like.

The anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit can effectively inhibit the TSLPR/IL7R binding on the surface of TSLP and CTLL2 reporter gene cells, and inhibit the release of CCL-17 induced by TSLP, and can demonstrate therapeutic effects on
5 inflammation in the body of an animal.

The anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, the pharmaceutical composition, or the kit can also be used in the diagnosis of the presence of relevant antigens (e.g., TSLP) in a sample.

The anti-TSLP antibody or the antigen-binding fragment thereof, the
10 immunoconjugate thereof, or the pharmaceutical composition of the present disclosure can be prepared into any dosage form known in the art, such as injection, suspension, solution, powder, emulsion, spray, tablet, pill, capsule, granule, paste, suppository, gel, etc.

The anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, or the pharmaceutical composition of the present disclosure may
15 be suitable for intravenous, intramuscular, intraarterial, intraarticular, subcapsular, subarachnoid, intraorbital, intracardiac, subcutaneous, parenteral, intraperitoneal, intraspinal, intranasal or epidermal administration (such as via injection or infusion). The anti-TSLP antibody or the antigen-binding fragment thereof, the immunoconjugate thereof, or the pharmaceutical composition of the present disclosure may be prepared into a form of a sterile
20 aqueous solution, a microemulsion, a liposome, or a powder.

The methods described herein include methods for the treatment of disorders associated with cancer. Generally, the methods include administering a therapeutically effective amount of engineered antibodies, the antigen-binding fragments thereof, the antigen-binding protein constructs (e.g., bispecific antibodies), or the antibody drug
25 conjugates as described herein, to a subject who is in need of, or who has been determined to be in need of, such treatment.

As used herein, the term “cancer” refers to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include all types of cancerous growths or oncogenic processes,
30 metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The term “tumor” as used herein refers to cancerous cells, e.g., a mass of cancerous cells. Cancers that can be treated or diagnosed using the methods described herein include malignancies of the various organ systems. In some embodiments, the agents described herein are designed for treating or diagnosing a

carcinoma in a subject. The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An

5 “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term “sarcoma” is art recognized and refers to malignant tumors of mesenchymal derivation. In some embodiments, the cancer is a chemotherapy resistant cancer.

In one aspect, the disclosure also provides methods for treating a cancer in a subject, methods of reducing the rate of the increase of volume of a tumor in a subject over time, 10 methods of reducing the risk of developing a metastasis, or methods of reducing the risk of developing an additional metastasis in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of a cancer. In some embodiments, the treatment can result in the reduction of in the number, severity, and/or duration of one or more symptoms of the cancer in a subject.

15 In one aspect, the disclosure features methods that include administering a therapeutically effective amount of antibodies, the antigen-binding fragments thereof, the antigen-binding protein constructs (e.g., bispecific antibodies), or an antibody drug conjugate described herein to a subject in need thereof, e.g., a subject having, or identified or diagnosed as having, a cancer.

20 In some embodiments, the compositions and methods disclosed herein can be used for treatment of patients at risk for a cancer. Patients with cancer can be identified with various methods known in the art.

An effective amount can be administered in one or more administrations. By way of example, an effective amount of an antibody, an antigen-binding fragment, or an antibody- 25 drug conjugate is an amount sufficient to ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of an autoimmune disease or a cancer in a patient or is an amount sufficient to ameliorate, stop, stabilize, reverse, slow and/or delay proliferation of a cell (e.g., a biopsied cell, any of the cancer cells described herein, or cell line (e.g., a cancer cell line)) *in vitro*. As is understood in the art, an effective amount of an antibody, antigen-binding 30 fragment, or antibody-drug conjugate may vary, depending on, *inter alia*, patient history as well as other factors such as the type (and/or dosage) of the composition used.

Effective amounts and schedules for administering the antibodies, antibody-encoding polynucleotides, antibody-drug conjugates, and/or compositions disclosed herein may be determined empirically, and making such determinations is within the skill in the art. Those

skilled in the art will understand that the dosage that must be administered will vary depending on, for example, the mammal that will receive the antibodies, antibody-encoding polynucleotides, antibody-drug conjugates, and/or compositions disclosed herein, the route of administration, the particular type of antibodies, antibody-encoding polynucleotides, antigen-binding fragments, antibody-drug conjugates, and/or compositions disclosed herein used and other drugs being administered to the mammal.

EXAMPLES

Hereinafter, the present disclosure is further described with reference to examples, but the scope of the present disclosure is not limited thereto. Those skilled in the art can make any adjustments, combinations, or modifications to each embodiment and example of the present disclosure without departing from the spirit or concept of the present disclosure, and the resulting solutions still fall within the scope of protection of the present disclosure.

Example 1. Proof of Concept

In order to verify the stronger blocking and inhibitory effects on type II immunity brought by simultaneously blocking IL-4R α and TSLP, this example employed two different functional experiments.

The production of chemokine CCL-17 from human PBMCs stimulated by IL-4/IL-13/TSLP mixed cytokines

Chemokine 17, also known as thymus and activation regulated chemokine (TARC, CCL17, or CCL-17), is mainly produced by endothelial cells, keratinocytes, and dendritic cells derived from monocytes, and expressed on epidermal keratinocytes, vascular endothelial cells, T cells, and dendritic cells, and recruits Th2 cells to the inflammation site by interacting with the chemokine receptor CCR4. The level of CCL-17 in the serum of healthy adults gradually decreases with age, and the concentration in the serum of healthy adults is below 450 pg/mL, while the level thereof in the serum of patients with atopic dermatitis is significantly increased. The concentration of CCL-17 in the serum of adult patients is about 1000-90000 pg/mL, which is 2-20 times higher than that of healthy individuals, and the serum level thereof is significantly positively correlated with disease recurrence.

This study utilized human peripheral blood mononuclear cells (PBMCs) to determine the release activity of CCL-17 induced by IL-4/IL-13 through IL-4R α and induced by TSLP

through TSLPR, as well as the synergistic effect among the three. Specifically, PBMCs (AllCell, #FPB004F-C) were thawed, and resuspended in the RPMI 1640 cell culture medium (Gibco, #22400-071), and the cells in the culture medium were added into wells of a 96-well plate at 4×10^5 cells per well. Different concentrations of IL-4 (R&D, #204-IL-050), IL-13 (R&D, #213-ILB-100/CF), and TSLP (Acro Biosystems, #TSP-H52Hb) were added to the cells. Then, the cells were cultured under the condition of 37°C and 5% CO₂ for 24 hours. The cell culture supernatant was collected and the release of expressed CCL17 in the supernatant was detected using the CCL17/TARC ELISA kit (R&D, #SDN00), and a dose-effect curve was plotted. As shown in **FIG. 1A**, stimulation of PBMCs with TSLP alone induced the release of CCL-17 at a very low concentration (~ 0.01 nM), and quickly reached a plateau, but the overall release was not high (< 100 pg/mL). However, when IL-4 was administered alone, the release of CCL-17 significantly increased with an increasing concentration of IL-4, and did not reach a plateau within the tested range (0.0001-1 nM). Under the stimulation of IL-13 alone, the release of CCL-17 reached a plateau at about 0.3 nM, but the overall release was not as high as IL-4, with a plateau of about 100 pg/mL. When the three cytokines were mixed for cell treatment, they showed a significant synergistic effect on the increased release of CCL-17 at lower concentrations (0.1-0.3 nM).

To better simulate the release of CCL-17 under physiological disease conditions, we then evaluated the inhibitory effect of the control IgG1 (whose heavy and light chain amino acid sequences are set forth in SEQ ID NOs: 72-73, respectively), an anti-TSLP antibody, an anti-IL-4R α antibody, and a combination of an anti-TSLP antibody and an anti-IL-4R α antibody on CCL-17 release, under the stimulation of the concentration with the strongest synergistic effect of mixed cytokines (0.3 nM) as determined above.

As shown in **FIG. 1B**, the inhibitory effect of the anti-IL-4R α antibody (Dupilumab) on CCL-17 release was significantly weaker than that of the anti-TSLP antibody (Tezepelumab) and other test groups. The combination group of both the anti-TSLP antibody and the anti-IL-4R α antibody showed a good inhibitory effect under the experimental conditions, and the CCL-17 release function was completely blocked at a lower concentration (0.06 nM), which was superior to the monotherapy groups.

The release test of IL-5 and IL-13 mediated by naïve T cells differentiated with TSLP-DC

TSLP is a pleiotropic cytokine produced by epithelial cells after being stimulated by foreign antigens, which can act on various immune cells, such as dendritic cells (DCs), T

cells, B cells, neutrophils, mast cells, eosinophils, and innate lymphoid cells, and promote their maturation. As shown in **FIG. 2A**, TSLP can strongly activate dendritic cells in allergic inflammation, which then drive naïve T cells to develop directionally into inflammatory effector Th2 cells and release a large amount of Th2 cytokines, e.g., IL-4, IL-5, and IL-13.

5 The IL-4 released by naïve T cells can further promote the maturation and differentiation of T cells into Th2 cells, ultimately mediating the massive production of Th2 cells and inducing local inflammatory reactions. For example, when skin epidermal cells are stimulated by antigens, inflammatory reactions may occur, mediating the onset of atopic dermatitis; when lung epithelial cells are stimulated by antigens, a large number of inflammatory reactions
10 may be caused, inducing asthma; after being stimulated by antigens, the epithelial cells of the digestive tract may mediate inflammatory reactions, leading to eosinophilic esophagitis and other disorders. Based on the above scientific mechanisms, this study used TSLP-mediated DC maturation to differentiate naïve T cells directionally into Th2 cells, and the differentiation function of TSLP was determined by detecting its release of Th2 cytokines.

