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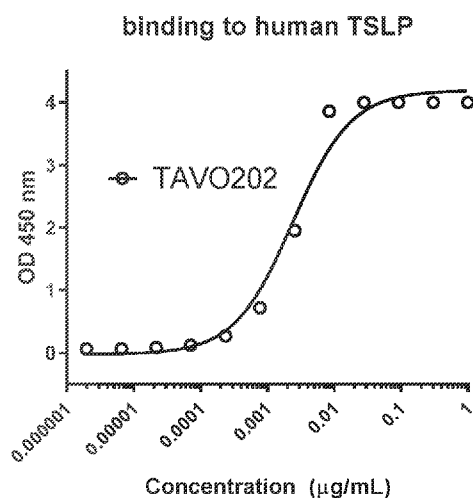


FIGURE 1A

(57) Abstract: The present disclosure is related to antibodies (*e.g.*, human antibodies) and antigen-binding fragments thereof that specifically bind and neutralize (human) thymic stromal lymphopoietin (TSLP). Such molecules may have modified F_c regions that increase antibody half-life, increase resistance to proteases, and/or reduce ability to interact with F_c gamma receptors. These molecules are useful for treatment of allergic and non-allergic inflammation associated with TSLP-related disorders and diseases.

AGENTS THAT INTERFERE WITH THYMIC STROMAL LYMPHOPOIETIN (TSLP)-RECEPTOR SIGNALING

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 62/873,051, filed on July 11, 2019, which is herein incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: TABI__002__01WO__SeqList__ST25; date recorded July 3, 2020; file size 49 kilobytes).

BACKGROUND OF THE DISCLOSURE

Thymic stromal lymphopoietin, TSLP, is an interleukin 7-like cytokine that plays a critical role in the regulation of immune responses and in the differentiation of hematopoietic cells. TSLP is widely expressed in epithelial cells of the lung, skin and gut, Hassall's corpuscles in the thymic medulla, mucosa-associated lymphoid tissues and tonsils (Liu, Soumelis et al. 2007, Rochman and Leonard 2008, Sokol, Barton et al. 2008). High TSLP expression levels are found in the heart, liver, spleen and prostate compared to expression levels in lung, skeletal muscle, kidney, spleen, ovary, small intestine and colon (Quentmeier, Drexler et al. 2001).

The epithelial cell-derived cytokine is produced in response to environmental and proinflammatory stimuli. It induces the release of T-cell attracting chemokines from monocytes and enhances maturation of myeloid dendritic cells. Within the thymus, TSLP activates myeloid and plasmacytoid dendritic cells, resulting in production of regulatory T cells. TSLP is important in regulating type 2 (Th2, T2) immunity through its activity on dendritic cells, T and B cells, and production of cytokines by antigen-specific Th2 cells (Ziegler, Roan et al. 2013).

TSLP signals through a heterodimeric receptor complex composed of the thymic stromal lymphopoietin receptor (TSLPR) and the IL-7R alpha-chain (Verstraete, van Schie et al. 2014). On binding its receptor complex, TSLP can activate multiple signal transduction pathways (Zhong, Sharma et al. 2014). Stimulation of IL7R/TSLPR complex by TSLP induces the phosphorylation and activation of Janus kinases (JAKs). Activated JAKs, in turn, regulate the

activity of multiple transcription (STAT) factors, which include STAT1, STAT3, STAT4, STAT5a, STAT5b and STAT6. Several other proteins such as AKT1, ERK1/2, JNKs, ribosomal protein S6 kinase and 4E-BP1 are also activated by TSLP stimulation. In addition, phosphoproteomic analyses reveal that TSLP can regulate phosphorylation of 226 proteins including kinases and protein phosphatases such as SHP-1 and SHP-2.

TSLP is known to be involved in the initiation of an inflammatory cascade and can contribute to development of chronic disease including allergic asthma, atopic dermatitis, allergic rhinitis, ulcerative colitis, and many chronic fibroproliferative disorders (Vannella, Ramalingam et al. 2016). Studies have suggested also that TSLP is involved in immune disorders including infection (Piliponsky et al. 2016), cancer (De Monte et al. 2011), autoimmunity (Moret et al. 2011); as well as in innate and adaptive immune responses (Ziegler, Roan, 2013; Headley et al. 2009).

TSLP is a master regulator of allergic inflammation, particularly type 2 inflammatory pathways, and therefore has been a promising target for treatment of asthma. Asthma is a disorder characterized by inflammation of the bronchial tubes with increased production of mucous inside the tubes. People with asthma experience symptoms such as coughing, wheezing, shortness of breath, chest tightness, pain or pressure due to chronic airway hyper-responsiveness, typically with eosinophil infiltration (Ohta, Nagase et al. 2017). Some patients with severe asthma have frequent exacerbations associated with persistent eosinophilic inflammation despite continuous treatment with high-dose inhaled glucocorticoids with or without oral glucocorticoids. A certain percentage of refractory asthma patients, those who do not respond to conventional treatment, have severe symptoms due most likely to a non-eosinophil, non-Th2 cell population. TSLP expression is higher in the airways of patients with asthma than in those of healthy controls, and its levels correlate with Th2 cytokine and chemokine expression and disease severity (Gauvreau, O'Byrne et al. 2014). More recently, Tezepelumab (AMG 157), a human anti-TSLP IgG₂ antibody that specifically binds human TSLP, is in clinical trials for uncontrolled asthma (Comeau, DESMEDT et al. 2006) (Comeau, Smothers et al. 2012) (Corren, Parnes et al. 2017).

Besides targeting TSLP, three mAbs directed toward IL-5 have been approved for patients with severe asthma and an eosinophilic phenotype, Mepolizumab and Reslizumab, which bind directly to IL-5, and Benralizumab which binds to the IL-5 receptor (Ortega, Liu et

al. 2014) (Castro, Zangrilli et al. 2015) (Bleecker, FitzGerald et al. 2016). Dupilumab, an antibody that binds to the IL-4 receptor alpha chain and prevents binding of both IL-4 and IL-13, has been approved for patients with moderate-to-severe eosinophilic asthma (Castro, Corren et al. 2018). Notably, all these therapeutic antibodies appear to be more efficacious in patients with increasing levels of T2 biomarkers relative to those with lower levels.

The prevalence of TSLP in other nonallergic diseases and several fibrotic conditions should also be considered as a justification for targeted therapy (Ying, Zhang 2015). In systemic sclerosis, an autoimmune disease, TSLP is highly expressed in perivascular areas and in immune cells of the skin. TSLP is upregulated in cutaneous epithelial cells, mast cells, and fibroblasts of patients with systemic sclerosis (Usategui, Criado et al. 2013). TSLP and its receptor are strongly expressed in lungs of patients with idiopathic pulmonary fibrosis, a severe fibrotic lung disease (Datta, Alexander et al. 2013). In a fibrotic skin disease, dermal fibroblasts produce high levels of TSLP in response to TGF β in keloidal tissues.

Further support for TSLP as a therapeutic target comes from the finding that TSLP has a role in the initiation and progression of a variety of tumors. These include solid tumors (such as breast, colon, and pancreatic) as well as hematological tumors (such as B cell acute lymphocytic leukemia (B-ALL)) (Corren, Ziegler 2019). Tumors displaying Th2-type responses generally have a worse prognosis than those tumors with predominantly Th-type responses. Tumor cells secrete IL-1 α and IL-1 β which are required for TSLP expression by the cancer associated fibroblasts, suggesting that the tumor and tumor microenvironment are important in induction of TSLP. Studies with human cervical carcinoma cells, gastric and ovarian cancers also provide evidence that there is TSLP mediated cross-talk between hematopoietic cells that infiltrate the tumor and stroma, and the tumor itself.

While TSLP has an important role in cancer, its function as a pro or anti-tumor factor is dependent on the type of tumor. TSLP in metastatic breast cancer is an area of intense study, but there have been contradictory findings in mouse models and human breast-tumor cell lines. Similarly, the role of TSLP in skin tumors is dichotomous. The role of TSLP in B-ALL is complicated by the presence of genetic lesions with mutations in genes encoding components of the TSLP signaling pathway which occur in 50-60% of patients with poor prognosis (Corren, Ziegler 2019).

These observations suggest an important role for TSLP in human disease, and disruption of TSLP and TSLP receptor signalling pathways may have clinical benefit for diseases that lack effective treatment. For asthma, as an example, mortality due to asthma related symptoms is around 250,000 per year globally, and the number is predicted to grow by more than 100 million by 2025 (Pawankar 2014). People with allergic asthma often have other conditions like diabetes, obesity, cardiovascular disease, gastro-oesophageal disease leading to more complicated and worse outcomes. About 70% of asthmatics also have allergies. Allergic diseases include anaphylaxis, food allergies, certain forms of asthma, rhinitis, conjunctivitis, angioedema, urticaria, atopic dermatitis, eczema, eosinophilic disorders, including eosinophilic esophagitis and drug and insect allergies (Pawankar 2014) (Kay 2001).

Owing to the high health care costs, morbidity, impact on quality of life, absenteeism, poorer work performance, increasing patient population with allergic and non-allergic diseases, there is undoubtedly a significant need to reduce Th2 cytokine related inflammation. Agents such as neutralizing antibody molecules that interfere with TSLP receptor binding and signalling, and have the desired efficacy and pharmacokinetic profiles, is an unmet need for inhibiting (both T2 high and T2 low) symptoms associated with chronic inflammatory conditions.

SUMMARY OF THE DISCLOSURE

The disclosure provides for monoclonal antibodies and antigen-binding fragments thereof that specifically bind and neutralize, inhibit, block, abrogate, reduce, or interfere with, at least one activity of thymic stromal lymphopoietin, TSLP. The activity of TSLP that can be neutralized, inhibited, blocked, abrogated, reduced or interfered, by the antibodies or fragments thereof as disclosed herein, includes, but is not limited by, neutralization of TSLP activation of its receptor complex, and the like. In embodiments, the antibodies are monoclonal antibodies, e.g., mouse monoclonal antibodies or humanized antibodies. In embodiments, the antibodies are antigen-binding antibody fragments.

The disclosure provides for a mouse monoclonal antibody to human TSLP, designated as TAVO202, comprising a heavy chain variable region sequence with amino acid sequence of SEQ ID NO. 1, and a light chain variable region sequence with amino acid sequence of SEQ ID NO. 2.

The disclosure provides for the heavy chain variable region of TAVO202 comprising three Complementarity Determining Regions (CDRs), designated as HCDR1, HCDR2 and HCDR3, with amino acid sequence set forth as SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5, respectively.

The disclosure provides for the light chain variable region of TAVO202 comprising three CDRs, designated as LCDR1, LCDR2 and LCDR3, with amino acid sequence set forth as SEQ ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8, respectively.

The disclosure provides for one humanized heavy chain variable region of TAVO202, designated as 202H2, with amino acid sequence set forth as SEQ ID NO. 9.

The disclosure provides for two humanized light chain variable region of TAVO202, designated as 202L3 and 202L4, with amino acid sequences set forth as SEQ ID NO. 10 and SEQ ID NO. 11, respectively.

In embodiments, the disclosure provides a humanized antibody or antigen-binding fragment thereof that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 according to SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8, respectively. In an embodiment, the humanized antibody comprises a heavy chain variable region having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO. 9. In an embodiment, the humanized antibody comprises a light chain variable region having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO. 10 or SEQ ID NO. 11.

As a non-limiting example, the disclosure provides for a first humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG2 Fc, designated as TAVO6264, comprising heavy chain sequence set forth as SEQ ID NO. 12 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a second humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG2 Fc, designated as TAVO6265, comprising heavy chain sequence set forth as SEQ ID NO. 12 and light chain sequence set forth as SEQ ID NO. 14.

The disclosure provides for a third humanized antibody for TAVO202 comprising

humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG1 Fc with L234F, L235E, D265A, F405L mutations, designated as TAVO7264, comprising heavy chain sequence set forth as SEQ ID NO. 15 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a fourth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG1 Fc with L234F, L235E, D265A, F405L mutations, designated as TAVO7265, comprising heavy chain sequence set forth as SEQ ID NO. 15 and light chain sequence set forth as SEQ ID NO. 14.

The disclosure provides for a fifth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG1 Fc with L234A, L235A, M428L, N434S mutations, designated as TAVO9764, comprising heavy chain sequence set forth as SEQ ID NO. 16 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a sixth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG1 Fc with L234A, L235A, M428L, N434S mutations, designated as TAVO9765, comprising heavy chain sequence set forth as SEQ ID NO. 16 and light chain sequence set forth as SEQ ID NO. 14.

The anti-TSLP monoclonal antibodies can be full length IgG₁, IgG₂, IgG₃, IgG₄ antibodies or may comprise only an antigen-binding portion including a F_{ab}, F_{(ab')₂}, or scFv fragment that specifically binds (e.g. human) TSLP, neutralizes, inhibits, blocks, abrogates, reduces, or interferes with at least one activity of TSLP. The antibody backbones may be modified to affect functionality, e.g. to eliminate residual effector functions.

The disclosure also provides for anti-TSLP monoclonal antibodies with an extended half-life when compared to the wild-type antibody. The extension of half-life can be realized by engineering the CH₂ and CH₃ domains of the antibody with any one set of mutations selected from M252Y/S254T/T256E, M428L/N434S, T250Q/M428L, N434A and T307A/E380A/N434A when compared to a parental wild-type antibody, residue numbering according to the EU Index.

The disclosure also provides for anti-TSLP monoclonal antibodies with enhanced

resistant to proteolytic degradation by a protease that cleaves the wild-type antibody between or at residues 222-237 (EU numbering). The resistance to proteolytic degradation can be realized by engineering E233P/L234V/L235A mutations in the hinge region with G236 deleted when compared to a parental wild-type antibody, residue numbering according to the EU Index.

The disclosure also provides for vectors comprising the polynucleotides of the disclosure.

The disclosure also provides for a method of producing the anti-TSLP monoclonal antibodies of the disclosure, comprising culturing the host cell of the disclosure under conditions that the antibody is expressed, and purifying the antibody.

The disclosure also provides for a pharmaceutical composition comprising the anti-TSLP antibodies or antigen-binding fragments thereof of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides for methods of detecting the binding of the anti-TSLP antibodies.

The disclosure also provides for methods of blocking the binding of TSLP to its receptor TLPR, the method comprises contacting the TSLPR with any one of the anti-TSLP antibodies or antigen binding fragments thereof provided herein.

The disclosure also provides for a method of treating asthma comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating COPD and idiopathic pulmonary fibrosis, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating symptoms of atopic dermatitis, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating symptoms of eczema, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating symptoms of other allergic states such as eosinophilic esophagitis, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating symptoms of inflammatory bowel diseases, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating fibrotic conditions such as systemic sclerosis, systemic idiopathic pulmonary fibrosis, and keloidal disease, comprising administering to a subject in need a therapeutically effective amount of the provided antibodies to TSLP.

The disclosure also provides for a method of treating cancers, i.e., breast, pancreatic, colorectal, lymphoblastic leukemia, head and neck cancer, comprising administering to a subject in need therapeutically effective amounts of the provided antibodies to TSLP.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Binding to (A) human and (B) mouse TSLP by mouse monoclonal anti-human TSLP antibody TAVO202.

FIG. 2: (A). The response of cell proliferation upon stimulation by human TSLP in BAF cells transfected with human TSLP receptor complex. (B). Dose-dependent neutralizing human TSLP-driven cell proliferation by TAVO202.

FIG. 3: (A). Dose-dependent STAT5 reporter gene expression upon stimulation by human TSLP in a luciferase reporter assay. (B). Dose-dependent neutralization of reporter gene expression by TAVO202 in human TSLP-driven reporter gene expression assay.

FIG. 4: Sequence alignments of heavy chain and light chain variable regions of TAVO202 with humanized V_H and V_L variants. Humanized antibodies can be formed by pairing the humanized V_H variant (202H2) and two humanized V_L variants (202L3 and 202L4) with different IgG Fc.

FIG. 5: SDS-PAGE analysis of example humanized anti-TSLP IgG antibodies under (A) non-reduced and (B) reduced conditions. (C). Size Exclusion Chromatography (SEC) analysis of example anti-TSLP IgG antibodies.

FIG. 6: Binding to (A) human and (B) cynomolgus TSLP by example humanized anti-TSLP IgG antibodies.

FIG. 7: Neutralizing human TSLP driven reporter gene activation by example humanized anti-TSLP IgG antibodies in STAT5 reporter gene expression assay.

FIG. 8: Neutralizing human TSLP driven CCL17 release from dendritic cells isolated from two donors by humanized anti-TSLP IgG antibodies.

FIG. 9: Binding to mouse FcRn at pH 6.0 by humanized anti-TSLP IgG antibody TAVO9765 with half-life extension Fc mutations and TAVO7265 lacking such mutations.

FIG. 10: PK profile of anti-TSLP antibody in a cynomolgus monkey. Anti-TSLP antibody with Fc mutations for half-life extension (TAVO9765) was administered as a 4 mg/kg intravenous dose into a cynomolgus monkey. The plasma concentration is displayed at time points up to day 35 post-dose.

DETAILED DESCRIPTION OF THE DISCLOSURE

Definitions

The terminology used herein is for describing embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. In describing and claiming the present disclosure, the following terminology will be used.