15 The specific experimental process was as follows. EasySep™ Human Myeloid DC Enrichment Kit (Stemcell, #19061) was used to isolate and purify initial myeloid DCs (mDCs) from human peripheral blood mononuclear cells (PBMCs). The obtained mDCs were inoculated into a 96-well cell culture plate at a cell density of 2×10^5 per well, and cultured at 37°C for 24 hours after being added with 50 ng/mL of human TSLP protein (Acro
20 Biosystems, #TSP-H52Hb). Stimulated mature mDCs were collected and washed twice with $1 \times$ PBS. EasySep™ Human Naïve CD4⁺ T Cell Isolation Kit (Stemcell, #19555) was used to isolate and extract CD4⁺ naïve T cells from PBMCs derived from another subject. The isolated naïve T cells and mature mDCs were mixed at a cell number ratio of 5:1 and inoculated in a 96-well cell culture plate. The cells were co-cultured at 37°C for 7 days. After
25 the co-culture, the cells were collected, stimulated with CD3/CD28 beads (Thermo, #11132D), and cultured at 37°C for 24 hours. Finally, the cell culture supernatant was collected. The release levels of IL-5 and IL-13 secreted by cells in the supernatant were detected using the corresponding ELISA kits (Mabtech, #3490-1H-20 for IL-5 detection and Mabtech, #3471-1H-20 for IL-13 detection, respectively). As shown in **FIG. 2B**, DCs
30 stimulated with TSLP were co-cultured with naïve T cells, which can significantly enhance the release of Th2 cytokines (e.g., IL-5/IL-13). In contrast, DCs stimulated with TSLP alone or CD4⁺ T cells alone only partially released or did not release IL-5 or IL-13.

As shown in **FIG. 2C**, for the release of IL-5, the inhibition levels of each test group were comparable, and the inhibition level of the anti-TSLP antibody (Tezepelumab) was

lower at low dose levels, indicating that inhibiting TSLP alone cannot completely inhibit the differentiation and maturation function of naïve T cells mediated by DCs. However, As shown in **FIG. 2D**, for the release of IL-13, administration of anti-IL-4R α antibody (Dupilumab) or the anti-TSLP antibody alone was almost unable to inhibit its release, while the combination administration of the anti-IL-4R α antibody and the anti-TSLP antibody could significantly inhibit its release in a dose-dependent manner. The above data demonstrated that simultaneously blocking TSLP and IL-4R α can significantly inhibit the effects of monocytes, especially antigen-presenting cells such as dendritic cells, on the activity, recruitment, and release of related cytokines and chemokines of Th2 cells. In addition, it was contemplated that an anti-IL-4R α /TSLP bispecific antibody may exert stronger efficacy in Th2-mediated inflammatory diseases.

Example 2. Molecular Structure Selection of Bispecific Antibodies

Design of Bispecific Molecular Structures

After identifying that the dual blockade of TSLP and IL-4R α has a stronger therapeutic effects on Th2- mediated inflammation than monoclonal antibodies, this study tested the effects of different molecular forms on biological functions by using benchmark sequences (the heavy and light chain amino acid sequences of Dupilumab are set forth in SEQ ID NO: 68 and SEQ ID NO: 69, respectively; the heavy and light chain amino acid sequences of Tezepelumab are set forth in SEQ ID NO: 65 and SEQ ID NO: 66, respectively). As shown in **FIG. 3**, three different forms of test molecules F-1, F-2, and F-3 (anti-IL-4R α -TSLP trap, 2+2, and 1+1, respectively) were constructed in the present disclosure for molecular form selection. In F-1, TSLPR and IL7Ra (the amino acid sequence of TSLPR is set forth in SEQ ID NO: 70; the amino acid sequence of IL7Ra is set forth in SEQ ID NO: 71) were linked to the C-terminal of the heavy chain of Dupilumab via (G₄S)₅, and the corresponding encoding gene sequences were synthesized and cloned into a pcDNA3.1 vector using a homologous recombinase from Nanjing Vazyme Biotech Co., Ltd. (Exnase[®]II, #C112-01), to obtain the corresponding heavy chain plasmids, and the light chain encoding gene sequence of Dupilumab was cloned into a pcDNA3.1 vector using the homologous recombinase to obtain the corresponding light chain plasmids. In F-2, Teze_scFV (the amino acid sequence of Teze_scFV is set forth in SEQ ID NO: 67) was linked to the C-terminal of the heavy chain of Dupilumab via (G₄S)₅, and the corresponding encoding gene sequence was synthesized and cloned into a pcDNA3.1 vector using the

homologous recombinase to obtain the corresponding heavy chain plasmid. The encoding gene sequence of the light chain of Dupilumab was cloned into a pcDNA3.1 vector using the homologous recombinase to obtain the corresponding light chain plasmid. In F-3, the encoding gene sequences of the heavy chain of Dupilumab and the heavy chain of Tezepelumab were cloned into the pcDNA3.1 vector using the homologous recombinase to obtain the corresponding heavy chain plasmids. The encoding gene sequences of the light chain of Dupilumab and the light chain of Tezepelumab were cloned into the pcDNA3.1 vector using the homologous recombinase to obtain the corresponding light chain plasmids.

10 *Bispecific antibody expression*

Expi293F cells were transfected with the above plasmids and the target proteins were obtained through purification. The specific operation was as follows:

Expi293F cells (purchased from Gibco) were cultured in the Expi293F medium (Gibco, #A14351-01), and the cell density (viability greater than 95%) was measured the day before transfection, and adjusted to 3×10^6 cells/mL with the fresh Expi293F medium for further culture. On the day of transfection, the cell density was adjusted to 3×10^6 cells/mL.

One tenth (1/10) of the final transfection volume of Opti-MEM medium (Gibco, #31985-070) was used as the transfection buffer. Each group of plasmids to be transfected was added thereto at 1 mg/L, with a molar ratio of light chain plasmid(s) to heavy chain plasmid(s) at 1:1. The plasmids were mixed well, into which PEI Max (Polysciences Inc., #24765-1) was added with a DNA: PEI mass ratio of 1:3, mixed well and incubated at room temperature for 20 minutes. Then the mixture was gently poured into Expi293F cell suspension under shaking, and the cells were placed on a shaker for culture under the conditions of 8% CO₂, 36.5°C, and 120 rpm.

After 16-18 hours of culturing, 2% (v/v) of Feed (100 g/L Phytone Peptone+100 g/L Difco Select Phytone) with a concentration of 200 g/L, a glucose solution with a final concentration of 5 g/L, and Valproic acid sodium salt (Merk, #P4543-100G) with a final concentration of 2.2 mM were added into the cell suspension. After gently mixing, and the culture was continued at 8% CO₂, 36.5°C, and 120 rpm for 7 days before the sample was collected by centrifugation. After centrifugation, a filtration was performed with a disposable vacuum filtration device of 0.22 μm pore size. An affinity capture was performed against the antibody using the HiTrap[®] MabSelect Prisma (GE Healthcare, #17549853) affinity chromatography column. Before purification, the pipeline and affinity chromatography

column were cleaned with 10-20 times the column volume of 0.1 M NaOH, and then washed with 10-20 times the column volume of distilled water. The chromatography column was then equilibrated with 5 times the column volume of 1 × PBS (Gibco). Afterwards, the filtered cell feed solution was loaded to the chromatography column, and then the chromatography column was washed with 10 times the column volume of 1 × PBS to remove non-specific binding proteins. The chromatography column was rinsed with 5 times the column volume of elution buffer (100 mM sodium citrate, pH 3.5), and the eluate was collected, the pH thereof was adjusted to 6.0 with 2 M Tris, filtered for sterilization, and sent to test by size exclusive chromatography (SEC) to confirm that the protein purity met the requirements.

Validation of bispecific antibody activity

This study used CHO cells overexpressing IL-4R α to test the blocking function of the above prepared bispecific antibodies against the IL-4R α /IL-4/IL-13 pathway. The specific experimental procedure was as follows: the cDNA encoding human IL-4R α was cloned onto the pCHO1.0 vector (Invitrogen) and transfected into CHO-S cells (Invitrogen) to produce CHO-S cells overexpressing human IL-4R α (CHO-S-human IL-4R α). The cells were counted and diluted to 2×10^6 cells/mL, added at 100 μ L/well to a U-shaped 96-well plate, centrifuged at 500 g for 5 minutes to remove the cell culture medium. The samples to be tested (each bispecific antibody) were diluted with FACS buffer at an initial concentration of 20 nM at a three-fold ratio. A total of 12 concentration gradients were prepared, and 50 μ L of each was added to the cells of the 96-well plate and mixed well. Biotinylated Human IL-4 protein (Acro Biosystems, #IL4-H82E0) was prepared at 50 ng/mL, and added at 50 μ L per well to the U-shaped plate. The cells were resuspended and incubated at 4°C for 30 minutes. Centrifugation was performed at 500 g for 5 minutes to remove the supernatant, and the cells were washed twice with FACS buffer. Centrifugation was performed at 500 g for 5 minutes to remove the FACS buffer, and 100 μ L of PE-Streptavidin secondary antibody (BioLegend, #554061) (diluted at 1:200 in FACS buffer) was added to each well, followed by an incubation at 4°C in dark for 30 minutes. After the incubation, centrifugation was performed at 500 g for 5 minutes to remove the supernatant, and the cells were washed three times with FACS buffer. Cells was resuspended in 200 μ L FACS buffer and detected by a flow cytometer.

The blocking function of each bispecific antibody against the TSLP/TSLPR pathway was evaluated using CTLL2-mCD127-hTSLPR-stat5-Luc2 reporter gene cells. The cDNA

encoding human TSLPR and IL7R was cloned onto the Plenti-IRES-Neo vector (Invitrogen), which was transfected into CTLL2-stat5-Luc2 cells (Invitrogen) to produce CTLL2-stat5-Luc2 cells overexpressing human TSLPR/IL7R. CTLL2-mCD127-hTSLPR-stat5-Luc2 reporter gene cells were cultured in the IMDM medium (Gibco, #12440-053) containing 10% FBS, 1% NEAA (Gibco, #11140-050), 400 µg/mL Hygromycin B (Invitrogen, #10687010), 500 µg/mL GENETICIN (Gibco, #10131-027), and 30 ng/mL rhuIL-2 (R&D, #202-IL). The cells were washed twice with the IMDM medium containing 10% FBS, 1% NEAA, and 0.5 ng/mL rhuIL-2, added to a 96-well plate at 1×10^5 cells per well, and then cultured overnight under the condition of 37°C and 5% CO₂. The antibody samples and the positive control antibody Tezepelumab (initial concentration of antibodies was 200 nM, which was diluted down 10 gradients at a three-fold ratio) were pre-incubated with 1 nM TSLP (Acro Biosystems, # TSP-H52Hb) at room temperature for 1 hour, respectively, added to the cells, and then cultured under the condition of 37°C and 5% CO₂ for 6 hours. After the culture was completed, Bio-Lite Luciferase Assay System (Vazyme, #DD1201-03) was used to detect the luminescence signal produced by the cells, and a dose-effect curve was plotted.