As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a cell” includes a combination of two or more cells, and the like.

“Antibodies” is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antibody fragments, bispecific or multi-specific antibodies, dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity. “Full length antibody molecules” are comprised of two heavy chains (HC) and two light chains inter-connected by disulfide bonds as well as multimers thereof (*e.g.* IgM). Each heavy chain is comprised of a heavy chain variable region (V_H) and a heavy chain constant

region (comprised of domains CH₁, hinge, CH₂ and CH₃). Each light chain is comprised of a light chain variable region (V_L) and a light chain constant region (C_L). The V_H and the V_L regions may be further subdivided into regions of hyper variability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each V_H and V_L is composed of three CDRs and four FR segments, arranged from amino-to-carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

“Complementarity determining regions (CDR)” are “antigen binding sites” in an antibody. CDRs may be defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the V_H (HCDR1, HCDR2, HCDR3) and three in the V_L (LCDR1, LCDR2, LCDR3) are based on sequence variability (Wu and Kabat 1970) (Kabat, National Institutes of *et al.* 1991); (ii) “Hypervariable regions,” “HVR,” or “HV,” three in the V_H (H1, H2, H3) and three in the V_L (L1, L2, L3) refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk 1987) (The International ImMunoGeneTics (IMGT) database (<http://www.imgt.org>) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.* (Lefranc, Pommie *et al.* 2003). The term “CDR,” “HCDR1,” “HCDR2,” “HCDR3,” “LCDR1,” “LCDR2” and “LCDR3” as used herein includes CDRs defined by any of the methods described supra, Kabat, Chothia or IMGT, unless otherwise explicitly stated in the specification.

Immunoglobulins may be assigned to five major classes, IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant region amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA₁, IgA₂, IgG₁, IgG₂, IgG₃ and IgG₄. Antibody light chains of any vertebrate species may assign to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant regions.

“Antibody fragments” refers to a portion of an immunoglobulin molecule that retains the heavy chain and/or the light chain antigen binding site, such as heavy chain complementarity determining regions (HCDR) 1, 2 and 3, light chain complementarity determining regions (LCDR) 1, 2 and 3, a heavy chain variable region (V_H), or a light chain variable region (V_L). Antibody fragments include well known F_{ab}, F_{(ab')₂}, F_d and F_v fragments as well as domain antibodies (dAb) consisting of one V_H domain. V_H and V_L domains may be linked together via a synthetic linker to form various types of single chain antibody designs where the V_H/V_L domains

may pair intramolecularly, or intermolecularly in those cases when the V_H and V_L domains are expressed by separate single chain antibody constructs, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Int. Patent Publ. Nos. WO1998/44001, WO1988/01649, WO1994/13804 and WO1992/01047.

“Monoclonal antibody” refers to an antibody population with single amino acid composition in each heavy and each light chain, except for possible well-known alterations such as removal of C-terminal lysine from the antibody heavy chain. Monoclonal antibodies typically bind one antigenic epitope, except that bispecific monoclonal antibodies bind two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multi-specific, or monovalent, bivalent or multivalent. A bispecific antibody is included in the term monoclonal antibody.

“Isolated antibody” refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody specifically binding for example TSLP is substantially free of antibodies that specifically bind antigens other than TSLP).

“Isolated antibody” encompasses antibodies that are isolated to a higher purity, such as antibodies that are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

“Humanized antibody” refers to an antibody in which the antigen binding sites are derived from non-human species and the variable region frameworks are derived from human immunoglobulin sequences. Humanized antibody may include substitutions in the framework so that the framework may not be an exact copy of expressed human immunoglobulin or human immunoglobulin germLine gene sequences.

“Human antibody” refers to an antibody having heavy and light chain variable regions in which both the framework and the antigen binding site are derived from sequences of human origin and is optimized to have minimal immune response when administered to a human subject. If the antibody contains a constant region or a portion of the constant region, the constant region also is derived from sequences of human origin.

The numbering of amino acid residues in the antibody constant region throughout the

specification is according to the EU index as described in Kabat *et al.*, unless otherwise explicitly stated (Kabat, National Institutes of *et al.* 1991).

Conventional one and three-letter amino acid codes are used herein as shown in **Table 1**.

Table 1.

Amino acid	Three-letter code	One-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Gln	E
Glutamine	Glu	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

A “variant” of a polypeptide (e.g., an antigen binding protein, or an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence.

Variants include antibodies and fragments thereof that have a recited percent identity to an antibody or fragment provided herein or to an antibody or fragment having a recited DNA or amino acid sequence.

The term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity,” “percent homology,” “sequence identity,” or “sequence homology” and the like mean the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (i.e., an “algorithm”). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al., 1988, SIAM J. Applied Math. 48:1073. In calculating percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences.

The constant region sequences of the mammalian IgG heavy chain are designated in sequence as CH1-hinge-CH2-CH3. The “hinge,” “hinge region” or “hinge domain” of an IgG is generally defined as including Glu216 and terminating at Pro230 of human IgG1 according to the EU Index but functionally, the flexible portion of the chain may be considered to include additional residues termed the upper and lower hinge regions, such as from Glu216 to Gly237 and the lower hinge has been referred to as residues 233 to 239 of the Fc region where FcγR binding was generally attributed. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds. Although boundaries may vary slightly, as numbered according to the EU Index, the CH1 domain is adjacent to the VH domain and amino terminal to the hinge region of an immunoglobulin heavy chain molecule and includes the first (most amino terminal) constant

region of an immunoglobulin heavy chain, *e.g.*, from about EU positions 118-215. The F_c domain extends from amino acid 231 to amino acid 447; the C_{H2} domain is from about Ala231 to Lys340 or Gly341 and the C_{H3} from about Gly341 or Gln342 to Lys447. The residues of the IgG heavy chain constant region of the C_{H1} region terminate at Lys. The F_c domain containing molecule comprises at least the C_{H2} and the C_{H3} domains of an antibody constant region, and therefore comprises at least a region from about Ala231 to Lys447 of IgG heavy chain constant region. The F_c domain containing molecule may optionally comprise at least portion of the hinge region.

“Epitope” refers to a portion of an antigen to which an antibody specifically binds. Epitopes typically consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be composed of contiguous and/or discontinuous amino acids that form a conformational spatial unit. For a discontinuous epitope, amino acids from differing portions of the linear sequence of the antigen come near in 3-dimensional space through the folding of the protein molecule. Antibody “epitope” depends on the methodology used to identify the epitope.

A “leader sequence” as used herein includes any signal peptide that can be processed by a mammalian cell, including the human B2M leader. Such sequences are well-known in the art.

The terms “peptide,” “polypeptide,” and “protein” are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The terms also include polypeptides that have co-translational (*e.g.*, signal peptide cleavage) and post-translational modifications of the polypeptide, such as, for example, disulfide-bond formation, glycosylation, acetylation, phosphorylation, proteolytic cleavage, and the like. Furthermore, as used herein, a “polypeptide” refers to a protein that includes modifications, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art) to the native sequence, if the protein maintains the desired activity. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of hosts that produce the proteins, or errors due to PCR amplification or other recombinant DNA methods.

The term “recombinant,” as used herein to describe a nucleic acid molecule, means a

polynucleotide of genomic, cDNA, viral, semisynthetic, and/or synthetic origin, which, by its origin or manipulation, is not associated with all or a portion of the polynucleotide sequences with which it is associated in nature. The term “recombinant,” as used with respect to a protein or polypeptide, refers to a polypeptide produced by expression from a recombinant polynucleotide. The term “recombinant,” as used with respect to a host cell or a virus, refers to a host cell or virus into which a recombinant polynucleotide has been introduced. Recombinant is also used herein to refer to, with reference to material (*e.g.*, a cell, a nucleic acid, a protein, or a vector) that the material has been modified by the introduction of a heterologous material (*e.g.*, a cell, a nucleic acid, a protein, or a vector).

The terms “polynucleotide,” “oligonucleotide,” “nucleic acid” and “nucleic acid molecule” are used interchangeably herein to include a polymeric form of nucleotides, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule.

“Vector” refers to a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers, that function to facilitate the duplication or maintenance of these polynucleotides in a biological system, such as a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The vector polynucleotide may be DNA or RNA molecules, cDNA, or a hybrid of these, single stranded or double stranded.

“Expression vector” refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

“Valent” refers to the presence of a specified number of binding sites specific for an antigen in a molecule. As such, the terms “monovalent,” “bivalent,” “tetravalent,” and “hexavalent” refer to the presence of one, two, four and six binding sites, respectively, specific for an antigen in a molecule.

As used herein, the term “heterologous” used about nucleic acid sequences, proteins or polypeptides, means that these molecules are not naturally occurring in the cell from which the heterologous nucleic acid sequence, protein or polypeptide was derived. For example, the

nucleic acid sequence coding for a human polypeptide that is inserted into a cell that is not a human cell is a heterologous nucleic acid sequence in that context. Whereas heterologous nucleic acids may be derived from different organism or animal species, such nucleic acid need not be derived from separate organism species to be heterologous. For example, in some instances, a synthetic nucleic acid sequence or a polypeptide encoded therefrom may be heterologous to a cell into which it is introduced in that the cell did not previously contain the synthetic nucleic acid. As such, a synthetic nucleic acid sequence or a polypeptide encoded therefrom may be considered heterologous to a human cell, *e.g.*, even if one or more components of the synthetic nucleic acid sequence or a polypeptide encoded therefrom was originally derived from a human cell.

A “host cell,” as used herein, denotes an *in vivo* or *in vitro* eukaryotic cell or a cell from a multicellular organism (*e.g.*, a cell line) cultured as a unicellular entity, which eukaryotic cells can be, or have been, used as recipients for a nucleic acid (*e.g.*, an expression vector that comprises a nucleotide sequence encoding a multimeric polypeptide of the present disclosure), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

A “recombinant host cell” (also referred to as a “genetically modified host cell”) is a host cell into which has been introduced a heterologous nucleic acid, *e.g.*, an expression vector. For example, a genetically modified eukaryotic host cell is genetically modified by introduction into a suitable eukaryotic host cell a heterologous nucleic acid, *e.g.*, an exogenous nucleic acid that is foreign to the eukaryotic host cell, or a recombinant nucleic acid that is not normally found in the eukaryotic host cell.

“Specific binding” or “specifically binds” or “binds” refer to an antibody binding to a specific antigen with greater affinity than for other antigens. Typically, the antibody “specifically binds” when the equilibrium dissociation constant (K_D) for binding is about 1×10^{-8} M or less, for example about 1×10^{-9} M or less, about 1×10^{-10} M or less, about 1×10^{-11} M or less, or about 1×10^{-12} M or less, typically with the K_D that is at least one hundred-fold less than its K_D for binding to a non-specific antigen (*e.g.*, BSA, casein). The K_D may be measured using standard procedures.

As used herein, the terms “treatment,” “treating,” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease.

“Treatment,” as used herein, covers any treatment of a disease in a mammal, *e.g.*, in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease.

The terms “individual,” “subject,” “host,” and “patient,” used interchangeably herein, refer to a mammal, including, but not limited to, murine (*e.g.*, rats, mice), lagomorphs (*e.g.*, rabbits), non-human primates, humans, canines, felines, ungulates (*e.g.*, equine, bovine, ovine, porcine, caprine), *etc.*

A “therapeutically effective amount” or “efficacious amount” refers to the amount of an agent, or combined amounts of two agents, that, when administered to a mammal or other subject for treating a disease, is sufficient to affect such treatment for the disease. The “therapeutically effective amount” will vary depending on the agent(s), the disease and its severity and the age, weight, *etc.*, of the subject to be treated.

Before the present disclosure is further described, it is to be understood that this disclosure is not limited to embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for describing embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

Composition of anti-TSLP antibody and antigen binding fragment

Herein is described monoclonal antibodies and antigen-binding fragments thereof that specifically bind and neutralize, inhibit, block, abrogate, reduce, or interfere with, at least one activity of TSLP, *e.g.*, a functional activity of TSLP to its receptor complex. The anti-TSLP antibodies and antigen binding fragments can be therapeutically administered to a subject to treat TSLP mediated diseases.

The disclosure provides for a mouse monoclonal antibody, designated as TAVO202, which was identified from a mouse hybridoma screening, that specifically binds and neutralizes, inhibits, blocks, abrogates, reduces, or interferes with, at least one activity of human thymic stromal lymphopoietin (TSLP). TAVO202 comprises a heavy chain variable region sequence with amino acid sequence of SEQ ID NO. 1, and a light chain variable region sequence with amino acid sequence of SEQ ID No. 2. The heavy chain variable region of TAVO202 comprises three Complementarity Determining Regions, designated as HCDR1, HCDR2 and HCDR3, with amino acid sequence set forth as SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5, respectively. The light chain variable region of TAVO202 comprises three Complementarity Determining Regions, designated as LCDR1, LCDR2 and LCDR3, with amino acid sequence set forth as SEQ ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8, respectively.

Heavy chain variable region sequence of anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 1)

QVQLQQSGAELVRPGSSVKISCKASGYTFSSYWVNWVKQRPGQGLEWIGQIYPGDGDT
DYNGKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGTYNNYYGTIDYWGQGT
SVTVSS

The sequence of three CDRs of heavy chain are underlined.

Light chain variable region sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 2)

EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWYQQKSETSPKPWIYGTSNLA SGVPV
RFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSYPLTFGGGTKLEIK

The sequence of three CDRs of light chain are underlined.

HCDR1 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 3)
GYTFSSYWVN

HCDR2 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 4)

QIYPGDGDTD

HCDR3 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 5)
 GTYYNNYYGTDY

LCDR1 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 6)
 SVSSSISSSNLH

LCDR2 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 7)
 GTSNLAS

LCDR3 sequence of Anti-human TSLP mouse monoclonal antibody TAVO202 (SEQ ID NO: 8)
 QQWSSYPLT

The mouse anti- human TSLP antibody TAVO202 can be humanized by grafting of mouse CDRs onto human germLine scaffolds. A few key mouse residues are preserved by back mutations to achieve higher stability and better expression while minimizing immunogenicity. For TAVO202, one humanized VH variant (202H2) is designed based on IGHV1-69*02 and two humanized VL variants are designed with 202L3 based on IGKV1-9*01 and 202L4 based on IGKV6-21*01 with a couple of back mutations in framework.

Based on the foregoing, the disclosure provides for one humanized heavy chain variable region of TAVO202, designated as 202H2, with amino acid sequence set forth as SEQ ID NO. 9.

Humanized heavy chain variable region 202H2 sequence (SEQ ID NO: 9)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSSYWVNWVRQAPGQGLEWIGQIYPGDGD
TDYAQKFQGRATLTADKSTSTAYMELSSLRSEDTAVYFCARGTYYNNYYGTDYWGQG
 TTVTVSS

The sequences of three CDRs of humanized heavy chain are underlined.

The disclosure provides for two humanized light chain variable region of TAVO202, designated as 202L3 and 202L4, with amino acid sequences set forth as SEQ ID NO. 10 and SEQ ID NO. 11, respectively.

Humanized light chain variable region 202L3 sequence (SEQ ID NO: 10)

DIQLTQSPSFLSASVGDRVTITCSVSSSISSSNLHWYQQKPGKAPKPWIYGTSNLASGVPS
RFSGSGSGTEYTLTISSLQPEDAATYYCQQWSSYPLTFGQGTKLEIK

The sequences of three CDRs of humanized light chain are underlined.

Humanized light chain variable region 202L4 sequence (SEQ ID NO: 11)

EIVLTQSPDFQSVTPKEKVTITCSVSSSISSSNLHWYQQKPDQSPKPWIYGTSNLASGVPS
RFSGSGSGTDYTLTINSLEAEDAATYYCQQWSSYPLTFGQGTKLEIK

The sequences of three CDRs of humanized light chain are underlined.

As a non-limiting example, by pairing the humanized 202H2 heavy chain variable region and the two humanized light chain variable regions 202L3 and 202L4 with different IgG Fc, six humanized antibodies for TAVO202 can be generated (Figure 4, Table 2).

Table 2. Exemplary antibodies

Humanized mAb	V _H	V _L	Fc
TAVO6264	202H2	202L3	IgG2
TAVO6265	202H2	202L4	IgG2
TAVO7264	202H2	202L3	IgG1 (L234F, L235E, D265A, F405L)
TAVO7265	202H2	202L4	IgG1 (L234F, L235E, D265A, F405L)

TAVO9764	202H2	202L3	IgG1 (L234A, L235A, M428L, N434S)
TAVO9765	202H2	202L4	IgG1 (L234A, L235A, M428L, N434S)

The disclosure provides for a first humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG2 Fc, designated as TAVO6264, comprising heavy chain sequence set forth as SEQ ID NO. 12 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a second humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG2 Fc, designated as TAVO6265, comprising heavy chain sequence set forth as SEQ ID NO. 12 and light chain sequence set forth as SEQ ID NO. 14.

The disclosure provides for a third humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG1 Fc with L234F, L235E, D265A, F405L mutations, designated as TAVO7264, comprising heavy chain sequence set forth as SEQ ID NO. 15 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a fourth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG1 Fc with L234F, L235E, D265A, F405L mutations, designated as TAVO7265, comprising heavy chain sequence set forth as SEQ ID NO. 15 and light chain sequence set forth as SEQ ID NO. 14.