The results showed that the bivalent form of anti-IL-4Rα antibody had significantly better blocking activity than the monovalent anti-IL-4Rα antibody, while the monovalent anti-TSLP molecule could achieve the blocking activity completely comparable to the bivalent form (as shown in **FIGS. 4A-4B**, wherein F-1, F-2, and F-3 are bispecific antibodies of anti-IL-4Rα-TSLP trap, 2+2, and 1+1 forms, respectively), while F-1 is a TSLP trap that blocked TSLP function, but its activity was not superior to that of anti-TSLP antibody Tezepelumab (as shown in **FIG. 4B**). Based on the above data, the 2+2 Morris format (IgG-ScFv) (Coloma, MJ, Morrison, SL, *Nat Biotechnol* 1997; 15: 159–63; Siwei Nie *et al.*, *Antib Ther.* 2020; 3(1): 18–62.) was selected as the final molecular form. However, considering that some antibodies may experience a decrease in activity when transformed into a scFv form, the TSLP antibody screening was conducted in the form of scFv, so as to obtain clones with high blocking activity.

Example 3. Preparation of Anti-IL-4Rα Antibody from Hybridoma and Humanization

30 Screening of anti-IL-4Rα antibody 10H4.6

The hybridoma technology was used in this study, involving using extracellular segment protein of human IL-4Rα (SinoBiologica, #10402-H08H) to immunize Balb/c mice (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.). When the

serum titer met the requirements, the spleens of the mice were isolated to prepare a B lymphocyte suspension, which was electro-fused with SP2/0 myeloma cells (ATCC).

The supernatant of the hybridoma was collected to screen for hybridoma cells specifically expressing the anti-IL-4R α antibody through fluorescence-activated cell sorting (FACS). The supernatant of positive clones was collected for the blocking experiment of IL-4R α and its ligand IL-4 at the cellular level, and the clones with strong blocking function were further tested for cell proliferation experiment using TF-1. One clone 10H4 was eventually obtained, which exhibited good blocking effects on IL-4 and IL-13.

Gene sequences of the antibody light and heavy chains were extracted from the hybridoma candidate clone 10H4, and constructed into the human-mouse chimeric antibody Ch10H4.6 according to the following steps: taking about 5×10^6 cells from each freshly cultured cell line, extracting RNA (Macherey-Nagel, #740984.250); obtaining cDNA by reverse transcription using PrimeScript II 1st Strand cDNA Synthesis Kit (Takara); designing upstream primers based on the base sequence located in the FR1 region at 5' end, and designing downstream primers based on the base sequence located in the constant region or FR4 region of the antibody to amplify gene fragments of the light chain and heavy chain variable regions in the antibody; linking them into the T vector (Mighty TA-cloning Kit, Takara), picking single clones for sequencing, and performing the analysis and alignment of the sequencing results using MEGA7 software.

After alignment, for the clones with the correct and paired variable region sequences of the light and heavy chains of the antibody, the gene fragments of the variable regions of the light and heavy chains thereof were linked into the pcDNA3.1 vector using the homologous recombinase (Exnase[®] II, #C112-01) from Nanjing Vazyme Biotech Co., Ltd., respectively. In particular, the heavy chain constant region was IgG1 LALA subtype (set forth in SEQ ID NO: 47), and the light chain constant region was human κ chain constant region (set forth in SEQ ID NO: 48). Expression plasmids for the light and heavy chain antibodies were obtained.

Then, the light chain plasmid and the heavy chain plasmid of the same antibody were mixed in a molar ratio of 1:1, and they were transfected into the 293F cells using polyethylene imine (PEI) (Polysciences, #23966). After 5-7 days of culturing, when the cell viability was below 60%, the cell culture supernatant was collected and purified using the Protein A affinity column to obtain the monoclonal antibody.

The activity of the final candidate molecule is shown in Table 2, where the blocking activity of the chimeric antibody Ch10H4.6 was comparable to that of the control antibody

Dupilumab.

Table 2: Inhibition of IL-4Rα chimeric antibody on IL-4/IL-13-induced TF-1 proliferation

Antibody	IC50 in TF-1 proliferation assay (nM)	
	Human IL-4	Human IL-13
Dupilumab	0.014	0.047
Ch10H4.6	0.016	0.010

Humanization of the anti-IL-4Rα antibody 10H4.6

5 Based on the software of Discovery Studio and PyMOL, the chimeric antibody Ch10H4.6 obtained via the screening above was humanized through the following steps: determining the CDR loop structure; finding the closest homologous sequences for each V/J region of the heavy and light chains in the human germline sequence database; screening for the most matched human germline sequences with the heavy and light chains, as well as the

10 lowest amount of reverse mutations; constructing the CDR regions of the chimeric antibody onto the human framework regions; identifying the amino acid positions in the framework regions that play a role in maintaining CDR functions by using sequence and structural features; performing a reverse mutation (returning to the input amino acid type) at the identified important sequence position(s); and synthesizing genes and preparing proteins.

15 After obtaining the protein, the equilibrium dissociation constants (K_D) of the chimeric antibody Ch10H4.6 and humanized antibody Hz10H4.6 binding to IL-4Rα were determined using the bio-layer interferometry technology with an Octet Red96 (ForteBio), and Dupilumab (DUP) was used as a control. BLI experiments was carried out according to existing methods (Estep, P *et al.*, High throughput solution Based measurement of antibody-antigen affinity and epitope binning. MAb, 2013, 5(2): pp. 270-8).

20 Briefly, AHQ (Pall, #1502051) sensor was equilibrated in the analysis buffer for 30 minutes, followed by a baseline measurement for 60 seconds. The purified antibody obtained as described above was immobilized on AHQ sensor. Then the sensor with loaded antibodies was exposed to human IL-4Rα or cynomolgus IL-4Rα, and the sensor was transferred to the

25 analysis buffer for dissociation rate measurement. The Octet Analysis Software was used to analyze K_D values. The test results of antibody affinity are shown in Table 3.

Table 3: Dissociation constant (K_D ; unit: M) of antigen-antibody binding detected by ForteBio

Antibody	Human IL-4Rα	Cynomolgus IL-4Rα
Ch10H4.6	1.76E-09	N.B.

Hz10H4.6	2.93E-09	N.B.
DUP	1.41E-09	N.B.

N.B.: no binding.

Example 4. Affinity Maturation of the Anti-IL-4R α Parent Antibody Hz10H4.6

Hz10H4.6 was engineered for achieving affinity maturation by using yeast display technology in this study. The main process included library construction, library screening, yeast cloning and identification, protein expression, monoclonal antibody property analysis, and *in vitro* function identification. Firstly, six CDRs in the light and heavy chains were mutated to construct six affinity-matured libraries, which were sorted using magnetic beads and flow cytometry. In the magnetic bead-based sorting, 0.3 nM Biotin-IL-4R α (Acro Biosystems, #ILR-H82E9) was used for screening the six libraries. In the flow cytometry-based sorting, an equilibrium screening method was used, and the antigen concentration was maintained at 0.3 nM. After multiple rounds of sorting, approximately 145 post-translational modification (PTM)-free molecules with high affinity at the yeast level were obtained. In order to further improve affinity, molecules from the aforementioned libraries were reconstructed by PCR to obtain a CDR combination library containing different mutants. The screening method for the combination library also used magnetic bead-based enrichment method and flow cytometry-based sorting method. In the magnetic bead-based enrichment method, the concentration of the antigen Biotin-IL-4R α was 0.3 nM. In the flow cytometry-based sorting method, the kinetic competition method was used with the addition of the parental antibody Hz10H4.6 at a concentration ratio of 1:3 to 1:10, i.e., 1 nM Biotin-IL4R α : 3 nM-10 nM Hz10H4.6 IgG. After multiple rounds of sorting and yeast monoclonal identification, molecules with relatively high MFI were selected as candidate molecules. About 200 candidate IgG molecules were constructed for expression and identification.

Preparation of IgG protein

After monoclonal analysis, clones with higher MFI fold increase were selected as candidate molecules, and yeast plasmids were extracted. Yeast plasmids were extracted using a kit (Tiangen, # DP112-02). Primers were design upstream of VH and VL, and downstream of CH1 and CL. The plasmids extracted from yeast were used as templates, and the resultant was linked into the pcDNA3.1 vector using the homologous recombinase (Exnase[®] II, #C112-01) from Nanjing Vazyme Biotech Co., Ltd. In particular, the heavy chain constant region was the IgG1 LALA subtype, and the light chain constant region was human κ chain constant region. Expression plasmids for light and heavy chains were obtained.

Then, the light chain plasmid and the heavy chain plasmid of the same antibody were mixed in a molar ratio of 1:1, and they were transfected into the 293F cells using polyethylene imine (PEI) (Polysciences, #23966). After 5-7 days of culturing, when the cell viability was below 60%, the cell culture supernatant was collected and purified using the
5 Protein A affinity column to obtain the monoclonal antibody.

Affinity detection

The affinity between the affinity-matured antibody molecule and human IL-4R α (SinoBiological, #10402-H08H) was determined using surface plasmon resonance (SPR).
10 Specifically, immobilization of anti-human Fc IgG: channels 1 and 2 of CM5 chip were selected for immobilization. 50 mM N-hydroxysuccinimide (NHS) and 200 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were freshly mixed well, and activated at a flow rate of 10 μ L/min for 420 seconds. Then, the anti-human Fc IgG was diluted in 10 mM sodium acetate (pH 5.0) to a diluted concentration of 20 μ g/mL. It was
15 injected into channels 3 and 4 of the chip to covalently couple the protein to the surface of the chip channels, with a coupling amount of approximately 8000-10000 RU. Then it was injected into 1 M ethanolamine at a flow rate of 10 μ L/min for 420 seconds to block the remaining activation sites. The coupled protein chip was equilibrated at a flow rate of 10 μ L/min.
20 Each cycle of affinity detection between affinity-matured candidate molecule antibodies and human IL-4R α included capturing antibodies, binding antigens, and chip regeneration. Firstly, the candidate molecule antibody was diluted to 2 μ g/mL, and captured in channel 4 of the chip at a flow rate of 10 μ L/min for 30 seconds. Then, the antigen human IL-4R α was diluted in a 2-fold concentration gradient (0, 0.5, 1, 2, 4, 8, and 16 nM) and
25 injected into channels 3 and 4 of the chip from the low to high concentration. The binding time of human IL-4R α was 180 seconds, and the dissociation time was 1200 seconds. Finally, the chip was regenerated by injecting 10 mM Glycine (pH 1.5) for 30 seconds. The test results of antibody affinity are shown in the following table.

Through comprehensive analysis from multiple aspects, four candidate molecules
30 were selected, namely H6, 1222D5 (hereinafter referred to as D5), 1222E2 (hereinafter referred to as E2), and 1180C3 (hereinafter referred to as C3). The affinity parameters of candidate molecules are shown in Table 4, which showed that the affinities of the candidate molecules were comparable to that of Dupilumab.

Table 4: Affinity test results of candidate antibodies against IL-4Rα

Antibody	Human IL-4Rα		
	K _a (1/Ms)	K _d (1/s)	K _D (M)
Dupilumab	8.414E+5	3.687E-5	4.382E-11
C3	8.134E+5	3.770E-5	4.634E-11
E2	8.439E+5	5.526E-5	6.547E-11
D5	7.931E+5	2.940E-5	3.708E-11
H6	3.575E+5	<1.000E-5	2.797E-11

Functional analysis in vitro

TF-1 human erythroleukemia cell line was used to detect the inhibitory activity of the
5 above four affinity-matured antibodies on the cell proliferation induced by IL-4 or IL-13
through IL-4Rα. Specifically, TF-1 cells were cultured in the RPMI 1640 (Gibco, #22400-
071) medium containing 10% FBS and 5 ng/mL GM-CSF (Acro Biosystems, #GMF-H4214).
The cells were washed twice with the RPMI 1640 medium containing 2% FBS, and added
into the wells of a 96-well plate at 1.8×10^4 cells per well. The aforementioned four
10 affinity-matured antibody samples, negative control IgG1, and positive control antibody
Dupilumab were added to the cells, respectively. The initial concentration of the antibodies
was 100 nM, which was serially diluted by five-fold, for a total of 10 gradients. 2 ng/mL of
IL-4 (R&D, #204-IL-050) or 50 ng/mL of IL-13 (Acro Biosystems, #IL3-H52H4) was added
thereto, and then the cells were cultured under the condition of 37°C and 5% CO₂ for 72
15 hours. After the culture was completed, cell proliferation was detected by using CellTiter-
Glo® (Promega, #G7572), and the IC₅₀ of the inhibitory effect of the antibodies on cell
proliferation was calculated. The results are shown in Table 5, and the antagonistic activity of
antibodies were further analyzed. The results showed that all of the candidate molecules and
Dupilumab could effectively inhibit the TF-1 cell proliferation induced by IL-4 and IL-13,
20 with comparable activity.

Table 5: Inhibitory effect of the candidate antibodies against IL-4Rα on IL-4/13 induced TF-1
cell proliferation

Antibody	IC50 in TF-1 proliferation assay (nM)	
	Human IL-4	Human IL-13
Dupilumab	0.36	0.89
C3	0.09	1.25
E2	0.07	0.17
D5	0.20	0.25
H6	0.07	0.30

Example 5. Preparation of Anti-TSLP Antibody from Hybridoma and Humanization***Antibody screening of anti-TSLP antibody 11H7E11***

This study utilized hybridoma technology to alternately immunize Balb/c mice
5 (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.) with human
TSLP protein (SinoBiological, #16135-H08H) and monkey TSLP protein (ACRO, #3385a-
9CHF1-QH). When the serum titer met the requirements, the spleens of the mice were
isolated to prepare a B lymphocyte suspension, which was electro-fused with SP2/0 myeloma
cells (ATCC). The fused cells were diluted to 1×10^4 - 2×10^4 cells/mL with a selective
10 culture medium (the 1640 culture medium containing 20% FBS, $1 \times$ HAT), seeded onto a 96-
well plate, and 100 μ L of the cell suspension was added into each well. Seven (7) days after
fusion, the medium was replaced with the screening culture medium (the 1640 culture
medium containing 10% FBS, $1 \times$ HT), cultured for 10 days (or longer, depending on the cell
growth status), and then the supernatant was collected for testing.

15 Hybridoma cells specifically expressing anti-TSLP antibodies were screened by
ELISA. Experimental steps are described as follows. Human TSLP protein was diluted to a
concentration of 1 μ g/mL using citrate-buffered saline, added into a 96-well ELISA plate at
100 μ L per well, and incubated overnight at 4°C. On the next day, the coated ELISA plate
was washed three times with the PBST buffer ($1 \times$ PBS, 0.05% Tween[®] 20), and patted to
20 dry. The blocking solution ($1 \times$ PBS, 0.05% Tween[®] 20, 1% BSA) was added to the 96-well
ELISA plate and incubated at 37°C for 1 hour. After blocking, the plate was washed three
times with the PBST buffer and patted to dry. 100 μ L of the hybridoma supernatant was
added into wells of the 96-well ELISA plate, respectively, and incubated at room temperature
for 2 hour. Afterwards, the plate was washed three times with the PBST buffer and patted to
25 dry. HRP Goat anti-mouse IgG (BioLegend, #405306, dilution ratio 1:3000) was diluted with
the blocking solution, added to the 96-well ELISA plate at 100 μ L per well, and incubated at
room temperature in dark for 1 hour. The plate was then washed three times with the PBST
buffer and patted to dry. 100 μ L TMB chromogenic solution was added into the 96-well
ELISA plate for color development, and the termination solution was used to terminate the
30 color development. OD450 absorbance value was measured using a microplate reader. The
supernatant of positive clones was collected for blocking tests of TSLPR and its ligand TSLP,
the clones with strong blocking function were subcloned with the limited dilution method,
and monoclonal cells were picked, e.g., from single colonies.

Subcloning steps of the limited dilution method are described below. A 96-well plate was prepared, and 200 μ L of the culture medium was added into each well thereof. The culture medium was the same as the screening medium described above except that HAT was replaced with HT (Gibco, #11067-030). The above screened positive clone cells were
5 prepared into cell suspensions, and 100 μ L of which were added into each well in the first row of the plate and mixed well, respectively. Then, 100 μ L of the first-row cell suspensions were added to the wells in the second row and mixed well before 100 μ L was added to the next row; and the above steps were repeated. The 96-well plate was kept statically for 30 minutes, and then the cell number was counted under a microscope. 20 mL of culture medium
10 was added per corresponding volume of 100 cells, mixed well and seeded onto the plate, with 200 μ L per well. After one week, by observing under the microscope, wells containing monoclonal cells were identified and labeled.

When the cell confluence in each well reached 50% or higher, through testing according to the high-throughput screening method described above, the target positive
15 clones were selected, which were further tested for their blocking function against TSLP and TSLPR, and a candidate clone 11H7E11 was obtained with strong blocking activity.

Gene sequences of the antibody light and heavy chains were extracted from the hybridoma candidate clone 11H7E11, and constructed into the human-mouse chimeric antibody 11H7E11 using the same method as described in Example 3 (with the heavy chain
20 constant region set forth in SEQ ID NO: 47, and the light chain constant region set forth in SEQ ID NO: 48). Meanwhile, the scFv antibody was constructed by incorporating a linker sequence (set forth in SEQ ID NO: 63) at the C-terminal of the VH (set forth in SEQ ID NO: 43) of the chimeric antibody to link it with the N-terminal of the VL (set forth in SEQ ID NO: 44) of the chimeric antibody. His tag was added to the C-terminal of the VL for subsequent
25 purification. According to the method described in Example 2, the encoding gene of the scFv antibody was cloned into the pcDNA3.1 vector and transfected into Expi293F cells. Then, cell culture and protein purification were performed to obtain scFv antibodies.

Affinity detection

30 The affinities of humanized molecules with human TSLP and monkey TSLP were determined using bio-layer interferometry (BLI). Half an hour before the experiment, a suitable number of AHC sensors (Sartorius, #18-5060) were soaked in SD buffer ($1 \times$ PBS, 0.1% BSA, 0.05% Tween[®] 20). The antibody and antigen were both diluted to 100 nM. The

SD buffer, the antibody, and the antigen were added into 96-well black polystyrene microplates (Greiner, #655209), respectively. The ForteBio Octet® Red96e system was used for detection, the plate was arranged, and the sensor position was selected according to the sample position. The operating parameters set in the instrument were as follows: baseline equilibrium 120 seconds, sampling for immobilizing antibody 100 seconds, baseline equilibrium 120 seconds, binding antigen 100 seconds, and dissociation 120 seconds, with a rotation speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, ForteBio Octet® analysis software was used to analyze affinity and kinetic parameters. The test results of the antibody affinity detection are shown in Table 6.

Table 6: Test results of affinity for candidate clones of anti-TSLP antibodies

Clone ID	Affinity					
	Human TSLP			Monkey TSLP		
	K _D (M)	K _{on} (1/Ms)	K _{dis} (1/s)	K _D (M)	K _{on} (1/Ms)	K _{dis} (1/s)
11h7E11	3.02E-10	6.63E+05	2.00E-04	6.74E-08	2.90E+05	1.95E-02
11h7E11 scFv	4.59E-10	4.36E+05	2.00E-04	1.06E-07	2.26E+05	2.39E-02
Tezepelumab	2.96E-10	6.75E+05	2.00E-04	1.15E-09	5.45E+05	6.25E-04

In vitro functional analysis

The blocking activity of the 11H7E11 chimeric antibody and scFv antibody against TSLP/TSLPR was detected using CTLL2-mCD127-hTSLPR-stat5-Luc2 reporter gene cells. The specific experimental method is described in Example 2, and the results are shown in FIG. 5A. The results showed that 11H7E11 chimeric antibody had better blocking activity than Tezepelumab.

In addition, *in vitro* functional analysis was conducted using antibodies against TSLP to inhibit the secretion of the chemokine CCL-17 from human mDCs induced by TSLP.