The disclosure provides for a fifth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L3 with human IgG1 Fc with L234A, L235A, M428L, N434S mutations, designated as TAVO9764, comprising heavy chain sequence set forth as SEQ ID NO. 16 and light chain sequence set forth as SEQ ID NO. 13.

The disclosure provides for a sixth humanized antibody for TAVO202 comprising humanized heavy chain variable region 202H2 and humanized light chain variable region 202L4 with human IgG1 Fc with L234A, L235A, M428L, N434S mutations, designated as

TAVO9765, comprising heavy chain sequence set forth as SEQ ID NO. 16 and light chain sequence set forth as SEQ ID NO. 14.

Anti-TSLP humanized antibody Heavy Chain based on 202H2 with IgG2 Fc (SEQ ID NO: 12)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSSYWVNWVRQAPGQGLEWIGQIYPGDGD
TDYAKQFOGRATLTADKSTSTAYMELSSLRSEDTAVYFCARGTYNNYYGTDYWGQG
TTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVRKCCVECPPCPAPP
 VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR
 EEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
 PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMMLDSGDSFFLYSKL
 TVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPGK

The sequence of variable domain of heavy chain is underlined.

Anti-TSLP humanized antibody Light Chain based on 202L3 (SEQ ID NO: 13)

DIQLTQSPSFLSASVGDRVTTITCSVSSSISSSNLHWYQQKPGKAPKRWIYGTSNLASGVPS
RFSGSGSGTEYTLTISSLOPEDAATYYCQOWSSYPLTFGQGTKLEIKRTVAAPSVFIFPPS
 DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL
 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

The sequence of variable domain of light chain is underlined.

Anti-TSLP humanized antibody Light Chain based on 202L4 (SEQ ID NO: 14)

EIVLTQSPDFQSVPKEKVTITCSVSSSISSSNLHWYQQKPDQSPKRWIYGTSNLASGVPS
RFSGSGSGTDYTLTINSLEAEDAATYYCQOWSSYPLTFGQGTKLEIKRTVAAPSVFIFPPS
 DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL
 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

The sequence of variable domain of light chain is underlined.

Anti-TSLP humanized antibody Heavy Chain based on 202H2 with IgG1 Fc with L234F, L235E, D265A, F405L mutations (SEQ ID NO: 15)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSSYWVNWVRQAPGQGLEWIGOIYPGDGD
TDYAQKFQGRATLTADKSTSTAYMELSSLRSED**TAVYFCARGTYYN**NYGTDYWGQG
TTVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP**TVTSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPS**SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP**
APEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFLL
YSKLTVDKSRWQQGNV**FSCSV**MHEALHNHYTQKSLSLSPGK

The sequence of variable domain of heavy chain is underlined. The L234F, L235E, D265A, F405L mutations are bolded.

Anti-TSLP humanized antibody Heavy Chain based on 202H2 with IgG1 Fc with L234A, L235A, M428L, N434S mutations (SEQ ID NO: 16)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSSYWVNWVRQAPGQGLEWIGOIYPGDGD
TDYAQKFQGRATLTADKSTSTAYMELSSLRSED**TAVYFCARGTYYN**NYGTDYWGQG
TTVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP**TVTSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPS**SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP**
APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFLL
YSKLTVDKSRWQQGNV**FSCSV**LHEALHSHYTQKSLSLSPGK

The sequence of variable domain of heavy chain is underlined. The L234A, L235A, M428L, N434S mutations are bolded.

The disclosure also provides for preparation of a bispecific antibody or multispecific antibody that can include the engagement of any two, three, or four TSLP epitopes by having Fab or scFv domains comprising the one humanized heavy chain variable region of TAVO202, designated as 202H2, with amino acid sequence set forth as SEQ ID NO. 9 with one of the two

humanized light chain variable region of TAVO202, designated as 202L3 and 202L4, with amino acid sequences set forth as SEQ ID NO. 10 and SEQ ID NO. 11, respectively. For instance, this can be a bispecific antibody that has Fab or scFv domains 202H2 with 202L3 and 202H2 with 202L4, or any other permutation of domains that can engagement two, three, or four epitopes.

The TSLP binding antibodies and fragments of the present disclosure encompass antigen-binding fragments that retain sufficient ability to specifically bind to TSLP. The TSLP binding fragments as used herein may include any 3 or more contiguous amino acids (e.g., 4 or more, 5 or more 6 or more, 8 or more, or even 10 or more contiguous amino acids) of the antibody and encompasses Fab, Fab', F(ab')₂, and F(v) fragments, or the individual light or heavy chain variable regions or portions thereof. These fragments lack the F_c fragment of an intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody. These fragments can be produced from intact antibodies using well known methods, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

The TSLP binding antibodies and fragments of the present disclosure may also encompass diabodies, which are bivalent antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites.

The TSLP binding antibodies and fragments of the present disclosure may also encompass single-chain antibody fragments (scFv) that bind to TSLP. An scFv comprises an antibody heavy chain variable region (V_H) operably linked to an antibody light chain variable region (V_L) wherein the heavy chain variable region and the light chain variable region, together or individually, form a binding site that binds TSLP. Such TSLP binding fragments can be prepared by methods known in the art such as, for example, the synthesis or PCR mediated amplification of the variable portions of the heavy and light chains of an antibody molecule and a flexible protein linker composed of the amino acids Gly and Ser. The resulting DNA fragment is cloned for expression in *E. coli* or mammalian cells. The expressed TSLP binding fragments are purified from the host cells.

The TSLP binding antibodies and fragments of the present disclosure encompass full length antibody comprising two heavy chains and two light chains. Exemplary human or humanized antibodies include IgG, IgM, IgE, IgA, and IgD antibodies. The present antibodies can be of any class (IgG, IgM, IgE, IgGA, IgD, etc.) or isotype. For example, a human antibody can comprise an IgG Fc domain, such as at least one of isotypes, IgG1, IgG2, IgG3 or IgG4.

In some instances, an IgG Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an IgG1 Fc sequence as SEQ ID NO: 17.

In some instances, an IgG Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an IgG2 Fc sequence as SEQ ID NO: 18.

In some instances, an IgG Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an IgG3 Fc sequence as SEQ ID NO: 19.

In some instances, an IgG Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an IgG4 Fc sequence as SEQ ID NO: 20.

A S228P mutation may be made into IgG4 antibodies to enhance IgG4 stability.

IgG₁ Fc (SEQ ID NO: 17):

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG₂ Fc (SEQ ID NO: 18):

TVERKCCVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVS LTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG₃ F_c (SEQ ID NO: 19):

RVELKTPLGDTTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPG
K

IgG₄ F_c (SEQ ID NO: 20)

RVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSVMSVHEALHNHYTQKSLSLSLGLK

The present anti-TSLP antibodies may comprise a modified F_c region, wherein the modified F_c region comprises at least one amino acid modification relative to a wild-type F_c region. In some embodiments, the present anti-TSLP antibodies are provided with a modified F_c region where a naturally-occurring F_c region is modified to extend the half-life of the antibody when compared to the parental wild-type antibody in a biological environment, for example, the serum half-life or a half-life measured by an in vitro assay.

Exemplary mutations that may be made singularly or in combinations are T250Q, M252Y, I253A, S254T, T256E, P257L, T307A, D376V, E380A, M428L, H433K, N434S, N434A, N434H, N434F, H435A and H435R mutations, or a fusion of an albumin-binding peptide onto the C terminus of the F_c) with enhanced circulating antibody half-life relative to a control without said F_c mutation.

In certain embodiments, the extension of half-life can be realized by engineering the M252Y/S254T/T256E mutations in IgG1 F_c as SEQ ID NO: 21, residue numbering according to the EU Index (Dall'Acqua, Kiener et al. 2006).

In certain embodiments, the extension of half-life can also be realized by engineering the M428L/N434S mutations in IgG1 F_c as SEQ ID NO: 22 (Zalevsky, Chamberlain et al. 2010).

In certain embodiments, the extension of half-life can also be realized by engineering the T250Q/M428L mutations in IgG1 Fc as SEQ ID NO: 23 (Hinton, Xiong et al. 2006).

In certain embodiments, the extension of half-life can also be realized by engineering the N434A mutations in IgG1 Fc as SEQ ID NO: 24 (Shields, Namenuk et al. 2001).

In certain embodiments, the extension of half-life can also be realized by engineering the T307A/E380A/N434A mutations in IgG1 Fc as SEQ ID NO: 25 (Petkova, Akilesh et al. 2006).

The effect Fc engineering on the extension of antibody half-life can be evaluated in PK studies in mice relative to antibodies with native IgG Fc.

IgG1 Fc with M252Y/S254T/T256E mutations (SEQ ID NO: 21)

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDLYITREPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG1 Fc with M428L/N434S mutations (SEQ ID NO: 22)

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

IgG1 Fc with T250Q/M428L mutations (SEQ ID NO: 23)

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK

IgG1 Fc with N434A mutations (SEQ ID NO: 24)

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHAHYTQKSLSLSPGK

IgG1 Fc with T307A/E380A/N434A mutations (SEQ ID NO: 25)

KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLAVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVAWESNGQPEN
 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHAHYTQKSLSLSPGK

In some embodiments, the present anti-TSLP antibodies are provided with a modified Fc region where a naturally-occurring Fc region is modified to enhance the antibody resistance to proteolytic degradation by a protease that cleaves the wild-type antibody between or at residues 222-237 (EU numbering).

In certain embodiments, the antibody or antigen binding fragment thereof further comprises a mutation in the lower hinge region of Fc (e.g., equivalent to residues 222-237 of IgG1 Fc) and allowing for increased protease resistance (e.g., resistance to degradation by MMP-3, MMP-7, MMP-12, MMP-13, cathepsin G, pepsin, IdeS, or GluV8) relative to the native IgG1 antibody in environments with endogenous proteolytic activity.

In certain embodiments, the resistance to proteolytic degradation can be realized by engineering E233P/L234V/L235A mutations in the hinge region with G236 deleted when compared to a parental wild-type antibody as SEQ ID NO: 26, residue numbering according to the EU Index (Kinder, Greenplate et al. 2013).

IgG1 Fc with E233P/L234V/L235A mutations and G236 deleted (SEQ ID NO: 26)

KVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
 FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
 PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
 YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK

In instances where effector functionality is not desired, the antibodies of the disclosure may further be engineered to introduce at least one mutation in the antibody F_c that reduces binding of the antibody to an activating Fcγ receptor (FcγR) and/or reduces F_c effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP).

F_c positions that may be mutated to reduce binding of the antibody to the activating FcγR and subsequently to reduce effector functions are those described for example in (Xu, Alegre et al. 2000) (Vafa, Gilliland et al. 2014) (Bolt, Routledge et al. 1993) (Chu, Vostiar et al. 2008) (Shields, Namenuk et al. 2001). Fc mutations with minimal ADCC, ADCP, CDC, Fc mediated cellular activation have been described also as sigma mutations for IgG1, IgG2 and IgG4 (Tam, McCarthy et al. 2017).

Exemplary mutations that may be made singularly or in combinations are K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, A330S and P331S mutations on IgG1, IgG2, IgG3 or IgG4.

Exemplary combination mutations that may be made to reduce ADCC are L234A/L235A on IgG1, V234A/G237A/P238S/H268A/V309L/A330S /P331S on IgG2, F234A/L235A on IgG4, S228P/F234A/L235A on IgG4, N297A on IgG1, IgG2, IgG3 or IgG4, V234A/G237A on IgG2, K214T/E233P/L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M on IgG1, H268Q/V309L/A330S/P331S on IgG2, S267E/L328F on IgG1, L234F/L235E/D265A on IgG1, L234A/L235A/G237A/P238S /H268A/A330S/P331S on IgG1, S228P/F234A/L235A/G237A/P238S on IgG4, and S228P/F234A/L235A/G236-deleted/G237A/P238S on IgG4. Hybrid IgG_{2/4} F_c domains may also be used, such as F_c with residues 117-260 from IgG2 and residues 261-447 from IgG4.

In some embodiments, the present anti-TSLP antibodies are provided with a modified F_c region where a naturally-occurring F_c region is modified to facilitate the generation of bispecific antibody by F_c heterodimerization.

In certain embodiments, the Fc heterodimerization can be realized by engineering F405L and K409R mutations on two parental antibodies and the generation of bispecific antibody in a process known as Fab arm exchange (Labrijn, Meesters et al. 2014).

In certain embodiments, the Fc heterodimerization can also be realized by Fc mutations to facilitate Knob-in-Hole strategy (see, e.g., Intl. Publ. No. WO 2006/028936). An amino acid with a small side chain (hole) is introduced into one Fc domain and an amino acid with a large side chain (knob) is introduced into the other Fc domain. After co-expression of the two heavy chains, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a “hole” with the heavy chain with a “knob” (Ridgway, Presta et al. 1996).

Exemplary Fc mutation pairs forming a knob and a hole are: T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S/L368A/Y407V.

In certain embodiments, the Fc heterodimerization can also be realized by Fc mutations to facilitate the electrostatically-matched interactions strategy (Gunasekaran, Pentony et al. 2010). Mutations can be engineered to generate positively charged residues at one Fc domain and negatively charged residues at the other Fc domain as described in US Patent Publ. No. US2010/0015133; US Patent Publ. No. US2009/0182127; US Patent Publ. No. US2010/028637 or US Patent Publ. No. US2011/0123532. Heavy chain heterodimerization can be formed by electrostatically-matched interactions between two mutated Fc.

Antibodies of the disclosure further comprising conservative modifications are within the scope of the disclosure.

“Conservative modifications” refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequences. Conservative modifications include amino acid substitutions, additions and deletions. Conservative substitutions are those in which the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, tryptophan),

aromatic side chains (e.g., phenylalanine, tryptophan, histidine, tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis ((MacLennan, Rice et al. 1998); (Sasaki and Sutoh 1998)). Amino acid substitutions to the antibodies of the disclosure may be made by known methods for example by PCR mutagenesis (US Patent No. 4,683,195). Alternatively, libraries of variants may be generated for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting antibody variants may be tested for their characteristics using assays described herein.

The antibodies of the disclosure may be post-translationally modified by processes such as glycosylation, isomerization, deglycosylation or non-naturally occurring covalent modification such as the addition of polyethylene glycol moieties (pegylation) and lipidation. Such modifications may occur in vivo or in vitro. For example, the antibodies of the disclosure may be conjugated to polyethylene glycol (PEGylated) to improve their pharmacokinetic profiles. Conjugation may be carried out by techniques known to those skilled in the art. Conjugation of therapeutic antibodies with PEG has been shown to enhance pharmacodynamics while not interfering with function (Leong, DeForge et al. 2001, Yang, Basu et al. 2003, Knight, Jordan et al. 2004).

Antibodies of the disclosure may be modified to improve stability, selectivity, cross-reactivity, affinity, immunogenicity or other desirable biological or biophysical property are within the scope of the disclosure. Stability of an antibody is influenced by a number of factors, including (1) core packing of individual domains that affects their intrinsic stability, (2) protein/protein interface interactions that have impact upon the HC and LC pairing, (3) burial of polar and charged residues, (4) H-bonding network for polar and charged residues; and (5) surface charge and polar residue distribution among other intra- and inter-molecular forces (Worn and Pluckthun 2001). Potential structure destabilizing residues may be identified based upon the crystal structure of the antibody or by molecular modelling in certain cases, and the effect of the residues on antibody stability may be tested by generating and evaluating variants harboring mutations in the identified residues. One of the ways to increase antibody stability is

to raise the thermal transition midpoint (T_m) as measured by differential scanning calorimetry (DSC). In general, the protein T_m is correlated with its stability and inversely correlated with its susceptibility to unfolding and denaturation in solution and the degradation processes that depend on the tendency of the protein to unfold (Remmele and Gombotz 2000). A number of studies have found correlation between the ranking of the physical stability of formulations measured as thermal stability by DSC and physical stability measured by other methods (Maa and Hsu 1996, Remmele, Nightlinger et al. 1997, Gupta and Kaisheva 2003, Bedu-Addo, Johnson et al. 2004, Zhang, Roy et al. 2004). Formulation studies suggest that a Fab T_m has implication for long-term physical stability of a corresponding mAb.

Antibodies of the disclosure may have amino acid substitutions in the F_c region that improve manufacturing and drug stability. An example for IgG₁ is H224S (or H224Q) in the hinge 221-DKTHTC-226 (Eu numbering) which blocks radically induced cleavage (Yates, Gunasekaran et al. 2010); and for IgG₄, the S228P mutation blocks half-antibody exchange (Angal, King et al. 1993, Labrijn, Buijsse et al. 2009).

Expression and purification of the anti-TSLP antibodies

The anti-TSLP antibodies and fragments of the disclosure can be encoded by a single nucleic acid (e.g., a single nucleic acid comprising nucleotide sequences that encode the light and heavy chain polypeptides of the antibody), or by two or more separate nucleic acids, each of which encode a different part of the antibody or antibody fragment.

As a non-limiting example, the disclosure provides nucleic acid sequence as SEQ ID NO: 27 which encodes anti-TSLP humanized antibody heavy chain based on 202H2 with IgG2 F_c , nucleic acid sequence as SEQ ID NO: 28 which encodes the anti-TSLP antibody light chain sequence based on 202L3, and nucleic acid sequence as SEQ ID NO: 29 which encodes the anti-TSLP antibody light chain sequence based on 202L4.