Briefly, the EasySep™ Human Myeloid DC Enrichment Kit (Stemcell, #19061) was used to isolate and purify primary myeloid DCs from human peripheral blood mononuclear cells (PBMCs). The obtained mDCs were seeded into a 96-well cell-culture plate at a cell density of 5×10^5 per well. The gradient-diluted antibody samples (with an initial concentration of 300 nM) and the human TSLP protein (Acro Biosystems, #TSP-H52Hb) with a final concentration of 50 ng/mL were pre-incubated at 37°C for 45 minutes, and then added to each cell-culture well with mDCs, respectively. The mDCs were stimulated *in vitro* and cultured for 24 hours under the condition of 37°C and 5% CO₂. The cell culture supernatant was collected and the content of CCL-17 in the supernatant was detected using the ELISA kit (R&D, #SDN00) for CCL-17/TARC. As shown in FIG. 5B, the results showed that the

11H7E11 chimeric antibody and scFv antibody had stronger blocking activity than Tezepelumab.

Humanization of the antibody

5 Based on the software of Discovery Studio and PyMOL, a chimeric antibody Ch11H7 (with the heavy chain constant region as IgG1 LALA subtype (set forth in SEQ ID NO: 47), and the light chain constant region as human κ chain constant region (set forth in SEQ ID NO: 48)) screened from hybridoma was humanized through the following steps: determining the CDR loop structure; finding the closest homologous sequences for each V/J region of the heavy and light chains in the human germline sequence database; screening for the most matched human germline sequences with the heavy and light chains, as well as the lowest amount of reverse mutations; constructing the CDR regions of the chimeric antibody onto the human framework regions; identifying the amino acid positions in the framework regions that play a role in maintaining CDR functions by using sequence and structural features; performing a reverse mutation (returning to the input amino acid type) at the identified important sequence position(s); synthesizing genes and preparing proteins

 After obtaining the antibody, the affinity between humanized molecules and human TSLP was measured using bio-layer interferometry (BLI), and the specific method thereof is described in Example 3.

20 As shown from the affinity data in Table 7, the humanized antibody Hz11H7 maintained an affinity that was comparable to that of the chimeric antibody.

Table 7: Dissociation constant (K_D ; unit: M) of antigen-antibody binding detected by ForteBio

Clone ID	Human TSLP		
	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
Ch11H7	2.17E-10	9.20E+05	2.00E-04
Hz11H7	2.42E-10	8.27E+05	2.00E-04

25 **Example 6. Construction, Expression and Purification of a Bispecific Antibody**

Construction of the bispecific antibody

 A bispecific antibody that targets both IL-4R α and TSLP was prepared, with a molecular structure of “2+2” in FIG. 3, according to Example 2.

 The schematic diagram of the structural form of an exemplary bispecific antibody is shown in FIG. 6A, including peptide chain # 1 and peptide chain # 2. The linear structures of

peptide chain # 1 and peptide chain # 2 are shown in **FIG. 6B**.

Peptide chain # 1 sequentially comprises, from the N-terminal to C-terminal, the heavy chain variable region of the anti-IL-4R α antibody, the CH1 and Fc domains of human IgG1, and the single-chain variable fragment (scFv, where VH and VL are derived from the anti-TSLP antibody) linked by an artificially synthesized peptide. Peptide chain # 2 sequentially comprises, from the N-terminal to the C-terminal, the light chain variable domain of the anti-IL-4R α antibody and immunoglobulin CL domain (human κ chain constant region). In particular, the scFv, formed by linking the VH and VL of the anti-TSLP antibody through linker 2 ($4 \times \text{G}_4\text{S}$), is linked to the C-terminal of the Fc domain of the anti-IL-4R α monoclonal antibody through linker 1 ($6 \times \text{G}_4\text{S}$).

Expression of the bispecific antibody

The constructed light chain plasmids and heavy chain plasmids were transfected into Expi293F cells, respectively. The target protein was obtained through purification, and the specific operation thereof is described in Example 2.

Briefly, the Expi293F cell density was adjusted to 3×10^6 cells/mL. The plasmids to be transfected (with a 1:1 molar ratio of light chain plasmids to heavy chain plasmids) were mixed into the transfection buffer containing polyethylene imine (PEI), and then the DNA and PEI were mixed in a mass ratio of 1:3. The mixture was added into the Expi293F cell suspension after being incubated for 20 minutes. The shaker culture conditions were 8% CO₂, 36.5°C, and 120 rpm.

After culturing for 16-18 h, 2% (v/v) of 200 g/L Feed (100 g/L Phytone Peptone + 100 g/L Difco Select Phytone), a glucose solution with a final concentration of 5 g/L, and Valproic acid sodium salt with a final concentration of 2.2 mM (Merk, #P4543-100G) were added into the cell suspension, and the samples were collected after another 7 days of culture by centrifugation. After centrifugation, a disposable vacuum filtration device of 0.22 μm pore size was used for filtration. The antibody was captured using the HiTrap[®] MabSelect Prisma (GE Healthcare, #17549853) affinity chromatography column. Before purification, the pipeline and affinity chromatography column were cleaned with 10-20 times the column volume of 0.1 M NaOH. Then, the pipeline and chromatography column were washed with distilled water in a volume of 10-20 times the column volume, and $1 \times \text{PBS}$ (Gibco) was used to equilibrate the chromatography column in a volume of 5 times the column volume. Afterwards, the filtered cell solution was loaded to the chromatography column, and then $1 \times$

PBS was used to wash the chromatography column in a volume of 10 times the column volume to remove non-specific binding proteins. The chromatographic column was rinsed with the elution buffer (100 mM sodium citrate, pH 3.5) in a volume of 5 times the column volume, the eluate was collected, the pH thereof was adjusted to 6.0 with 2 M Tris, filtered to remove microbes, and sent to test via SEC, to confirm that the protein purity met the requirements.

Example 7. Binding Kinetics of Bispecific Antibodies to an Antigen

The affinities between the bispecific antibody candidate molecules and human IL-4Rα (SinoBiological, #10402-H08H) were determined using surface plasmon resonance (SPR).

Specifically, channels 1 and 2 of CM5 chip were selected for immobilization of anti-human Fc IgG. 50 mM N-hydroxysuccinimide (NHS) and 200 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were freshly mixed well, and activated at a flow rate of 10 μL/min for 420 seconds. Then, the anti-human Fc IgG was diluted in 10 mM sodium acetate (pH 5.0) to a diluted concentration of 20 μg/mL. It was injected into channels 3 and 4 of the chip to covalently couple the protein to the surface of the chip channels, with a coupling amount of approximately 8000-10000 RU. Then it was injected into 1 M ethanolamine at a flow rate of 10 μL/min for 420 seconds to block the remaining activation sites. The coupled protein chip was equilibrated at a flow rate of 10 μL/min.

Each cycle of affinity detection between affinity-matured candidate molecules and human IL-4Rα included capturing antibodies, binding antigens, and chip regeneration. Firstly, the candidate molecule antibody was diluted to 2 μg/mL, and captured in channel 4 of the chip at a flow rate of 10 μL/min with a capture time of 30 seconds. Then, the antigen human IL-4Rα was diluted in a 2-fold concentration gradient (0, 0.5, 1, 2, 4, 8, and 16 nM) and injected into channels 3 and 4 of the chip from the low to high concentration. The binding time of human IL-4Rα was 180 seconds, and the dissociation time was 1200 seconds. Finally, the chip was regenerated by injecting 10 mM Glycine (pH 1.5) for 30 seconds. The test results of antibody affinity were shown in the following table.

Table 8: Affinity test results of bispecific antibodies

Antibody	Human IL-4Rα		
	K _a (1/Ms)	K _d (1/s)	K _D (M)
Dupilumab	8.414E+5	3.687E-5	4.382E-11
H6-11H7	7.650E+5	6.864E-5	8.972E-11

C3-11H7	8.439E+5	5.526E-5	6.547E-11
E2-11H7	9.577E+5	7.852E-5	8.199E-11
D5-11H7	7.573E+5	2.509E-5	3.313E-11

The affinities of bispecific antibody molecules to human TSLP and monkey TSLP were determined using bio-layer interferometry (BLI). The experimental method is described in Example 5. The antibody affinity detection of bispecific antibodies at the anti-TSLP end was similar to that of the monoclonal antibody, and the dissociation constant (K_D) values of all of the four candidate molecules to TSLP were about $2.50E-10$ M, which was comparable to that of Tezepelumab.

Example 8. Optimization of Linker for Bispecific Antibodies

As shown in Example 6 above, bispecific antibodies in the present disclosure were constructed by linking a scFv, which is formed by linking the heavy chain variable region to the light chain variable region of the anti-TSLP antibody through linker 2 ($4 \times G_4S$), to the C-terminal of the Fc domain of IL-4R α monoclonal antibody molecule through linker 1 ($6 \times G_4S$).

To test the stability of candidate molecules, the candidate molecules were subjected to accelerated thermostability test at 40°C for two weeks and then subjected to capillary electrophoresis (CE) and mass spectrometry to detect aggregates, fragmentations, and post-translational modifications. According to the CE-SDS results (**FIG. 7A**), the proportion of aggregates increased by 5.1%, the proportion of fragmentation products increased by 9.2%, and the S residue in linker 1 ($6 \times G_4S$) underwent approximately 10% O-glycosylation. Based on the above results, it was concluded that the excessively long length of linker 1 may cause aggregation and fragmentation during the accelerated thermostability test at 40°C for 2 weeks. At the same time, it was contemplated that replacing the G_4S repeats in linker 1 to repeats of GGGGG (SEQ ID NO: 64) may avoid heterogeneity caused by O-glycosylation of S residues.

Therefore, the linker length was optimized by replacing $6 \times G_4S$ with GGGGG (G_5), $3 \times (G_5)$, and $4 \times (G_5)$, to generate H6-5G-hz11H7-2-scFv, H6-15G-hz11H7-2-scFv, and H6-20G-hz11H7-2-scFv, respectively. Biological functional testing *in vitro* was performed according to the method described in Example 2, and the results showed that different linker lengths had no effect on the blocking function (**FIGS. 8A-8B**). Afterwards, the molecule H6-11H7 with the shortest linker (H6-5G-hz11H7-2-scFv) was selected for a 40°C two-week

accelerated thermostability test, and the results showed that the molecule did not show any significant increase in aggregates or fragmentation products (**FIG. 7B**).

Example 9. Experiment of the Inhibition of Bispecific Antibodies on TF-1 Cell

5 Proliferation Induced by hIL-4 and hIL-13

TF-1 human erythroleukemia cell line was used to detect the inhibitory activity of the bispecific antibodies on the cell proliferation induced by IL-4 or IL-13 through IL-4R α . Specifically, TF-1 cells were cultured in the RPMI 1640 (Gibco, #2240-071) medium containing 10% FBS and 5 ng/mL GM-CSF (Acro Biosystems, #GMF-H4214). The cells
10 were washed twice with the RPMI 1640 medium containing 2% FBS and added to wells of a 96-well plate at 1.8×10^4 cells per well. The bispecific antibody samples (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv), negative control IgG1 (the heavy and light chain amino acid sequences thereof are set forth in SEQ ID NOs: 72 and 73, respectively) and positive control antibody Dupilumab were added to the
15 cells. The initial concentration of the antibodies was 100 nM, which was diluted 5-fold, in 10 gradients, into which 2 ng/mL IL-4 (R&D, #204-IL-050) or 50 ng/mL IL-13 (Acro Biosystems, #IL3-H52H4) was further added, and the culture was performed under the condition of 37°C and 5% CO₂ for 72 hours. After the culture, cell proliferation was detected by using CellTiter-Glo® (Promega, #G7572), and a dose-effect curve was plotted (**FIGS. 9A-**
20 **9B**), so as to analyze the antagonistic activity of antibodies. The results showed that all of bispecific antibodies and Dupilumab could effectively inhibit TF-1 cell proliferation induced by IL-4 and IL-13. Specifically, **FIG. 9A** shows the results of the inhibitory activity of bispecific antibodies and Dupilumab on IL-4-induced TF-1 cell proliferation, and the IC₅₀ of H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv inhibiting IL-4-induced TF-1 cell proliferation was about 0.32 nM, 0.16 nM, 0.09 nM,
25 and 0.17 nM, respectively, while the IC₅₀ of Dupilumab was about 0.27 nM. **FIG. 9B** shows the results of the inhibitory activity of bispecific antibodies and Dupilumab on IL-13-induced TF-1 cell proliferation, and the IC₅₀ values of H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv inhibiting IL-13-induced TF-1 cell
30 proliferation were about 0.31 nM, 0.27 nM, 0.15 nM, and 0.31 nM, respectively, while the IC₅₀ of Dupilumab was about 0.37 nM.

Example 10. CTLL2 Reporter Gene Cell Activity Experiment of Bispecific Antibodies

Binding to TSLP

CTLL2-mCD127-hTSLPR-stat5-Luc2 reporter gene cells were used to detect the effect of bispecific antibodies on blocking the binding of TSLP to TSLPR/IL7R receptors on the surface of CTLL2 cells. The test method is described in Example 2. The bispecific antibody samples (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv) and positive control antibody Tezepelumab (with the initial antibody concentration of 300 nM, diluted 3-fold, in total of 10 gradients) were pre-incubated with 1 nM TSLP (Acro Biosystems, #TSP-H52Hb) at room temperature for 1 hour, and added to the cells. The cells were cultured under the condition of 37°C and 5% CO₂ for 6 hours. After the culture was completed, Bio-Lite Luciferase Assay System (Vazyme, #DD1201-03) was used to detect the luminescence signals generated by the cells, and a dose-effect curve was plotted (FIG. 10), so as to analyze the antagonistic activity of antibodies. The results showed that all of the tested bispecific antibodies and Tezepelumab effectively inhibited the binding of TSLP to TSLPR/IL7R on the surface of CTLL2 reporter gene cells. According to the results in FIG. 10, the IC₅₀ values of H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv inhibiting the activity of the CTLL2 reporter gene cells were about 0.65 nM, 0.49 nM, 0.61 nM, and 0.51 nM, respectively, which were significantly lower than that of the control antibody Tezepelumab (with IC₅₀ of about 2.69 nM). The results indicate that the tested bispecific antibodies exhibited superior inhibition on the activity of the CTLL2 reporter gene cells by binding to TSLP, as compared to the control antibody Tezepelumab.

Example 11. Inhibition of Bispecific Antibodies on the Release of CCL-17 from PBMCs Induced by hIL-4, hIL-13, and hTSLP

PBMCs were used to determine the activity of bispecific antibodies (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv) to antagonize IL-4/IL-13-induced CCL-17 release through IL-4R α . Specifically, PBMC cells (AllCell, #FPB004F-C) were thawed, re-suspended with the RPMI 1640 cell culture medium (Gibco, #22400-071), and cells in the culture medium were added into the wells of a 96-well plate at 4×10^5 cells per well. Antibodies were added to the cells (antibody dilution method: initial concentration was 100 nM, diluted eight-fold in cell culture medium, 8 concentration gradients in total), and 0.03 nM of IL-4 (R&D, #204-IL-050) and 0.3 nM of IL-13 (R&D, #213-ILB-100/CF) were added thereto. Then, the cells were cultured under the condition of

37°C and 5% CO₂ for 24 hours, and the cell culture supernatant was collected and detected for the release of expressed CCL-17 in the supernatant using the CCL17/TARC ELISA kit (R&D, #SDN00), and a dose-effect curve was plotted so as to analyze the antagonistic activity of antibodies. As shown in **FIG. 11A**, the results showed that the bispecific antibodies inhibited the release of CCL-17 from PBMCs induced by IL-4/IL-13. The IC₅₀ of the control antibody Dupilumab inhibiting the release of CCL-17 from PBMCs induced by IL-4/IL-13 was about 0.09 nM, and the IC₅₀ of the Dupilumab and Tezepelumab combination group was about 0.07 nM. In contrast, the IC₅₀ of bispecific antibodies H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, D5-5G-hz11H7-2-scFv inhibiting the release of CCL-17 from PBMCs induced by IL-4/IL-13 were about 0.18 nM, 0.10 nM, 0.08 nM, and 0.14 nM, respectively.

The bispecific antibodies also significantly inhibited the release of CCL-17 from PBMCs induced by TSLP. The concentration of TSLP (Acro Biosystems, #TSP-H52Hb) added for detecting was 0.3 nM, and the remaining steps are described above. As shown in **FIG. 11B**, the bispecific antibodies H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, and D5-5G-hz11H7-2-scFv effectively inhibited the release of CCL-17 from PBMCs induced by TSLP, with the inhibitory activities (IC₅₀ thereof were about 0.12 nM, 0.07 nM, 0.08 nM, and 0.18 nM, respectively) significantly superior to those of the control antibody Tezepelumab, as well as Dupilumab and Tezepelumab combination group (IC₅₀ thereof were about 0.21 nM and 0.49 nM, respectively).

Likewise, the bispecific antibodies also showed a significant inhibitory effect on the release of CCL-17 from PBMCs simultaneously induced by IL-4, IL-13 and TSLP. The concentrations of IL-4, IL-13, and TSLP added for detecting were the same as the above test concentrations. As shown in **FIG. 11C**, the bispecific antibodies H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, and D5-5G-hz11H7-2-scFv effectively inhibited the release of CCL-17 from PBMCs induced by IL-4, IL-13 and TSLP, with the inhibitory activities (IC₅₀ thereof were about 0.57 nM, 0.22 nM, 0.25 nM, and 0.25 nM, respectively) significantly superior to those of the control antibody Dupilumab, as well as Dupilumab and Tezepelumab combination group (IC₅₀ thereof were about 3.07 nM and 0.58 nM, respectively).

The experimental results of the above Examples show that compared with the existing anti-IL-4R α monoclonal antibodies and anti-TSLP monoclonal antibodies, the bispecific antibodies of the present disclosure have comparable or superior effects to the currently approved drugs Dupilumab and Tezepelumab.

Example 12. The Therapeutic Effect of Bispecific Antibodies on Asthmatic Mice

A mouse asthma model was established as follows. IL-4/IL-4R α humanized transgenic mice (purchased from Biocytogen JiangSu Co., Ltd.) were selected, and injected intraperitoneally on days 0, 7, and 14 with 50 μ g ovalbumin (Sigma, #A5503) dissolved in 0.2 mL of solvent (saline containing 2% Al₂O₃ as an adjuvant), to sensitize the IL-4/IL-4R α humanized mouse. On days 21-24, 200 μ g of ovalbumin (OVA) dissolved in 1 $\times$ PBS was used daily to induce nasal sensitization and stimulation in mice after anesthesia.

This experiment included 7 groups in total, including the blank control group, model control IgG group, Dupilumab group, and bispecific antibody groups (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, and D5-5G-hz11H7-2-scFv, respectively), with 5 mice in each group (3 mice in the blank control group). The mice were administered intraperitoneally on days 20 and 23 (the blank control group and model control IgG group were injected with an equal amount of IgG antibodies (purchased from Equitech-Bio, #SLH56)), and the bronchoalveolar lavage fluid (BALF) of mice was collected on day 25 for flow cytometry detection.

BALF was centrifuged at 500 g for 5 minutes, washed twice with FACS buffer after the supernatant was discarded, and then the prepared antibodies (PE-Cy7 anti-CD45 (BioLegend, #103114), AF700 anti-Siglec F (BioLegend, #565183), APC anti-Ly6G (BioLegend, #127610), BUV395 anti-CD11b (BioLegend, #563553), AF488 anti-F4/80 (BioLegend, #123120), BV421 anti-Ly6C (BioLegend, #128032), BB700 anti-CD11C (BioLegend, #745852), PE Dazzel anti-MHC-II (BioLegend, #107648), BV605 anti-CD3 (BioLegend, #100237), and PE anti-B220 (BioLegend, #103208)) were added thereto, incubated at 4 $^{\circ}$ C for 30 minutes. The cells were washed twice with FACS buffer, and the number of different immune cells was detected using flow cytometry.