Nucleotide sequence for anti-TSLP humanized antibody heavy chain based on 202H2 with IgG2 F_c (SEQ ID NO: 27)

caagtgcagctggtgcagtcgagccgaggtgaagaagcccggtccagcgtgaaggtgagctgcaaagcctccggctacaccttca
gcagctactgggtgaactgggtcagacaagccccggccaaggactggagtggtggccagatctatccggcgacggcgacaccga

ttacgccagaaggtccaaggcagagccacactgaccgccgacaagtccaccagcaccgcctacatggagctgagctctctgaggagcg
 aggataccgccgtgtacttctgcgctagaggcacctactacaacaactactacggcaccgactactggggccaaggcaccaccgtgaccg
 tgagcagcgccagcaccaagggcccatccgtcttccccctggccccttgctccagaagcacctccgagagcacagccgccctcggatgt
 ctggtgaaagactacttccccgagcctgtgaccgtgagctggaaacagcggcgccctgacaagcggcgtgcataccttctcctgcccgtgctgc
 agagcagcgccctgtactccctgtccagcgtggtgaccgtgcccagcagcaatttcggcaccagacctacacctgtaacgtggatcaca
 agccctccaacaccaaagtggacaagaccgtggagagggaagtgtgtgtggaatgcccccttgctcctgccccctcccgtggctggcccca
 gcgtgttctcttccccccaagcccaaggacacctcatgatcagcagaacacccgaggtgacctgcgtcgtggtggacgtgtccacga
 ggaccccgaggtgcagttcaactggtagcgtggacggcggtggaggtgcacaacgccaagaccaagcccagggaggagcagttcaattcc
 acctcaggggtggtgagcgtgctgaccgtggtgcaccaggactggctgaacggcaaggagtacaagtgaaggtgagcaacaagggcc
 tgcccccccccatcgaaaagaccatttccaaaaccaaaggccagcccagggagccccaggtgtacacactgccccccagcagagagga
 gatgacaaagaaccaggtgagcctgacatgcctggtgaagggtttaccctagcgacatcgctgtggagtgggagagcaacggccagc
 ccgagaacaactacaagacaacccctcccatgctggattccgatggctccttcttctgtactccaagctgaccgtggacaagagcaggtg
 gcagcagggcaacgtgttctctgttccgtgatgcatgaggccctgcacaaccactacaccagaagtccctgagcctgagccccggcaa
 g

Nucleotide sequence for anti-TSLP humanized antibody light chain based on 202L3 (SEQ ID NO: 28)

gatatccagctgaccagagccccagcttctgagcgtagcgtgggagacagagtgaccatcacatgcagcgtgtccagcagcatcagc
 agcagcaatctgcactggtagcagcagaagcccggcaaggcccccaagccttgatctacggaaccagcaatctggccagcggcgtgc
 ctgacagatttccggatccggaagcggcaccgagtagacactgaccatcagctctctgcagcccgaagacgccgtacctactactgcca
 gcagtggagcagctacctctgaccttggccaaggcaccgaagctggagatcaagcgtacggtggctgcaccatctgttctcatcttccg
 ccatctgatgagcagttgaaatctggaactgcctctgtgtgtgctgctgaataacttctatcccagagaggccaaagtacagtggaggtg
 gataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctga
 cgtgagcaaaagcagactacgagaaacaaaagtctacgcctgcgaagtacccatcagggcctgagctcgcgccgtcacaagagcttc
 aacaggggagagtgt

Nucleotide sequence for anti-TSLP humanized antibody light chain based on 202L4 (SEQ ID NO: 29)

gagatcgtgtgaccagagccccgattccagtcctgacccccaaaggagaaggtagaccattacatgctccgtgagcagcagcatcagc
 agcagcaatctgcactggtagcagcagaagcccgatcagagccccaaagccttgatttacggcacaagcaatctggccagcggagtgcc

ctccagattcagcggcagcgggaagcggcaccgactacacactgaccatcaactctctggaggccgaggatgccgccactactactgcc
agcagtgaggagcagctacccctctgaccttcggccaaggcaccaagctggaaatcaagcgtacgggtggctgcaccatctgtcttcatcttcccg
ccatctgatgagcagttgaaatctggaactgcctctgtgtgtgcctgctgaataacttctatccagagaggccaaagtacagtggaaagtg
gataacgcccctccaatcgggtaactccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagcacccctga
cgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtacccatcagggcctgagctcgcgccgtcacaagagcttc
aacaggggagagtgt

The nucleic acids can be inserted into vectors, *e.g.*, nucleic acid expression vectors and/or targeting vectors. Such vectors can be used in various ways, *e.g.*, for the expression of anti-TSLP binding antibody or antibody fragment in a cell or transgenic animal. Vectors are typically selected to be functional in the host cell in which the vector will be used. A nucleic acid molecule encoding anti-TSLP binding antibody or fragment may be amplified / expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether the anti-TSLP binding antibody or fragment is to be post-translationally modified (*e.g.*, glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host cells are preferable. Expression vectors typically contain one or more of the following components: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element.

In most cases, a leader or signal sequence is engineered at the N-terminus of the anti-TSLP antibodies or fragments to guide its secretion. The secretion of anti-TSLP antibodies or fragments from a host cell will result in the removal of the signal peptide from the antibody or fragment. Thus, the mature antibody or fragment will lack any leader or signal sequence. In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various pre-sequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a signal peptide, or add pro-sequences, which also may affect glycosylation.

The disclosure further provides a cell (*e.g.*, an isolated or purified cell) comprising a nucleic acid or vector of the disclosure. The cell can be any type of cell capable of being

transformed with the nucleic acid or vector of the disclosure so as to produce a polypeptide encoded thereby. To express the anti-TSLP binding antibodies or fragments, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences.

Methods of introducing nucleic acids and vectors into isolated cells and the culture and selection of transformed host cells *in vitro* are known in the art and include the use of calcium chloride-mediated transformation, transduction, conjugation, triparental mating, DEAE, dextran-mediated transfection, infection, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, direct microinjection into single cells, and electroporation.

After introducing the nucleic acid or vector of the disclosure into the cell, the cell is cultured under conditions suitable for expression of the encoded sequence. The antibody, antigen binding fragment, or portion of the antibody then can be isolated from the cell.

In certain embodiments, two or more vectors that together encode anti-TSLP binding antibodies, or antigen binding fragments thereof, can be introduced into the cell.

Purification of anti-TSLP binding antibodies or fragments which have been secreted into the cell media can be accomplished using a variety of techniques including affinity, immunoaffinity or ion exchange chromatography, molecular sieve chromatography, preparative gel electrophoresis or isoelectric focusing, chromatofocusing, and high-pressure liquid chromatography. For example, antibodies comprising a F_c region may be purified by affinity chromatography with Protein A, which selectively binds the F_c region.

Modified forms of an antibody or antigen binding fragment may be prepared with affinity tags, such as hexahistidine or other small peptide such as FLAG (Eastman Kodak Co., New Haven, Conn.) or myc (Invitrogen) at either its carboxyl or amino terminus and purified by a one-step affinity column. For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of polyhistidine-tagged selective binding agents. In some instances, more than one purification step may be employed.

Modified forms of an antibody or antigen binding fragment may be prepared with affinity tags, such as hexahistidine or other small peptide such as FLAG (Eastman Kodak Co., New

Haven, Conn.) or myc (Invitrogen) at either its carboxyl or amino terminus and purified by a one-step affinity column. For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of polyhistidine-tagged selective binding agents. In some instances, more than one purification step may be employed.

TSLP

Thymic stromal lymphopoietin, TSLP, is an epithelial cell derived protein belonging to a cytokine family that is implicated in allergic inflammation (Cianferoni and Spergel 2014). It is produced mainly by non-hematopoietic cells such as fibroblasts, epithelial cells and different - like cells, and plays an important role in the maturation of T cell populations through activation of antigen presenting cells. TSLP is produced in response to proinflammatory stimuli and drives allergic inflammatory responses mainly by influence on dendritic cells for T helper (Th) 2 cytokine production.

Human TSLP has two isoforms. The long form of TSLP, lftTSLP, is expressed in several tissues including heart, liver and prostate and induces release of T-cell attracting chemokines from monocytes and enhances the maturation of CD11c-dendritic cells. It can induce allergic inflammation by directly activating mast cells (Reche, Soumelis *et al.* 2001, Zhang and Zhou 2012). Isoform 2 is the short form of TSLP, sftTSLP, and is the predominant form in keratinocytes of oral mucosa, skin and in salivary glands, and may function as an antimicrobial peptide (Bjerkkan, Sonesson *et al.* 2016). Human TSLP is mapped to chromosome 5q22.1 (Quentmeier, Drexler *et al.* 2001).

The amino acid sequence of lftTSLP is provided as SEQ ID No. 30, of which the first 28 amino acids constitute the signal peptide and Y29 to Q159 is the mature form. The amino acid sequence of sftTSLP is provided as SEQ ID No. 31.

Amino acid sequence for long form TSLP lftTSLP (SEQ ID NO: 30)

MFPFALLYVLSVSFRKIFILQLVGLVLTVDFTNCDFEKIKAAAYLSTISKDLITYMSGTKST
EFNNTVSCSNRPHCLTEIQSLTFNPTAGCASLAKEMFAMKTKAALAIWCPGYSETQINA
TQAMKKRRKRKVTNNKCLEQVSQQLQGLWRRFNRPLLKQQ

Amino acid sequence for short form TSLP sFTSLP (SEQ ID NO: 31)

MFAMKTKAALAIWCPGYSETQINATQAMKKRRKRKVTTNKCLEQVSQQLQGLWRRFNR
PLLKQQ

TSLP receptor (TSLPR) binds TSLP with low affinity and is a member of the hematopoietin receptor family (Park, Martin *et al.* 2000). A combination of IL-7 receptor alpha chain (IL-7R α) and TSLPR is required for high affinity binding of TSLP to form the ternary complex (Verstraete, van Schie *et al.* 2014). The ternary complex is necessary for cell proliferation and signalling.

After binding of TSLP, STAT5 phosphorylation is induced resulting in expression of downstream transcription factors. Although initial results suggest that TSLP engagement on TSLPR complex activates STAT5 without detectable JAK activation, recent results demonstrate that JAK1 and JAK2, which bind to IL-7R α and TSLPR chain respectively, is required for TSLP-mediated STAT5 activation (Rochman, Kashyap *et al.* 2010). Other signalling pathways stimulated by many cytokines, such as activation of ERK1,2 and p70S6K, are not involved in TSLP activity (Quentmeier, Drexler *et al.* 2001).

The homology between mouse and human TSLP is 43% and 39% for the TSLPR, with no cross reactivity between the species. Despite the poor amino acid sequence identity between murine and human TSLP and murine and human TSLPR, the fact that high affinity binding of TSLP to its receptor in both species requires IL-7R α suggest that human TSLP and TLPLR are orthologs to mouse TSLP and TSLPR. Thus, mouse models have been used for various biological studies, including use of a chimeric human TSLP and human TSLPR transgenic mouse strain (Francis, Milford *et al.* 2016).

TSLP expression is linked to many disease states including asthma (Ying, O'Connor *et al.* 2005), inflammatory arthritis (Koyama, Ozawa *et al.* 2007), inflammatory bowel disease (Park, Jeong *et al.* 2017), atopic dermatitis (Ebner, Nguyen *et al.* 2007), eczema, eosinophilic esophagitis and other allergic states (Soumelis and Liu 2004), (Soumelis, Reche *et al.* 2002). Understanding the mechanism of TSLP production and those potential substances that block the production may enable better methods to prevent or treat these conditions. Thus, targeting TSLP

may inhibit multiple biologic pathways involved in asthma and Th2 cytokine mediated inflammatory disorders (Gauvreau , O'Byrne *et al.* 2014).

Binding and functional activity of anti-TSLP antibodies

The present disclosure encompasses anti-TSLP monoclonal antibodies or antibody fragments that bind selectively to TSLP with greater affinity than to other antigens. The anti-TSLP monoclonal antibodies and fragments may bind selectively to human TSLP, but also bind detectably to non-human TSLP. Alternatively, or additionally, TSLP binding antibodies and antibody fragments may have the same or substantially the same potency against recombinant human TSLP and endogenous human TSLP.

In vitro and cell-based assays are well described in the art for use in determining binding of TSLP antibodies to its target. For example, the binding of TSLP to their receptors may be determined by immobilizing a TSLP binding antibody, sequestering TSLP with the immobilized antibody and determining whether the TSLP is bound to the antibody, and contacting a soluble form of receptor with the bound TSLP/antibody complex and determining whether the soluble receptor is bound to the complex. The protocol may also include contacting the soluble receptors with the immobilized antibody before the contact with TSLP antibody, to confirm that the soluble receptor does not bind to the immobilized antibody. This protocol can be performed using a Biacore® instrument for kinetic analysis of binding interactions. Such a protocol can also be employed to determine whether an antibody or other molecule permits or blocks the binding of TSLP to their receptors.

For other TSLP /receptor binding assays, the permitting or blocking of TSLP binding to its receptor may be determined by comparing the binding of TSLP to its receptor in the presence or absence of TSLP antibodies or TSLP binding fragments thereof. Blocking is identified in the assay readout as a designated reduction of TSLP binding to its receptor in the presence of anti-TSLP antibodies or TSLP binding fragments thereof, as compared to a control sample that contains the corresponding buffer or diluent but not an TSLP antibody or TSLP binding fragment thereof. The assay readout may be qualitatively viewed as indicating the presence or absence of blocking or may be quantitatively viewed as indicating a percent or fold reduction in binding due to the presence of the antibody or fragment. When an TSLP binding antibody or TSLP binding

fragment substantially blocks TSLP binding to its receptor, the TSLP binding to its receptor is reduced by at least 10-fold, alternatively at least about 20-fold, alternatively at least about 50-fold, alternatively at least about 100-fold, alternatively at least about 1000-fold, alternatively at least about 10000-fold, or more, compared to binding of the same concentrations of TSLP and its receptor in the absence of the antibody or fragment.

Preferred anti- TSLP antibodies for use in accordance with the disclosure generally bind to human TSLP with high affinity (*e.g.*, as determined with BIACORE), such as for example with an equilibrium binding dissociation constant (KD) for TSLP of about 10 nM or less, about 5 nM or less, about 1 nM or less, about 500 pM or less, or more preferably about 250 pM or less, about 100 pM or less, about 50 pM or less, about 25 pM or less, about 10 pM or less, about 5 pM or less, about 3 pM or less about 1 pM or less, about 0.75 pM or less, about 0.5 pM or less, or about 0.3 pM or less.

Antibodies or fragments of the present disclosure may, for example, bind to TSLP with an EC50 of about 10 nM or less, about 5 nM or less, about 2 nM or less, about 1 nM or less, about 0.75 nM or less, about 0.5 nM or less, about 0.4 nM or less, about 0.3 nM or less, or even about 0.2 nM or less, as determined by enzyme linked immunosorbent assay (ELISA).

Preferably, the antibody or antibody fragment of the present disclosure does not cross-react with any target other than TSLP. For example, the present antibodies and fragments may bind to TSLP, but do not detectably bind to, or have at least about 100 times (*e.g.*, at least about 150 times, at least about 200 times, or even at least about 250 times) greater selectivity in its binding of TSLP relative to its binding to other proteins.

The key amino acid residues (epitope) bound by the TSLP binding antibody or fragment described in this disclosure may be determined using a peptide array, such as for example, a PepSpot™ peptide array (JPT Peptide Technologies, Berlin, Germany), wherein a scan of twelve amino-acid peptides, spanning the entire TSLP amino acid sequence, each peptide overlapping by 11 amino acid to the previous one, is synthesized directly on a membrane. Alternatively, or in addition, antibody competition experiments may be performed and such assays are well known in the art.

The present disclosure also encompasses neutralizing antibodies or neutralizing fragments thereof which bind to TSLP so as to neutralize its biological activity. Neutralization

of biological activity of TSLP can be assessed by assays for one or more indicators of biological activity, such as TSLP stimulated reporter gene expression in a reporter assay. Neutralization of biological activity of TSLP can also be assessed *in vivo* by animal models of asthma or atopic dermatitis. Preferably the TSLP binding antibodies and fragments of the present disclosure neutralize the biological activity of TSLP connected with the signalling function of their receptors bound by the TSLP.

The present antibodies or fragments may be neutralizing antibodies or fragments which bind specifically to TSLP epitopes that affects biological activity of TSLP. The present antibodies or fragments can bind to neutralization-sensitive epitopes of TSLP. When a neutralization-sensitive epitope of TSLP is bound by one of the present antibodies or fragments, the result is a loss of biological activity containing the epitope.

In certain embodiments, an antibody or fragment thereof of the present disclosure can neutralize, inhibit, block, abrogate, reduce or interfere with, an activity of TSLP by binding to an epitope of TSLP that is directly involved in the targeted activity of TSLP. In another embodiment, an antibody or fragment thereof of the disclosure can neutralize, inhibit, block, abrogate, reduce or interfere with, an activity of TSLP by binding to an epitope of TSLP that is not directly involved in the targeted activity of TSLP, but the antibody or fragment binding thereto sterically or conformationally inhibits, blocks, abrogates, reduces or interferes with, the targeted activity of TSLP. In yet another embodiment, an antibody or fragment thereof of the disclosure binds to an epitope of TSLP that is not directly involved in the targeted activity of TSLP (*i.e.*, a non-blocking antibody), but the antibody or fragment binding thereto results in the enhancement of the clearance of TSLP.