As shown in **FIGS. 12A-12D**, after sensitization and stimulation with OVA, the number of lymphocytes (CD45+%) in the model control IgG group was higher than that in the blank control group (**FIG. 12A**). Compared with the model control IgG group, the number of CD45 positive cells (CD45+%), as well as the numbers of eosinophils (**FIG. 12B**), monocytes (**FIG. 12C**), and neutrophils (**FIG. 12D**) were significantly reduced in the peripheral blood of mice in the Dupilumab and bispecific antibody groups.

Example 13. Pharmacokinetic Study of Bispecific Antibodies in Mice

The pharmacokinetics of bispecific antibodies were detected by intravenous injection (I.V.) in mice. Each group included 9 BALB/c mice weighing around 20 g. Each mouse was intravenously injected with 10 mg/kg of the bispecific antibodies, and blood samples were collected from the orbit at 5 minutes, 0.5 hour, 2 hours, 6 hours, 2 days, 4 days, 7 days, 14 days, and 21 days after a single dose. After natural coagulation of blood, serum was collected by centrifugation. The method for determining the serum drug concentration of antibodies is as follows. The antigen human IL-4R α -his protein (Acro Biosystems, #ILR-H5221) was diluted to 1 μ g/mL with a coating solution (prepared by dissolving one package of carbonate powder (Thermo, # 23282) in 400 mL of ultrapure water, mixing well, and making up to 500 mL), added to a 96-well microplate (Thermo, #442404) at 100 μ L per well, and incubated overnight at 4°C. After discarding the coating solution, the plate was washed 3 times with 1 $\times$ PBST. 200 μ L of blocking solution (PBST solution containing 2% BSA) was added to each well to block at room temperature for 1 hour. After discarding the blocking solution, the plate was washed 3 times with 1 $\times$ PBST, and the diluted mouse serum was added thereto. The plate was incubated at room temperature for 2 hours. After discarding the solution in the microplate, it was washed 5 times with 1 $\times$ PBST. The diluted Goat anti-human IgG-Fc-HRP (BETHYL, #A80-104P) was added thereto at 100 μ L per well, and incubated at room temperature for 1 hour. After discarding the solution in the microplate, it was washed 5 times with 1 $\times$ PBST. 100 μ L of TMB chromogenic solution (Solarbio, #PR1200) was added to each well, incubated for 5-10 minutes of color development, and 50 μ L of termination solution (Solarbio, #C1058) was added to each well for termination. The OD450nm and OD620nm values were measured using a microplate reader. The changes in blood drug concentration at different time points in the four groups of mice administered with bispecific antibodies (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, and D5-5G-hz11H7-2-scFv; administered to mice at 10 mg/kg) are shown in **FIG. 13**. The pharmacokinetic parameters were calculated using non-compartmental model by Excel PKSolver, and the main drug exposure parameters ($T_{1/2}$, C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, CL, and VSS) are shown in Table 9.

Table 9: Pharmacokinetic parameters of candidate molecules in mice

Antibody	C_{max} (μ g/mL)	AUC_{0-t} (μ g $\cdot$ h/mL)	$AUC_{0-\infty}$ (μ g $\cdot$ h/mL)	$MRT_{0-\infty}$ (h)	$T_{1/2}$ (h)	CL (mL/kg/h)	Vss (mL/kg)
H6-5G-hz11H7-2-scFv	205	14544	17026	237	193	0.59	139
C3-5G-hz11H7-2-scFv	196	9924	10208	116	95	1	114
E2-5G-hz11H7-2-scFv	189	5196	5326	75	68	1.88	140

D5-5G-hz11H7- 2-scFv	182	15526	16918	204	139	0.59	120
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Example 14. Construction and Pharmacokinetic Study of YTE Molecules

The site-directed mutagenesis of three sites in the Fc region of the bispecific antibodies (H6-5G-hz11H7-2-scFv, C3-5G-hz11H7-2-scFv, E2-5G-hz11H7-2-scFv, and D5-5G-hz11H7-2-scFv) in the above Examples was performed (M252Y/S254T/T256E (EU Numbering), i.e., YTE mutation) to further enhance their binding ability to human FcRn, so as to prolong their half-lives *in vivo*. Similar to the method described in Example 6, Freedom[®] CHO-S[®] kit (Invitrogen) was used to produce CHO-S cell lines expressing the above mutated antibodies. The transfected cells were subjected to two rounds of pressure screening to obtain a cell pool with high expression of antibodies. Afterwards, the cell pool was expanded through the culture to express a large number of antibodies, and the cell supernatant was collected and purified using a Protein A column to achieve a purity greater than 95% for the antibodies. The obtained antibodies were named as H6-11H7-YTE, D5-11H7-YTE, C3-11H7-YTE, and E2-11H7-YTE (the amino acid sequences of peptide chain # 1 thereof, as shown in **FIG. 6B**, are set forth in SEQ ID NOs: 74-77, respectively).

ForteBio was used to detect the affinity between the above antibodies and human FcRn. As expected, the YTE mutation increased the affinity by more than 10 times under specific conditions.

The pharmacokinetics of bispecific antibodies were detected by intravenous injection (I.V.) using FcRn humanized transgenic mice (purchased from Biocytogen JiangSu Co., Ltd.). Specifically, each group included 6 FcRn humanized mice weighing around 20 g. Each mouse was intravenously injected with 10 mg/kg of the bispecific antibody D5-5G-hz11H7-2-scFv or D5-11H7-YTE, and blood samples were collected from the orbit at 5 minutes, 2 hours, 6 hours, 24 hours, 48 hours, 4 days, 7 days, 14 days, 21 days, and 28 days after a single dose. After natural coagulation of blood, serum was collected by centrifugation.

The method for determining the serum drug concentration of antibodies is as follows. The antigen human IL-4Rα-His protein (Acro Biosystems, #ILR-H5221) was diluted to 1 μg/mL with a coating solution (prepared by dissolving one package of carbonate powder (Thermo, # 23282) in 400 mL of ultrapure water, mixing well, and making up to 500 mL), added to a 96-well microplate (Thermo, #442404) at 100 μL per well, and incubated overnight at 4°C. After discarding the coating solution, the plate was washed 3 times with 1× PBST. 200 μL of blocking solution (PBST solution containing 2% BSA) was added to each

well to block at room temperature for 1 hour. After discarding the blocking solution, the plate was washed 3 times with 1× PBST, and the diluted standard (D5-5G-hz11H7-2-scFv), quality control samples (standards with different dilution ratios, used for quality control among experiments) and samples to be tested (D5-11H7-YTE) were added thereto at 100 μL per well, and incubated at room temperature in dark for 2 hours. After discarding the solution in the microplate, it was washed 5 times with 1× PBST. The diluted Goat anti-human IgG-Fc-HRP (diluted in 1:80000, BETHYL, #A80-104P) was added thereto at 100 μL per well, and incubated at room temperature for 1 hour (avoiding light). After discarding the solution in the microplate, it was washed 5 times with 1× PBST. TMB chromogenic solution (Solarbio, #PR1200) was added at 100 μL per well for 5-10 minutes for color development at room temperature in dark, and termination solution (Solarbio, #C1058) was then added and shook gently for 10 seconds. The OD450nm and OD620nm values were measured within 30 minutes. The changes in blood drug concentration at different time points in mice administered with samples to be tested (administered to mice at 10 mg/kg) are shown in **FIG. 14**. The pharmacokinetic parameters were calculated using non-compartmental model by Excel PKSolver, and the main drug exposure parameters ($T_{1/2}$, C_{max} , AUC_{0-4} , $AUC_{0-\infty}$, CL, and VSS) are shown in Table 10.

Table 10. Pharmacokinetic parameters of D5-5G-hz11H7-2-scFv and D5-11H7-YTE molecules in FcRn humanized mice

ID	C_{max} (μg/mL)	AUC_{0-4} (μg*h/mL)	$AUC_{0-\infty}$ (μg*h/mL)	$T_{1/2}$ (h)	CL (mL/kg/h)	Vss (mL/kg)
D5-5G-hz11H7-2-scFv	162	3022	3032	118	3.3	151
D5-11H7-YTE	193	8819	9705	217	1.0	253

The other YTE molecules constructed in this Example (including H6-11H7-YTE, C3-11H7-YTE, and E2-11H7-YTE) exhibited pharmacokinetic properties that were comparable to those of D5-11H7-YTE.

OTHER EMBODIMENTS

Embodiment 1. An isolated anti-TSLP antibody or an antigen-binding fragment thereof, comprising: HCDR1 set forth in SEQ ID NO: 37, HCDR2 set forth in SEQ ID NO: 38, and HCDR3 set forth in SEQ ID NO: 39; and/or LCDR1 set forth in SEQ ID NO: 40, LCDR2 set forth in SEQ ID NO: 41, and LCDR3 set forth in SEQ ID NO: 42.

Embodiment 2. The anti-TSLP antibody or the antigen-binding fragment thereof according to embodiment 1, comprising: a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43 or 45; and a VL having at
5 least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44 or 46.

Embodiment 3. The anti-TSLP antibody or the antigen-binding fragment thereof according to embodiment 1 or 2, wherein compared with the amino acid sequence set forth in
10 SEQ ID NO: 43 or 45, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto mainly exist or all exist in the FR region; or compared with the amino acid sequence set forth in SEQ ID NO: 44 or 46, the different amino acids in the amino acid sequences with at least 80%, at least 85%, at least 90%, at least
15 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto mainly exist or all exist in the FR region.

Embodiment 4. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-3, comprising: (1) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or
20 higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43, and a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44; or (2) a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino
25 acid sequence set forth in SEQ ID NO: 45, and a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 46; wherein, compared with the amino acid sequence set forth in any one of SEQ ID NOs: 43, 44, 45, and 46, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least
30 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto all exist in the FR region.

Embodiment 5. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-4, comprising: (1) VH set forth in SEQ ID NO: 43,

and VL set forth in SEQ ID NO: 44; or (2) VH set forth in SEQ ID NO: 45, and VL set forth in SEQ ID NO: 46.

Embodiment 6. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5, wherein the anti-TSLP antibody or the antigen-binding fragment thereof is an anti-TSLP scFv.

Embodiment 7. The anti-TSLP antibody or the antigen-binding fragment thereof according to embodiment 6, wherein the anti-TSLP scFv comprises a linker, which comprises an amino acid sequence of (GGGGS)_n, n is an integer from 1 to 5, preferably n is 2, 3, or 4.