In certain embodiments, the antibody or antigen-binding fragment thereof: a) inhibits binding of TSLP and TSLPR; b) reduces the expression level of an TSLP-dependent gene; c) inhibits TSLP-induced cell proliferation; d) inhibits TSLP-induced STAT5 activation; and/or e) inhibits TSLP-induced CCL17 production from primary human dendritic cells.

Pharmaceutical Compositions

TSLP binding antibodies and antibody fragments for use according to the present disclosure can be formulated in compositions, especially pharmaceutical compositions, for use in

the methods herein. Such compositions comprise a therapeutically or prophylactically effective amount of an TSLP binding antibody or antibody fragment of the disclosure in admixture with a suitable carrier, *e.g.*, a pharmaceutically acceptable agent. Typically, TSLP binding antibodies and antibody fragments of the disclosure are sufficiently purified for administration to an animal before formulation in a pharmaceutical composition.

Pharmaceutically acceptable agents include carriers, excipients, diluents, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, and surfactants.

The composition can be in liquid form or in a lyophilized or freeze-dried form and may include one or more lyoprotectants, excipients, surfactants, high molecular weight structural additives and/or bulking agents.

Compositions can be suitable for parenteral administration. Exemplary compositions are suitable for injection or infusion into an animal by any route available to the skilled worker, such as intraarticular, subcutaneous, intravenous, intramuscular, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intralesional, intrarectal, transdermal, oral, and inhaled routes.

Pharmaceutical compositions described herein can be formulated for controlled or sustained delivery in a manner that provides local concentration of the product (*e.g.*, bolus, depot effect) sustained release and/or increased stability or half-life in a particular local environment.

Methods of Use

The present antibodies and fragments are useful for the prophylaxis and treatment of TSLP-mediated diseases or medical conditions, *e.g.*, asthma, allergic rhinitis, atopic dermatitis, allergies, and certain cancers.

One aspect of the disclosure provides a method of treating moderate to severe uncontrolled asthma, including asthma that is not Th2 driven, by administering to a subject in need thereof a therapeutically effective amount of any one of the subject antibody or antigen binding fragment thereof and the subject pharmaceutical composition.

Another aspect of the disclosure provides a method of treating a fibrotic disease and related condition, including systemic sclerosis, systemic idiopathic pulmonary fibrosis, and keloidal disease, by administering to a subject in need thereof a therapeutically effective amount of any one of the subject antibody or antigen binding fragment thereof and the subject pharmaceutical composition.

Another aspect of the disclosure provides a method of treating chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, and refractory asthma, by administering to a subject in need of a therapeutically effective amount of any one of the subject antibody or antigen binding fragment thereof and the subject pharmaceutical composition.

Another aspect of the disclosure provides a method of treating disorders associated with inflammatory diseases, including rhinitis, atopic dermatitis, eczema, food allergies, and inflammatory bowel disease; the method comprising administering to a subject in need of a therapeutically effective amount of any one of the subject antibody or antigen-binding fragments thereof and the subject pharmaceutical composition.

Another aspect of the disclosure provides a method of treating certain cancers, including lung, breast, pancreatic, colorectal, lymphoblastic leukemia, head and neck carcinomas; the method comprising administering to a subject in need of a therapeutically effective amount of any one of the subject antibody or antigen-binding fragments thereof and the subject pharmaceutical composition.

An aspect of the disclosure also provides uses of any of the antibodies or antigen binding fragments thereof provided herein, in a the manufacture of a medicament for blocking the binding of TSLP to its receptor TLPR, and/or a medicament for the prophylaxis and treatment of TSLP-mediated diseases or medical conditions such as those provided herein. An aspect of the disclosure also provides for use of the provided antibodies or antigen binding fragments thereof for blocking the binding of TSLP to its receptor TLPR and/or for in methods for the prophylaxis and treatment of TSLP-mediated diseases or medical conditions such as those provided herein. For example, in embodiments, the disclosure provides uses the antibodies provided herein in methods for treating asthma, COPD, idiopathic pulmonary fibrosis, atopic dermatitis, eczema, eosinophilic esophagitis, inflammatory bowel disease, systemic sclerosis, systemic idiopathic pulmonary fibrosis, keloidal disease, or cancer.

In addition to therapeutic uses, the present antibodies and fragments can be used in diagnostic methods to detect TSLP (for example, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (Rizos C.V.), an radioimmunoassay (RIA) or tissue immunohistochemistry.

A method for detecting TSLP in a biological sample can comprise the steps of contacting a biological sample with one or more of the present antibodies or fragments and detecting either the antibody or fragment bound to TSLP or unbound antibody or fragment, to thereby detect TSLP in the biological sample. The antibody or fragment can be directly or indirectly labelled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

EXAMPLES

The following examples are provided to describe the present disclosure in greater detail. They are intended to illustrate, not to limit, the present disclosure.

Example 1: TSLP binding affinity for mouse anti-TSLP antibody TAVO202

TAVO202 was identified as a mouse anti-human TSLP antibody by hybridoma screening. ELISA-based binding assay was employed to evaluate TAVO202 binding to recombinant human TSLP. In this assay, 1 µg/mL recombinant human TSLP (R&D Systems) were coated on ELISA plate. Increasing concentrations of TAVO202 antibodies were applied on the plate and their binding to the recombinant human TSLP were detected by HRP-conjugated anti-mouse secondary antibody. It was observed that TAVO202 dose-dependently bound recombinant human TSLP with EC50 at 2.4 ng/mL (Figure 1A).

The binding of TAVO202 to mouse TSLP was also evaluated in similar ELISA assay by coating the plate with mouse TSLP (R&D Systems). TAVO202 did not show significant binding affinity to mouse TSLP (Figure 1B).

Example 2: *in vitro* assays for TSLP neutralization by TAVO202

A TSLP-mediated cell proliferation assay was developed to assess functional activity of TAVO202. In this assay, human IL-7 receptor alpha (IL-7R α) and TSLP receptor (TSLPR) were co-transfected into BAF3 mouse pro-B cells. Recombinant human TSLP was added to the transfected cells and cell proliferation was quantitated with a cell proliferation assay two days later. It was observed that recombinant human TSLP could stimulate the proliferation of the transfected BAF3 cell line in a dose-dependent manner with EC50 at 0.18 ng/mL (Figure 2A).

To assess the functional activity of TAVO202, increasing concentrations of TAVO202 along with 0.5 ng/mL human TSLP were applied with BAF3 cells co-transfected with IL-7R α and TSLPR. It was observed that TAVO202 could dose-dependently neutralize TSLP activity in stimulating transfected BAF3 cell proliferation with IC50 at 6.6 ng/mL (Figure 2B).

Besides the cell proliferation assay, a TSLP-driven reporter gene expression assay was also developed to assess the functional activity of TAVO202. STAT5 activation is a downstream event that occurs after TSLP binds to and activates its receptor complex (TSLPR:IL-7R α). In this assay, plasmids expressing TSLPR, IL-7R α and a STAT5 luciferase reporter construct were transiently transfected into HEK293T cells. One day after transfection, recombinant human TSLP was added and TSLP-driven luciferase reporter gene expression was quantitated 24 hours later. It was observed that human TSLP could dose-dependently drive reporter gene expression with EC50 at 1.4 ng/mL (Figure 3A).

To assess the functional activity of TAVO202, increasing concentrations of TAVO202 along with 10 ng/mL human TSLP were applied with HEK293T cells co-transfected with IL-7R α , TSLPR and STAT5 luciferase reporter gene. It was observed that TAVO202 dose-dependently neutralized TSLP activity in stimulating reporter gene expression with IC50 of 20 ng/mL (Figure 3B).

Example 3: Humanization of mouse anti-human TSLP antibody TAVO202

The mouse anti- human TSLP antibody TAVO202 was humanized by grafting of mouse CDRs onto human germLine scaffolds. A few key mouse residues were preserved by back mutations to achieve higher stability and better expression while minimizing immunogenicity.

For TAVO202, one humanized V_H variant (202H2) was designed based on IGHV1-69*02 and two humanized V_L variants were designed with 202L3 and 202L4 based on IGKV1-9*01 and IGKV6-21*01 respectively with a couple of back mutations (Figure 4). By combination of the humanized V_H variant with the two humanized V_L variants, two humanized TAVO202 antibodies with IgG2 Fc, designated as TAVO6264 and TAVO6265, were generated with 202H2 variant pairing with 202L3 and 202L4 respectively (Figure 4). Likewise, two humanized TAVO202 antibodies with IgG1 Fc with L234F, L235E, D265A, F405L mutations, designated as TAVO7264 and TAVO7265, were also generated with 202H2 variant pairing with 202L3 and 202L4 respectively. Besides, two humanized TAVO202 antibodies with IgG1 Fc with L234A, L235A, M428L, N434S mutations, designated as TAVO9764 and TAVO9765, were also generated with 202H2 variant pairing with 202L3 and 202L4 respectively (Figure 4).

Example 4: Expression and purification of humanized TAVO202

Plasmids encoding heavy chains and light chains of TAVO6264, TAVO6265, TAVO7264, TAVO7265, TAVO9764, TAVO9765 were co-transfected into Expi293F cells following the transfection kit instructions (Thermo Scientific). Cells were spun down five days post transfection, and the supernatant were passed through a 0.2 µm filter. The purification of expressed antibodies in supernatant was carried out by affinity chromatography over protein A agarose column (GE Healthcare Life Sciences). The purified antibodies were buffer-exchanged into DPBS, pH7.2 by dialysis, and protein concentrations were determined by UV absorbance at 280 nm.

The purified humanized TAVO202 antibodies were subjected to SDS-PAGE analysis and an example for TAVO7264 and TAVO7265 was shown in Figure 5A and 5B. Under the reduced condition, all four antibodies showed heavy chains and light chains with the expected molecular weight. Under the non-reduced condition, all four antibodies migrated as a major protein band with a molecular weight around 150 kDa. The purified humanized TAVO202 antibodies were also assessed by Size Exclusion Chromatography (SEC). Figure 5C revealed SEC profiles of example antibodies TAVO7264 and TAVO7265.

Example 5: Binding to TSLP by humanized TAVO202

ELISA-based binding assays were employed to evaluate humanized TAVO202 antibodies binding to recombinant human TSLP antigen. In this assay, 1 µg/mL recombinant human TSLP (R&D Systems) were coated on ELISA plate. Increasing concentrations of humanized TAVO202 antibodies were applied on the plate and their binding to the recombinant human TSLP were detected by HRP-conjugated anti-mouse secondary antibody. As an example, it was observed that TAVO6264 and TAVO6265 dose-dependently bound recombinant human TSLP with similar potency and comparable to the mouse antibody TAVO202 (Figure 6A).

The binding of humanized TAVO202 antibodies on Cynomolgus monkey TSLP were also evaluated in similar ELISA assay by coating the plate with Cynomolgus monkey TSLP. None of TAVO7264 or TAVO7265 antibodies could bind to monkey TSLP (Figure 6B).

To further measure the binding of humanized antibodies to immobilized recombinant TSLP, surface plasmon resonance (SPR) binding assays will be performed using Biacore. This assay not only can measure binding affinities, but also kinetic rate constants and thermodynamics of binding.

Example 6: *In vitro* assay for TSLP neutralization by humanized TAVO202 antibodies

The TSLP-driven STAT5 luciferase reporter gene expression assay was employed to assess the functional activity of humanized TAVO202 antibodies. Increasing concentrations of TAVO6264 and TAVO6265 along with 10 ng/mL human TSLP were applied with HEK293T cells co-transfected with IL-7R α , TSLPR and STAT5 luciferase reporter gene. It was observed that both TAVO6264 and TAVO6265 could dose-dependently neutralize TSLP activity in stimulating reporter gene expression with IC₅₀ comparable to TAVO202 (Figure 7).

Example 7: Inhibition of TSLP-mediated dendritic cell activation by humanized TAVO202

TSLP can stimulate the activation of primary human CD1c⁺ blood dendritic cells with the increased secretion of CCL17 chemokine. An ex vivo assay was set up to evaluate the humanized TAVO202 antibodies in neutralizing TSLP-driven CCL17 release from activated dendritic cells. CD1c⁺ dendritic cells were isolated from donor peripheral blood mononuclear

cells (PBMC) by anti-CD1c⁺ antibody coated magnetic beads selection (Miltenyi Biotec). The isolated cells were incubated with recombinant human TSLP and the release of CCL17 was quantitated by ELISA. It was observed that 15 ng/mL recombinant human TSLP could stimulate several folds of enhanced CCL17 release from activated dendritic cells after 48 hours incubation. Both TAVO7264 and TAVO7265 could block TSLP-driven CCL17 release from activated dendritic cells when added at 1 ug/mL along with TSLP. Figure 8 showed results from assays with PBMC isolated from two different donors.

Example 8: Fc engineering of humanized antibodies for extended half-life and reduced effector functions

To improve the PK profile of humanized antibodies, Fc mutations can be introduced to IgG1 antibody to extend antibody half-life. Specifically, M428L/N434S mutations have been demonstrated to extend antibody half-life by increasing FcRn binding affinity (Booth, Ramakrishnan et al. 2018). Furthermore, L234A/L235A Fc mutations can abolish the ADCC and CDC effector functions of IgG1 antibody (Hezareh, Hessel et al. 2001). Therefore, a humanized IgG1 heavy chain based on 202H1 with L234A/L235A/M428L/N434S (AALS) mutations was generated which comprises sequence set forth as SEQ ID NO. 16. By pairing this Fc-engineered 202H1 heavy chain with either humanized 202L3 or 202L4 light chain, two humanized TAVO202 antibodies with extended half-life and reduced effector functions, were generated and designated as TAVO9764 and TAVO9765, respectively (Figure 4).

To study whether the Fc engineered antibody has improved FcRn binding affinity, the binding by TAVO9765 and TAVO7265 to mouse FcRn were assessed in ELISA-based binding assay. 1 µg/mL recombinant mouse FcRn (R&D Systems) were coated on ELISA plate. Increasing concentrations of TAVO9765 and TAVO7265 antibodies were applied on the plate and their binding to the recombinant FcRn under pH 6.0 were detected by HRP-conjugated anti-human secondary antibody. It was observed that TAVO9765, which has the Fc M428L/N434S mutations, could bind FcRn with about 10-fold better potency than TAVO7265 which is lacking such half-life extension mutations (Figure 9).

To determine whether the M428L/N434S mutations could extend circulating half-life of an anti-TSLP antibody, TAVO9765 was tested in a cynomolgus monkey PK model. TAVO9765

was administered as an intravenously infusion at 4 mg/kg into a male naïve cynomolgus monkey at a volume of 1.0 mL/kg for 3 minutes based on the body weight on day 0. Whole blood was collected into EDTA-K2 collection tubes at pre-dose, and at 1h, 2h, and on days 2, 5, 9, 12, 16, 21, 24, 26, 29, 32, 35 post-dose. Plasma was separated by centrifugation at 3500xg for 10 minutes at 4°C, and then transferred to microfuge tubes for storage at -80°C. Plasma samples were measured by a standard ELISA method to detect human IgG. Goat anti-human IgG was the capture antibody and HRP conjugated goat anti-human IgG (H+L) was the detection antibody. Dilution factor for capture and detection antibodies was 13,333 and 30,000, respectively. PK data was analyzed for half-life, C_0 , AUC, V_d , and clearance using Winnonlin 6.4 software. The PK profile of plasma concentration vs time for TAVO9765 is shown in Figure 10. PK parameters for TAVO9765 were as follows: C_0 : 131405 ng·mL⁻¹, half-life: 26.131 d, AUC_{0-∞} : 1268621 ng·d·mL⁻¹, AUC_{0-inf} : 2128728 ng·d·mL⁻¹, clearance : 1.879 mL·kg⁻¹·d⁻¹, V_d : 71.049 mL·kg⁻¹.

REFERENCES

- Angal, S., D. J. King, M. W. Bodmer, A. Turner, A. D. Lawson, G. Roberts, B. Pedley and J. R. Adair (1993). "A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody." Mol Immunol **30**(1): 105-108.
- Bedu-Addo, F. K., C. Johnson, S. Jeyarajah, I. Henderson and S. J. Advant (2004). "Use of biophysical characterization in preformulation development of a heavy-chain fragment of botulinum serotype B: evaluation of suitable purification process conditions." Pharm. Res. **21**: 1353-1361.
- Bjerkkan, L., A. Sonesson and K. Schenck (2016). "Multiple Functions of the New Cytokine-Based Antimicrobial Peptide Thymic Stromal Lymphopoietin (TSLP)." Pharmaceuticals **9**(3): 41.
- Bleecker, E. R., J. M. FitzGerald, P. Chanez, A. Papi, S. F. Weinstein, P. Barker, S. Sproule, G. Gilmartin, M. Aurivillius, V. Werkstrom and M. Goldman (2016). "Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial." Lancet **388**(10056): 2115-2127.
- Bolt, S., E. Routledge, I. Lloyd, L. Chatenoud, H. Pope, S. D. Gorman, M. Clark and H. Waldmann (1993). "The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties." Eur J Immunol **23**(2): 403-411.
- Booth, B. J., B. Ramakrishnan, K. Narayan, A. M. Wollacott, G. J. Babcock, Z. Shriver and K. Viswanathan (2018). "Extending human IgG half-life using structure-guided design." MAbs **10**(7): 1098-1110.
- Castro, M., J. Corren, I. D. Pavord, J. Maspero, S. Wenzel, K. F. Rabe, W. W. Busse, L. Ford, L. Sher, J. M. FitzGerald, C. Katelaris, Y. Tohda, B. Zhang, H. Staudinger, G. Pirozzi, N. Amin, M. Ruddy, B. Akinlade, A. Khan, J. Chao, R. Martincova, N. M. H. Graham, J. D. Hamilton, B. N. Swanson, N. Stahl, G. D. Yancopoulos and A. Teper (2018). "Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma." New England Journal of Medicine **378**(26): 2486-2496.

Castro, M., J. Zangrilli, M. E. Wechsler, E. D. Bateman, G. G. Brusselle, P. Bardin, K. Murphy, J. F. Maspero, C. O'Brien and S. Korn (2015). "Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials." Lancet Respir Med **3**(5): 355-366.

Chothia, C. and A. M. Lesk (1987). "Canonical structures for the hypervariable regions of immunoglobulins." J Mol Biol **196**(4): 901-917.

Chu, S. Y., I. Vostiar, S. Karki, G. L. Moore, G. A. Lazar, E. Pong, P. F. Joyce, D. E. Szymkowski and J. R. Desjarlais (2008). "Inhibition of B cell receptor-mediated activation of primary human B cells by coengagement of CD19 and FcγRIIb with Fc-engineered antibodies." Mol Immunol **45**(15): 3926-3933.

Cianferoni, A. and J. Spergel (2014). "The importance of TSLP in allergic disease and its role as a potential therapeutic target." Expert Rev Clin Immunol **10**(11): 1463-1474.

Comeau, M. R., T. N. DESMEDT and D. R. Fitzpatrick (2006). Methods and compositions for treating allergic inflammation. **WO 2006023791 A2**.

Comeau, M. R., J. F. Smothers, B. R. P. Yoon and C. Mehlin (2012). Methods of treating TSLP-related inflammatory disorders. **US 8,163,284 B2**

Corren, J., J. R. Parnes, L. Wang, M. Mo, S. L. Roseti, J. M. Griffiths and R. van der Merwe (2017). "Tezepelumab in Adults with Uncontrolled Asthma." New England Journal of Medicine **377**(10): 936-946.

Corren, J. and Ziegler, S.F. (2019). "TSLP: from allergy to cancer." Nature Immunology **20**:1603-1609.

Dall'Acqua, W. F., P. A. Kiener and H. Wu (2006). "Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn)." J Biol Chem **281**(33): 23514-23524.

Datta, A, Alexander, R, Sulikowski M.G, et.al. (2013) "Evidence for a functional thymic stromal lymphopoietin signaling axis in fibrotic lung disease." J Immunol **191**:4867-79.

De Monte L, et al. (2011). "Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer." J Exp Med **208**(3):469-478.

- Ebner, S., V. A. Nguyen, M. Forstner, Y. H. Wang, D. Wolfram, Y. J. Liu and N. Romani (2007). "Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells." J Allergy Clin Immunol **119**(4): 982-990.
- Francis, O. L., T. A. Milford, S. R. Martinez, I. Baez, J. S. Coats, K. Mayagoitia, K. R. Concepcion, E. Ginelli, C. Beldiman, A. Benitez, A. J. Weldon, K. Arogyaswamy, P. Shiraz, R. Fisher, C. L. Morris, X. B. Zhang, V. Filippov, B. Van Handel, Z. Ge, C. Song, S. Dovat, R. J. Su and K. J. Payne (2016). "A novel xenograft model to study the role of TSLP-induced CRLF2 signals in normal and malignant human B lymphopoiesis." Haematologica **101**(4): 417-426.
- Gauvreau, G. M., P. M. O'Byrne, L.-P. Boulet, Y. Wang, D. Cockcroft, J. Bigler, J. M. FitzGerald, M. Boedigheimer, B. E. Davis, C. Dias, K. S. Gorski, L. Smith, E. Bautista, M. R. Comeau, R. Leigh and J. R. Parnes (2014). "Effects of an Anti-TSLP Antibody on Allergen-Induced Asthmatic Responses." New England Journal of Medicine **370**(22): 2102-2110.
- Gunasekaran, K., M. Pentony, M. Shen, L. Garrett, C. Forte, A. Woodward, S. B. Ng, T. Born, M. Retter, K. Manchulenko, H. Sweet, I. N. Foltz, M. Wittekind and W. Yan (2010). "Enhancing antibody Fc heterodimer formation through electrostatic steering effects: applications to bispecific molecules and monovalent IgG." J Biol Chem **285**(25): 19637-19646.
- Gupta, S. and E. Kaisheva (2003). "Development of a multidose formulation for a humanized monoclonal antibody using experimental design techniques." AAPS PharmSci **5E8**: 2003.
- Headley MB, Zhou B, Shih WX, Aye T, Comeau MR, Ziegler SF. (2009) "TSLP conditions the lung immune environment for the generation of pathogenic innate and antigen-specific adaptive immune responses." J Immunol. 182(3):1641–1647.
- Hezareh, M., A. J. Hessel, R. C. Jensen, J. G. van de Winkel and P. W. Parren (2001). "Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1." J Virol **75**(24): 12161-12168.
- Hinton, P. R., J. M. Xiong, M. G. Johlfs, M. T. Tang, S. Keller and N. Tsurushita (2006). "An engineered human IgG1 antibody with longer serum half-life." J Immunol **176**(1): 346-356.
- Kabat, E. A., H. National Institutes of and U. Columbia (1991). Sequences of proteins of

immunological interest. Bethesda, MD, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health.

Kay, A. B. (2001). "Allergy and Allergic Diseases." New England Journal of Medicine **344**(1): 30-37.

Kinder, M., A. R. Greenplate, K. D. Grugan, K. L. Soring, K. A. Heeringa, S. G. McCarthy, G. Bannish, M. Perpetua, F. Lynch, R. E. Jordan, W. R. Strohl and R. J. Brezski (2013).

"Engineered protease-resistant antibodies with selectable cell-killing functions." J Biol Chem **288**(43): 30843-30854.

Knight, D. M., R. E. Jordan, M. Kruszynski, S. H. Tam, J. Giles-Komar, G. Treacy and G. A. Heavner (2004). "Pharmacodynamic enhancement of the anti-platelet antibody fab abciximab by site-specific pegylation." Platelets **15**(7): 409-418.

Koyama, K., T. Ozawa, K. Hatsushika, T. Ando, S. Takano, M. Wako, F. Suenaga, Y. Ohnuma, T. Ohba, R. Katoh, H. Sugiyama, Y. Hamada, H. Ogawa, K. Okumura and A. Nakao (2007). "A possible role for TSLP in inflammatory arthritis." Biochemical and Biophysical Research Communications **357**(1): 99-104.

Labrijn, A. F., A. O. Buijsse, E. T. van den Bremer, A. Y. Verwilligen, W. K. Bleeker, S. J. Thorpe, J. Killestein, C. H. Polman, R. C. Aalberse, J. Schuurman, J. G. van de Winkel and P. W. Parren (2009). "Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo." Nat Biotechnol **27**(8): 767-771.

Labrijn, A. F., J. I. Meesters, B. E. de Goeij, E. T. van den Bremer, J. Neijssen, M. D. van Kampen, K. Strumane, S. Verploegen, A. Kundu, M. J. Gramer, P. H. van Berkel, J. G. van de Winkel, J. Schuurman and P. W. Parren (2014). "Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange." Proc Natl Acad Sci U S A **110**(13): 5145-5150.

Lefranc, M. P., C. Pommie, M. Ruiz, V. Giudicelli, E. Foulquier, L. Truong, V. Thouvenin-Contet and G. Lefranc (2003). "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains." Dev Comp Immunol **27**(1): 55-77.

Leong, S. R., L. DeForge, L. Presta, T. Gonzalez, A. Fan, M. Reichert, A. Chuntharapai, K. J. Kim, D. B. Tumas, W. P. Lee, P. Gribling, B. Snedecor, H. Chen, V. Hsei, M. Schoenhoff, V.

- Hale, J. Deveney, I. Koumenis, Z. Shahrokh, P. McKay, W. Galan, B. Wagner, D. Narindray, C. Hebert and G. Zapata (2001). "Adapting pharmacokinetic properties of a humanized anti-interleukin-8 antibody for therapeutic applications using site-specific pegylation." Cytokine **16**(3): 106-119.
- Liu, Y. J., V. Soumelis, N. Watanabe, T. Ito, Y. H. Wang, W. Malefyt Rde, M. Omori, B. Zhou and S. F. Ziegler (2007). "TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation." Annu Rev Immunol **25**: 193-219.
- Maa, Y. F. and C. C. Hsu (1996). "Aggregation of recombinant human growth hormone induced by phenolic compounds." Int. J. Pharm. **140**: 155-168.
- MacLennan, D. H., W. J. Rice, A. Odermatt and N. M. Green (1998). "Structure-function relationships in the Ca(2+)-binding and translocation domain of SERCA1: physiological correlates in Brody disease." Acta Physiol Scand Suppl **643**: 55-67.
- Moret F.M, Hack C.E, van der Wurff-Jacobs KM, Radstake TR, Lafeber FP, van Roon JA. (2014) "Thymic stromal lymphopoietin, a novel proinflammatory mediator in rheumatoid arthritis that potently activates CD1c+ myeloid dendritic cells to attract and stimulate T cells." Arthritis Rheumatol. **66**(5):1176–1184.
- Ortega, H. G., M. C. Liu, I. D. Pavord, G. G. Brusselle, J. M. FitzGerald, A. Chetta, M. Humbert, L. E. Katz, O. N. Keene, S. W. Yancey and P. Chanaz (2014). "Mepolizumab treatment in patients with severe eosinophilic asthma." N Engl J Med **371**(13): 1198-1207.
- Park, J. H., D. Y. Jeong, L. Peyrin-Biroulet, M. Eisenhut and J. I. Shin (2017). "Insight into the role of TSLP in inflammatory bowel diseases." Autoimmun Rev **16**(1): 55-63.
- Park, L. S., U. Martin, K. Garka, B. Gliniak, J. P. Di Santo, W. Muller, D. A. Largaespada, N. G. Copeland, N. A. Jenkins, A. G. Farr, S. F. Ziegler, P. J. Morrissey, R. Paxton and J. E. Sims (2000). "Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor." J Exp Med **192**(5): 659-670.
- Pawankar, R. (2014). "Allergic diseases and asthma: a global public health concern and a call to action." World Allergy Organization Journal **7**(1): 1-3.
- Petkova, S. B., S. Akilesh, T. J. Sproule, G. J. Christianson, H. Al Khabbaz, A. C. Brown, L. G.

- Presta, Y. G. Meng and D. C. Roopenian (2006). "Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease." Int Immunol **18**(12): 1759-1769.
- Quentmeier, H., H. G. Drexler, D. Fleckenstein, M. Zaborski, A. Armstrong, J. E. Sims and S. D. Lyman (2001). "Cloning of human thymic stromal lymphopoietin (TSLP) and signaling mechanisms leading to proliferation." Leukemia **15**(8): 1286-1292.
- Piliponsky AM, Lahiri A, Truong, P et al. (2016) "Thymic stromal lymphopoietin improves survival and reduces inflammation in sepsis." Am J Respir Cell Mol Biol. **55**(2): 264–274.
- Reche, P. A., V. Soumelis, D. M. Gorman, T. Clifford, M. Liu, M. Travis, S. M. Zurawski, J. Johnston, Y. J. Liu, H. Spits, R. de Waal Malefyt, R. A. Kastelein and J. F. Bazan (2001). "Human thymic stromal lymphopoietin preferentially stimulates myeloid cells." J Immunol **167**(1): 336-343.
- Remmele, R. L. and W. R. Gombotz (2000). "Differential scanning calorimetry: A practical tool for elucidating stability of liquid biopharmaceuticals." Biopharm. **13**: 36-46.
- Remmele, R. L., N. S. Nightlinger, S. Srinivasan and W. R. Gombotz (1997). "Interleukin-1 receptor (IL-1R) liquid formulation development using differential scanning calorimetry." Pharm. Res. **15**: 200-208.
- Ridgway, J. B., L. G. Presta and P. Carter (1996). "'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization." Protein Eng **9**(7): 617-621.
- Rizos C.V., F. M. E. M. S. (2017). "The role of angiopoietin-like 3 in the metabolism of lipoproteins: therapeutic perspectives." Hell J Atheroscler **8**: 61-66.
- Rochman, Y., M. Kashyap, G. W. Robinson, K. Sakamoto, J. Gomez-Rodriguez, K. U. Wagner and W. J. Leonard (2010). "Thymic stromal lymphopoietin-mediated STAT5 phosphorylation via kinases JAK1 and JAK2 reveals a key difference from IL-7-induced signaling." Proc Natl Acad Sci U S A **107**(45): 19455-19460.
- Rochman, Y. and W. J. Leonard (2008). "Thymic stromal lymphopoietin: a new cytokine in asthma." Curr Opin Pharmacol **8**(3): 249-254.
- Sasaki, N. and K. Sutoh (1998). "Structure-mutation analysis of the ATPase site of

Dictyostelium discoideum myosin II." Adv Biophys **35**: 1-24.

Shields, R. L., A. K. Namenuk, K. Hong, Y. G. Meng, J. Rae, J. Briggs, D. Xie, J. Lai, A. Stadlen, B. Li, J. A. Fox and L. G. Presta (2001). "High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R." J Biol Chem **276**(9): 6591-6604.

Sokol, C. L., G. M. Barton, A. G. Farr and R. Medzhitov (2008). "A mechanism for the initiation of allergen-induced T helper type 2 responses." Nat Immunol **9**(3): 310-318.

Soumelis, V. and Y. J. Liu (2004). "Human thymic stromal lymphopoietin: a novel epithelial cell-derived cytokine and a potential key player in the induction of allergic inflammation." Springer Semin Immunopathol **25**(3-4): 325-333.

Soumelis, V., P. A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, K. Smith, D. Gorman, S. Zurawski, J. Abrams, S. Menon, T. McClanahan, R. de Waal-Malefyt Rd, F. Bazan, R. A. Kastelein and Y. J. Liu (2002). "Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP." Nat Immunol **3**(7): 673-680.

Tam, S. H., S. G. McCarthy, A. A. Armstrong, S. Somani, S. J. Wu, X. Liu, A. Gervais, R. Ernst, D. Saro, R. Decker, J. Luo, G. L. Gilliland, M. L. Chiu and B. J. Scallion (2017). "Functional, Biophysical, and Structural Characterization of Human IgG1 and IgG4 Fc Variants with Ablated Immune Functionality." Antibodies (Basel) **6**(3).

Usategui A, Criado G, Izquierdo E et al. (2013) "A profibrotic role for thymic stromal lymphopoietin in systemic sclerosis." Ann Rheum Dis **72**:2018-23.

Vafa, O., G. L. Gilliland, R. J. Brezski, B. Strake, T. Wilkinson, E. R. Lacy, B. Scallion, A. Teplyakov, T. J. Malia and W. R. Strohl (2014). "An engineered Fc variant of an IgG eliminates all immune effector functions via structural perturbations." Methods **65**(1): 114-126.

Vannella, K.M, et al. (2016). "Combinatorial targeting of TSLP, IL-25, and IL-33 in type 2 cytokine-driven inflammation and fibrosis." Sci. Transl. Med. **8**(337):337ra65.

Verstraete, K., L. van Schie, L. Vyncke, Y. Bloch, J. Tavernier, E. Pauwels, F. Peelman and S. N. Savvides (2014). "Structural basis of the proinflammatory signaling complex mediated by

TSLP." Nat Struct Mol Biol **21**(4): 375-382.

Worn, A. and A. Pluckthun (2001). "Stability engineering of antibody single-chain Fv fragments." J Mol Biol **305**(5): 989-1010.

Wu, T. T. and E. A. Kabat (1970). "An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity." J Exp Med **132**(2): 211-250.

Xu, D., M. L. Alegre, S. S. Varga, A. L. Rothermel, A. M. Collins, V. L. Pulito, L. S. Hanna, K. P. Dolan, P. W. Parren, J. A. Bluestone, L. K. Jolliffe and R. A. Zivin (2000). "In vitro characterization of five humanized OKT3 effector function variant antibodies." Cell Immunol **200**(1): 16-26.

Yang, K., A. Basu, M. Wang, R. Chintala, M. C. Hsieh, S. Liu, J. Hua, Z. Zhang, J. Zhou, M. Li, H. Phyu, G. Petti, M. Mendez, H. Janjua, P. Peng, C. Longley, V. Borowski, M. Mehlig and D. Filpula (2003). "Tailoring structure-function and pharmacokinetic properties of single-chain Fv proteins by site-specific PEGylation." Protein Eng **16**(10): 761-770.