Embodiment 8. The anti-TSLP antibody or the antigen-binding fragment thereof according to embodiment 6 or 7, wherein the anti-TSLP scFv comprises: (1) VH set forth in SEQ ID NO: 43, VL set forth in SEQ ID NO: 44, and a linker set forth in SEQ ID NO: 63; or (2) VH set forth in SEQ ID NO: 45, VL set forth in SEQ ID NO: 46, and a linker set forth in SEQ ID NO: 63.

Embodiment 9. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5, wherein the anti-TSLP antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region and a light chain constant region, wherein the heavy chain constant region is selected from the constant regions of human IgG1, IgG2, IgG3, and IgG4, or the variants thereof, the light chain constant region is selected from the constant regions of human κ and λ chains, or the variants thereof.

Embodiment 10. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5 and 9, the anti-TSLP antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43 or 45, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44 or 46, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48.

Embodiment 11. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5 and 9-10, the anti-TSLP antibody comprises a heavy chain and a light chain, wherein (1) the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 43, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 44, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48; or (2) the heavy chain comprises a VH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 45, and a CH having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 47; and the light chain comprises a VL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 46, and a CL having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity to the amino acid sequence set forth in SEQ ID NO: 48; wherein, compared with the amino acid sequence set forth in any one of SEQ ID NOs: 43, 44, 45, and 46, the different amino acids in the amino acid sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or higher sequence identity thereto all exist in the FR region.

Embodiment 12. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5 and 9-11, the anti-TSLP antibody comprises a heavy chain and a light chain, wherein (1) the heavy chain comprises a VH set forth in SEQ ID NO: 43, and a CH set forth in SEQ ID NO: 47; and the light chain comprises a VL set forth in SEQ ID NO: 44, and a CL set forth in SEQ ID NO: 48; or (2) the heavy chain comprises a VH set forth in SEQ ID NO: 45, and a CH set forth in SEQ ID NO: 47; and the light chain comprises a VL set forth in SEQ ID NO: 46, and a CL set forth in SEQ ID NO:

48.

Embodiment 13. The anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-5 and 9-12, wherein the antigen-binding fragment is selected from a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, and nanobody.

Embodiment 14. A polynucleotide encoding the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13.

Embodiment 15. An expression vector, comprising the polynucleotide according to embodiment 14.

Embodiment 16. A host cell integrated with the polynucleotide according to embodiment 14 or the expression vector according to embodiment 15.

Embodiment 17. An immunoconjugate, comprising the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13.

Embodiment 18. A pharmaceutical composition, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13, or the immunoconjugate according to embodiment 17, and an optional pharmaceutically acceptable excipient.

Embodiment 19. A kit, comprising: the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13, the immunoconjugate according to embodiment 17, or the pharmaceutical composition according to embodiment 18.

Embodiment 20. Use of the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13, the immunoconjugate according to embodiment 17, the pharmaceutical composition according to embodiment 18, or the kit according to embodiment 19 in the preparation of an antibody or antigen-binding fragment thereof.

Embodiment 21. Use of the anti-TSLP antibody or the antigen-binding fragment thereof according to any one of embodiments 1-13, the immunoconjugate according to embodiment 17, the pharmaceutical composition according to embodiment 18, or the kit according to embodiment 19 in the preparation of a medicament for treating or preventing a disease or disorder (e.g., a cancer or autoimmune disease).

Embodiment 22. The use according to embodiment 21, wherein the disease or

disorder is type II and mixed allergic disease, e.g., asthma and/or atopic dermatitis.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other
5 aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that binds to TSLP (thymic stromal lymphopoietin) comprising:

5 a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2, and 3, wherein the VH CDR1 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR1 amino acid sequence, the VH CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR2 amino acid sequence, and the VH CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VH CDR3 amino acid sequence; and

10 a light chain variable region (VL) comprising CDRs 1, 2, and 3, wherein the VL CDR1 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR1 amino acid sequence, the VL CDR2 region comprises an amino acid sequence that is at least 80% identical to a selected VL CDR2 amino acid sequence, and the VL CDR3 region comprises an amino acid sequence that is at least 80% identical to a selected VL
15 CDR3 amino acid sequence,

wherein the selected VH CDRs 1, 2, and 3 amino acid sequences and the selected VL CDRs, 1, 2, and 3 amino acid sequences are one of the following:

(1) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 37, 38, 39, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth
20 in SEQ ID NOs: 40, 41, 42, respectively;

(2) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 86, 38, 39, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 40, 41, 42, respectively;

(3) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 25 117, 118, 119, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 120, 121, 122, respectively.

2. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 37, 38, 39,
30 respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 40, 41, 42, respectively, wherein the VH CDR1 is determined according to AbM definition, wherein the VH CDR2, VH CDR3, and VL CDRs 1, 2, 3, are determined according to Kabat definition.

3. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 86, 38, 39, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in
5 SEQ ID NOs: 40, 41, 42, respectively, according to Kabat definition.

4. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 117, 118, 119, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth
10 in SEQ ID NOs: 120, 121, 122, respectively, according to Chothia definition.

5. An antibody or antigen-binding fragment thereof that binds to TSLP comprising a heavy chain variable region (VH) comprising an amino acid sequence that is at least 90% identical to a selected VH sequence, and a light chain variable region (VL) comprising
15 an amino acid sequence that is at least 90% identical to a selected VL sequence, wherein the selected VH sequence and the selected VL sequence are one of the following:

- (1) the selected VH sequence is SEQ ID NO: 43, and the selected VL sequence is SEQ ID NO: 44; and
- (2) the selected VH sequence is SEQ ID NO: 45, and the selected VL sequence is
20 SEQ ID NO: 46.

6. The antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein the antibody or antigen-binding fragment specifically binds to human TSLP and/or monkey TSLP.
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7. The antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment is a human or humanized antibody or antigen-binding fragment thereof.

30 8. The antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the antigen-binding fragment is selected from a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, scFv, and nanobody.

9. An antibody or antigen-binding fragment thereof that binds to TSLP comprising a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 that are identical to VH CDR1, VH CDR2, and VH CDR3 of a selected VH sequence; and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 that are identical to VL CDR1, VL CDR2, and VL CDR3 of a selected VL sequence, wherein the selected VH sequence and the selected VL sequence are one of the following:

- (1) the selected VH sequence is SEQ ID NO: 43, and the selected VL sequence is SEQ ID NO: 44; and
- (2) the selected VH sequence is SEQ ID NO: 45, and the selected VL sequence is SEQ ID NO: 46.

10. An antibody or antigen-binding fragment thereof that cross-competes with the antibody or antigen-binding fragment thereof of any one of claims 1-9.

11. The antibody or antigen-binding fragment thereof of any one of claims 1-10, wherein the antibody or antigen-binding fragment thereof is a bispecific or a multi-specific antibody or an antigen-binding fragment thereof.

12. A nucleic acid comprising a polynucleotide encoding a polypeptide comprising:

- (1) an immunoglobulin heavy chain or a fragment thereof comprising a VH comprising CDRs 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 37, 38, 39, respectively; SEQ ID NOs: 86, 38, 39, respectively; or SEQ ID NOs: 117, 118, 119, respectively; and wherein the VH, when paired with a light chain variable region (VL) comprising the amino acid sequence set forth in SEQ ID NO: 44 or 46, binds to TSLP;
- (2) an immunoglobulin light chain or a fragment thereof comprising a VL comprising CDRs 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 40, 41, 42, respectively; or SEQ ID NOs: 120, 121, 122, respectively; and wherein the VL, when paired with a VH comprising the amino acid sequence set forth in SEQ ID NO: 43 or 45, binds to TSLP.

13. The nucleic acid of claim 12, wherein the VH when paired with a VL specifically binds to human TSLP.

14. The nucleic acid of claim 12 or 13, wherein the immunoglobulin heavy chain or

the fragment thereof is a human or humanized immunoglobulin heavy chain or a fragment thereof.

15 15. The nucleic acid of any one of claims 12-14, wherein the nucleic acid encodes a single-chain variable fragment (scFv), a bispecific or a multi-specific antibody or an antigen-binding fragment thereof.

16. The nucleic acid of any one of claims 12-15, wherein the nucleic acid is cDNA.

10 17. A vector comprising one or more of the nucleic acids of any one of claims 12-16, or a nucleic acid encoding the antibody or antigen-binding fragment thereof of any one of claims 1-11.

15 18. A cell comprising the vector of claim 17.

19. The cell of claim 18, wherein the cell is a CHO cell.

20 20. A cell comprising one or more of the nucleic acids of any one of claims 12-16, or a nucleic acid encoding the antibody or antigen-binding fragment thereof of any one of claims 1-11.

21. A method of producing an antibody or an antigen-binding fragment thereof, or an antigen-binding protein construct, the method comprising

25 (a) culturing the cell of any one of claims 18-20 under conditions sufficient for the cell to produce the antibody or the antigen-binding fragment thereof, or the antigen-binding protein construct; and

 (b) collecting the antibody or the antigen-binding fragment thereof, or the antigen-binding protein construct produced by the cell.

30 22. An antibody-drug conjugate comprising a therapeutic agent covalently bound to the antibody or antigen-binding fragment thereof of any one of claims 1-11.

23. The antibody drug conjugate of claim 22, wherein the therapeutic agent is a cytotoxic or cytostatic agent.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the antibody or antigen-binding fragment thereof of any one of claims 1-11, or the antibody-drug conjugate of any one of claims 22 or 23.

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25. A kit comprising the antibody or antigen-binding fragment thereof of any one of claims 1-11, or the antibody-drug conjugate of any one of claims 22 or 23.

26. A method of treating a subject having an immune disorder or a cancer, the method comprising administering a therapeutically effective amount of a composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-11, the antibody-drug conjugate of claim 22 or 23, or the pharmaceutical composition of claim 24, to the subject.

27. The method of claim 26, wherein the immune disorder is allergy, asthma, or atopic dermatitis.

28. The method of claim 26, wherein the immune disorder is a type II or mixed allergic disease.

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29. The method of any one of claims 26-28, wherein the subject is a human or a non-human animal.