Yates, Z., K. Gunasekaran, H. Zhou, Z. Hu, Z. Liu, R. R. Ketchum and B. Yan (2010). "Histidine residue mediates radical-induced hinge cleavage of human IgG1." J Biol Chem **285**(24): 18662-18671.

Ying G, Zhang y, Tang G, Chen S. (2015) "Functions of thymic stromal lymphopoietin in non-allergic diseases." Cell Immunol 295:144-9.

Ying, S., B. O'Connor, J. Ratoff, Q. Meng, K. Mallett, D. Cousins, D. Robinson, G. Zhang, J. Zhao, T. H. Lee and C. Corrigan (2005). "Thymic Stromal Lymphopoietin Expression Is Increased in Asthmatic Airways and Correlates with Expression of Th2-Attracting Chemokines and Disease Severity." The Journal of Immunology **174**(12): 8183-8190.

Zalevsky, J., A. K. Chamberlain, H. M. Horton, S. Karki, I. W. Leung, T. J. Sproule, G. A. Lazar, D. C. Roopenian and J. R. Desjarlais (2010). "Enhanced antibody half-life improves in vivo activity." Nat Biotechnol **28**(2): 157-159.

Zhang, Y., S. Roy, L. S. Jones, S. Krishnan, B. A. Kerwin, B. S. Chang, M. C. Manning, T. W. Randolph and J. F. Carpenter (2004). "Mechanism for benzyl alcohol-induced aggregation of

recombinant human interleukin-1 receptor antagonist in aqueous solution." J. Pharm. Sci. **93**: 3076-3089.

Zhang, Y. and B. Zhou (2012). "Functions of Thymic Stromal Lymphopoietin in Immunity and Disease." Immunologic Research **52**(3): 211-223.

Zhong, J., J. Sharma, R. Raju, S. M. Palapetta, T. S. K. Prasad, T.-C. Huang, A. Yoda, J. W. Tyner, D. van Bodegom, D. M. Weinstock, S. F. Ziegler and A. Pandey (2014). "TSLP signaling pathway map: a platform for analysis of TSLP-mediated signaling." Database : the journal of biological databases and curation **2014**: bau007-bau007.

Ziegler, S. F., F. Roan, B. D. Bell, T. A. Stoklasek, M. Kitajima and H. Han (2013). "The biology of thymic stromal lymphopoietin (TSLP)." Advances in pharmacology (San Diego, Calif.) **66**: 129-155.

WHAT IS CLAIMED:

1. An isolated antibody or an antigen-binding fragment thereof that specifically binds to thymic stromal lymphopoietin TSLP and neutralizes, inhibits, blocks, abrogates, reduces, or interferes with an activity of TSLP.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or fragment is a mouse monoclonal antibody or antibody fragment with the heavy chain variable region set forth as SEQ ID NO: 1 and the light chain variable region set forth as SEQ ID NO: 2.
3. The antibody or antigen-binding fragment of claim 1, wherein the antibody or fragment comprises complementarity determining region (CDR) sequences HCDR1, HCDR2, HCDR3 of the heavy chain as according to SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, respectively.
4. The antibody or antigen-binding fragment of claim 1, wherein the antibody or fragment comprises complementarity determining region (CDR) sequences LCDR1, LCDR2, LCDR3 of the light chain as according to SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively.
5. The antibody or antigen-binding fragment of claim 1, wherein the antibody or fragment is a humanized antibody or antibody fragment.
6. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody or fragment comprises a heavy chain variable region set forth as SEQ ID NO: 9.
7. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody or fragment comprises a light chain variable region set forth as SEQ ID NO: 10.
8. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody or fragment comprises a light chain variable region set forth as SEQ ID NO: 11.
9. The antibody or antigen-binding fragment of claim 5, wherein the antibody or fragment comprises complementarity determining region (CDR) sequences HCDR1, HCDR2, HCDR3 of the heavy chain according to SEQ ID NO: 3, SEQ ID NO: 4 and

- SEQ ID NO: 5, respectively, and LCDR1, LCDR2, LCDR3 of the light chain according to SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively, and wherein the antibody or antigen-binding fragment comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO: 9 and a light chain variable region having at least 80% sequence identity to SEQ ID NO: 10 or 11.
10. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody comprises a human IgG heavy chain sequence set forth as SEQ ID NO: 12.
 11. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody comprises a human light chain sequence set forth as SEQ ID NO: 13.
 12. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody comprises a human light chain sequence set forth as SEQ ID NO: 14.
 13. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody comprises a human IgG heavy chain sequence set forth as SEQ ID NO: 15.
 14. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody comprises a human IgG heavy chain sequence set forth as SEQ ID NO: 16.
 15. The antibody or antigen-binding fragment of claims 1 to 14, wherein the antibody is an IgG₁, IgG₂, IgG₃ or IgG₄ isotype.
 16. The antibody or antigen-binding fragment of claims 1 to 14, wherein the antibody has one or more F_c mutations that extend the half-life of the engineered antibody compared to the parental wild-type antibody.
 17. The antibody or antigen-binding fragment of claims 1 to 14, wherein the antibody has one or more F_c mutations that enhance the engineered antibody resistance to proteolytic degradation by a protease compared to the parental wild-type antibody.
 18. The antibody or antigen-binding fragment of claims 1 to 14, wherein the antibody has one or more F_c mutations that reduce or eliminate the effector functions of engineered antibody compared to the parental wild-type antibody.
 19. The antibody or antigen-binding fragment of claims 1 to 14, wherein the antibody has L234A, L235A, M428L and N434S F_c mutations that extend the half-life and reduce the effector functions of the engineered antibody, residue numbering according to the EU Index, compared to the parental wild-type antibody.

20. The antibody or antigen-binding fragment of claim 5, wherein the humanized antibody or fragment comprises a human IgG1 heavy chain with L234A, L235A, M428L, N434S Fc mutations set forth as SEQ ID NO: 16.
21. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment blocks the binding of human TSLP to its receptor complex.
22. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment neutralizes, reduces, or interferes the functional activity of TSLP to its receptor complex.
23. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment neutralizes the TSLP-driven reporter gene activation in STAT5 reporter gene assay.
24. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment neutralizes the TSLP-driven proliferation of cells expressing TSLP receptor complex in cell proliferation assay.
25. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment inhibits the TSLP-driven cytokine release from human dendritic cells in cytokine release assay.
26. The antibody or antigen-binding fragment of claims 1 to 20, wherein the antibody or fragment inhibits the TSLP-driven asthma and lung inflammation in animal asthma model.
27. An isolated polynucleotide encoding the anti-TSLP IgG antibody thereof as in claims 1-20.
28. A vector comprising the polynucleotide of claim 27.
29. The vector of claim 28, which is an expression vector.
30. A host cell comprising the vector of claim 28 or 29.
31. A method of producing the anti-TSLP IgG antibody or fragment as in claims 1-20, comprising culturing the host cell of claim 29 in conditions wherein the anti-TSLP IgG antibody or fragment is expressed, and isolating the anti-TSLP IgG antibody or fragment.

32. A method of measuring the half-life of the anti-TSLP IgG antibody or fragment as in claims 1-20.
33. A method of measuring the resistance to proteolytic degradation of the anti-TSLP IgG antibody or fragment as in claims 1-20.
34. A method of measuring the effector functions of the anti-TSLP IgG antibody or fragment as in claims 1-20.
35. A method for treating a TSLP mediated disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of the anti-TSLP IgG antibody or fragment according to claims 1-20.
36. The method according to claim 35, wherein the TSLP mediated disease or disorder is asthma, including moderate to severe uncontrolled asthma.
37. The method according to claim 35, wherein the TSLP mediated disease or disorder is a fibrotic disease and related condition, including systemic sclerosis, systemic idiopathic pulmonary fibrosis, and keloidal disease.
38. The method according to claim 35, wherein the TSLP mediated disease or disorder is chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, and refractory asthma.
39. The method according to claim 35, wherein the TSLP mediated disease or disorder is an inflammatory disease, including rhinitis, atopic dermatitis, eczema, systemic sclerosis, food allergies, and inflammatory bowel disease.
40. The method according to claim 35, wherein the TSLP mediated disease or disorder is certain cancers, including lung, breast, pancreatic, colorectal, lymphoblastic leukemia, head and neck carcinomas.
41. The method according to any one of claims 35-40, further comprising administering a second agent to the subject in need of treatment.
42. The method of claim 41, wherein the second agent is a selected from the group consisting of corticosteroid, bronchodilator, antihistamine, anti-leukotriene, PDE-4 inhibitor, anti-cancer drugs, immunomodulatory drugs, and cytokine therapy drugs.

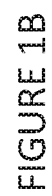
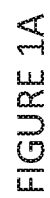


FIGURE 2

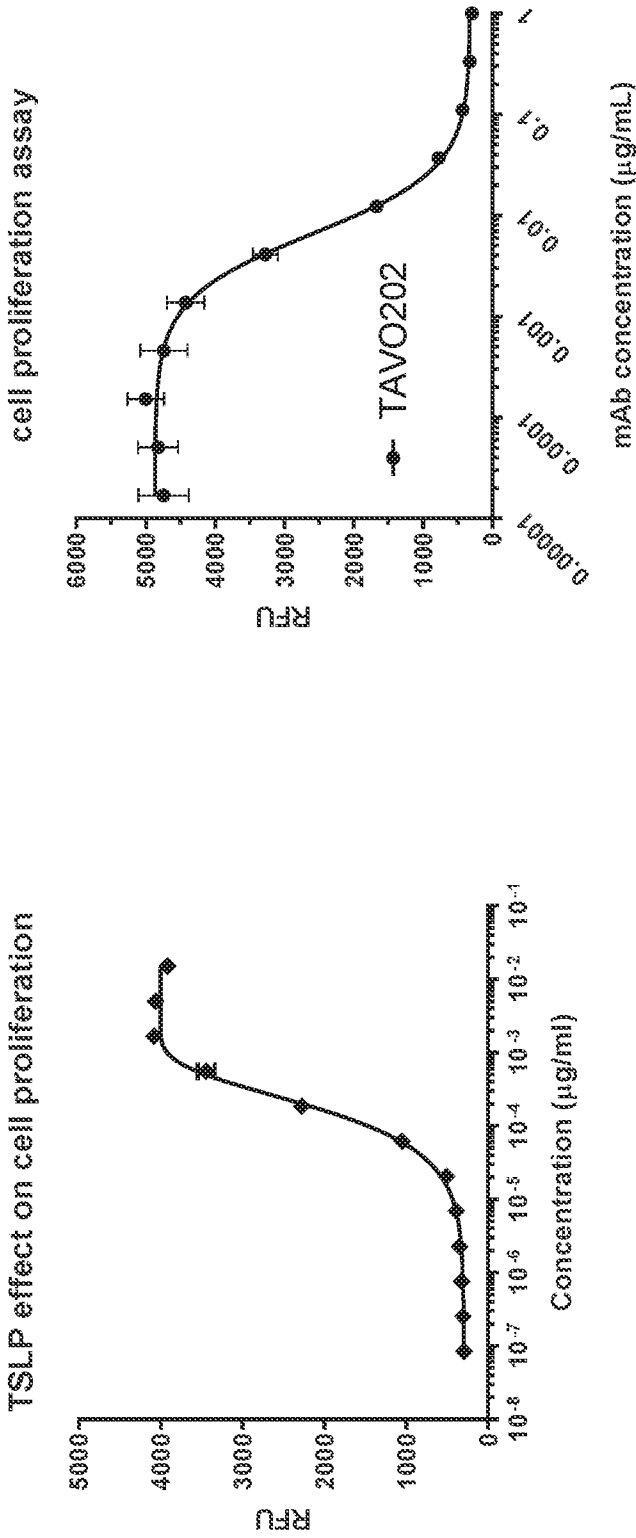


FIGURE 2B

FIGURE 2A

FIGURE 3

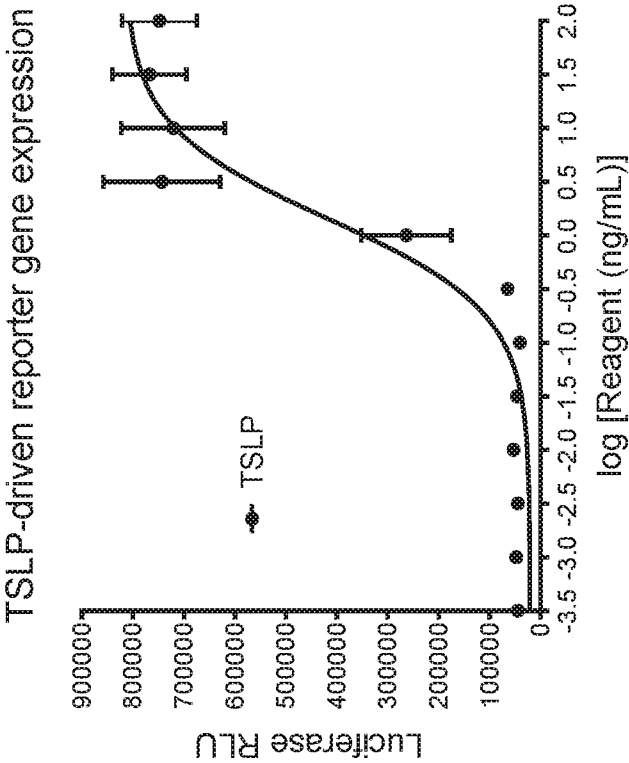


FIGURE 3A

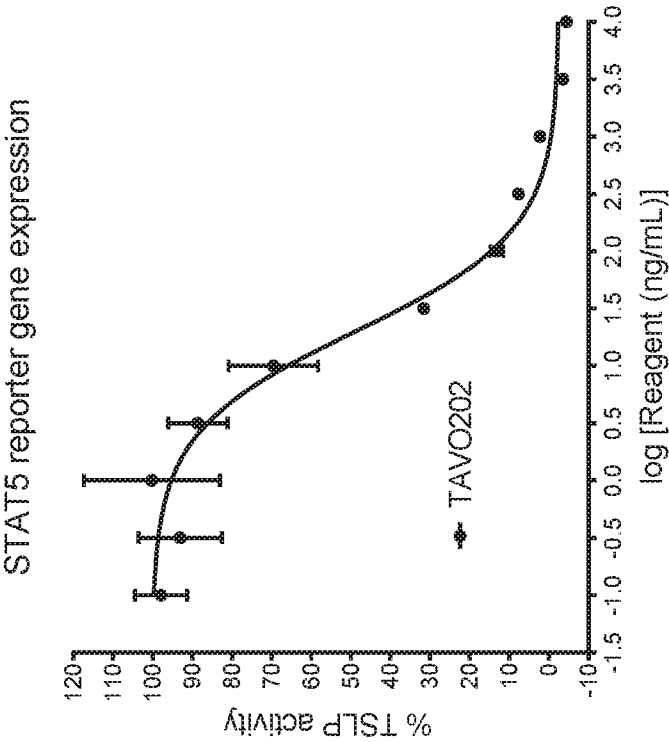
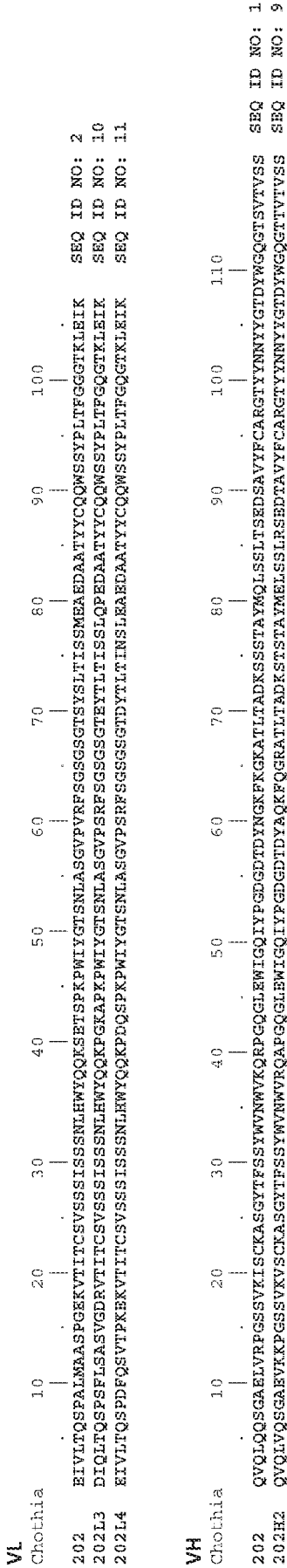


FIGURE 3B

FIGURE 4



CDRs are underscored

FIGURE 5

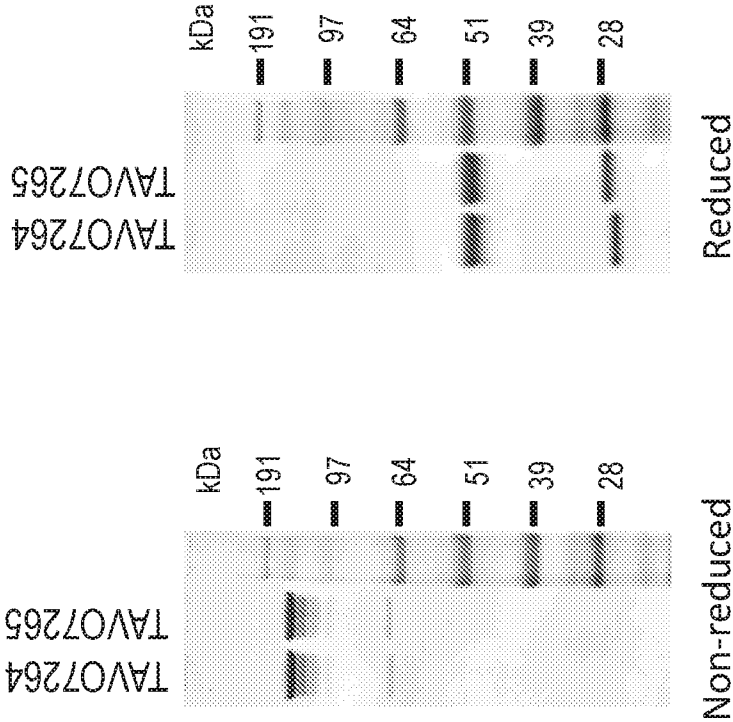


FIGURE 5A

FIGURE 5B

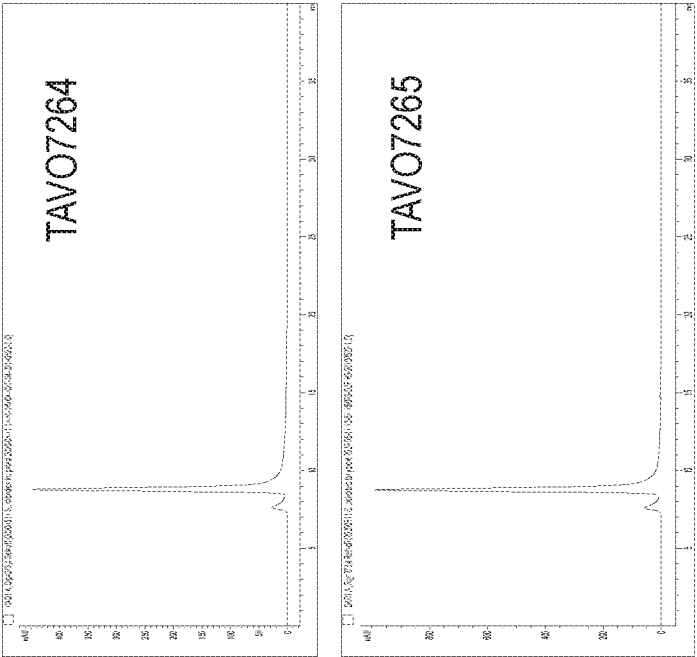


FIGURE 5C

FIGURE 6

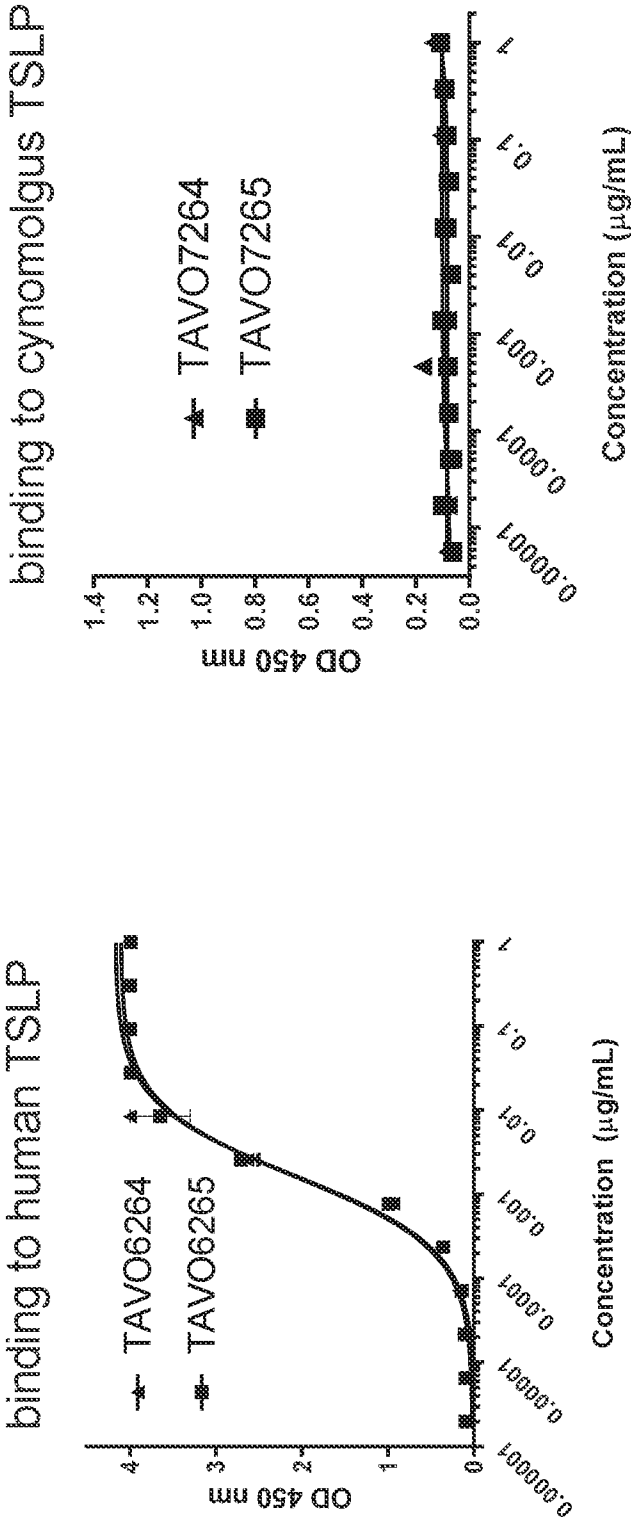


FIGURE 6A

FIGURE 6B

FIGURE 7

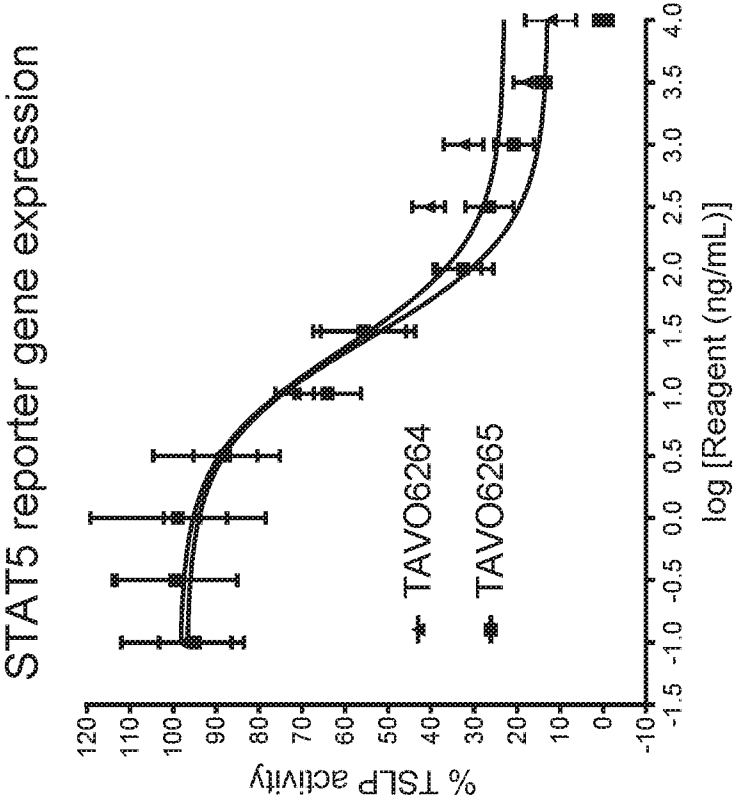


FIGURE 8

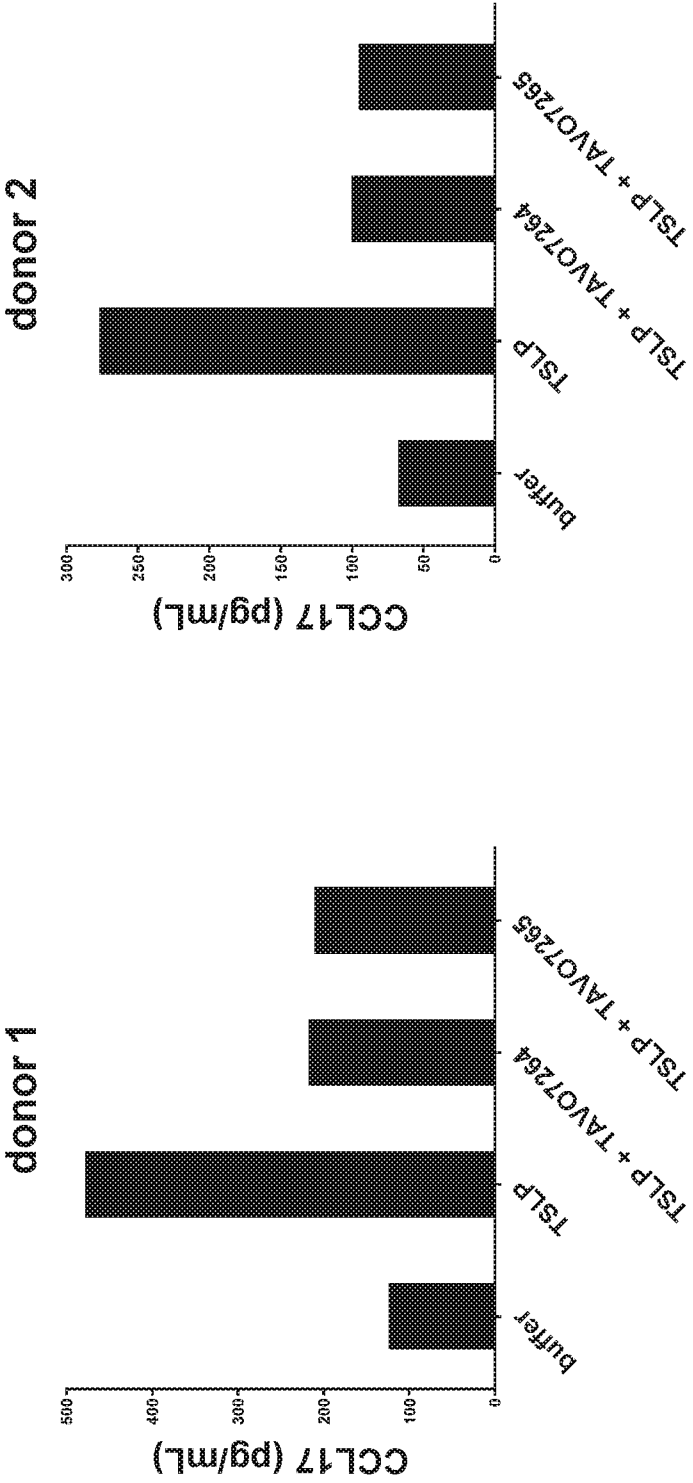


FIGURE 9

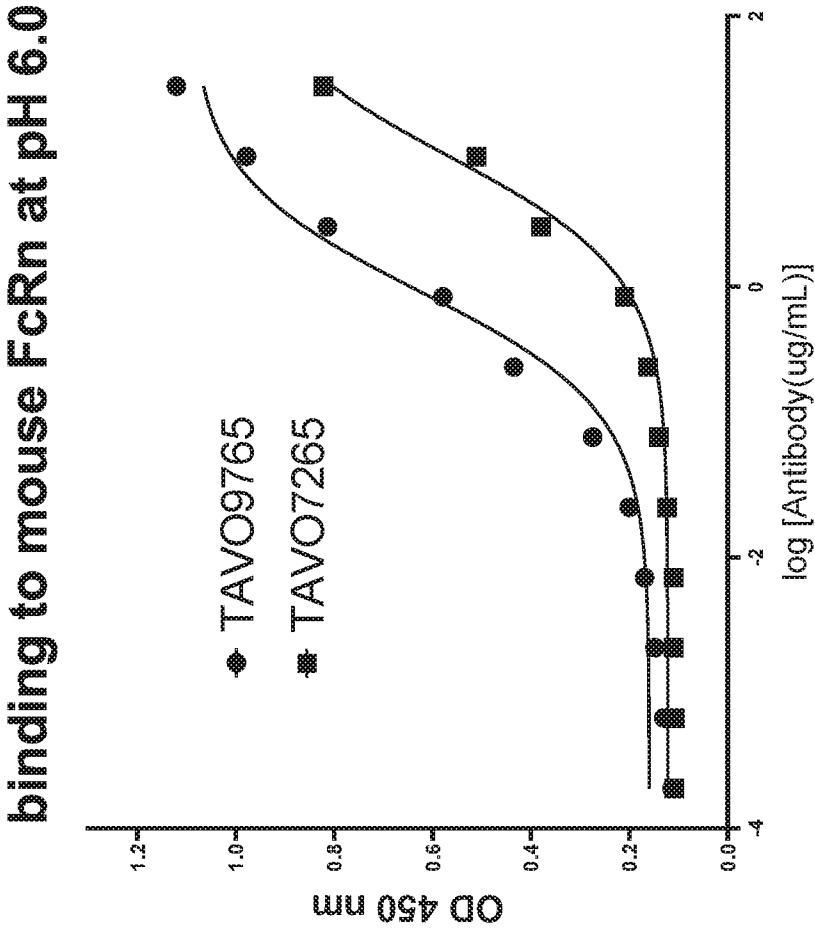
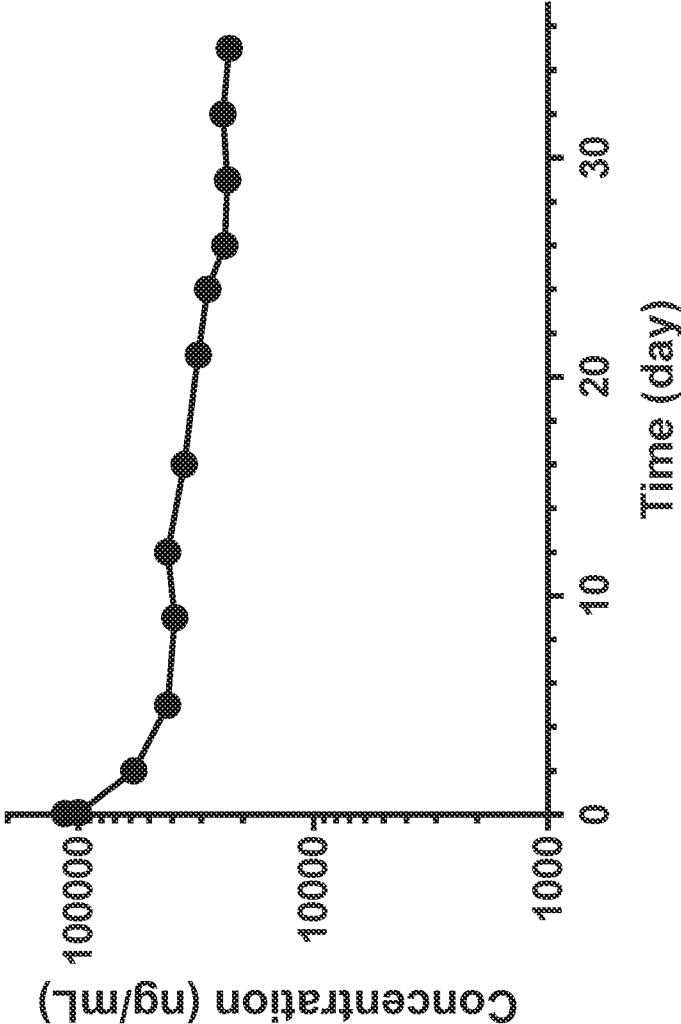


FIGURE 10
PK profile of anti-TSLP antibody
in cynomolgus monkey



Parameter	Quantity	Unit
C_0	131405	ng / mL
$t_{1/2}$	26.131	day
$AUC_{(0-t)}$	1268621	ng·day / mL
$AUC_{(0-inf)}$	2128728	ng·day / mL
CL	1.875	mL / (kg·day)
V_d	71.049	mL / kg

Cynomolgus monkey i.v. dosed with 4 mg/kg, anti-TSLP antibody

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/041642

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-20 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/041642

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 21-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/041642

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; C07K 16/24 (2020.01)

CPC - C07K 16/24; C07K 2317/565; C07K 2317/76 (2020.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2018/0327489 A1 (NOVARTIS AG) 15 November 2018 (15.11.2018) entire document	1, 5, 15-18 ----- 4, 19
Y	US 2008/0050368 A1 (CELNIKER et al) 28 February 2008 (28.02.2008) entire document	4
Y	US 2018/0044427 A1 (JANSSEN BIOTECH, INC.) 15 February 2018 (15.02.2018) entire document	19
A	US 2017/0320960 A1 (ABBVIE STEMCENTRX LLC) 09 November 2017 (09.11.2017) entire document	1-20
A	US 2016/0069900 A1 (AYANOGLU et al) 10 March 2016 (10.03.2016) entire document	1-20
A	US 2017/0210797 A1 (BIRD ROCK BIO, INC.) 27 July 2017 (27.07.2017) entire document	1-20
A	US 2005/0208042 A1 (CO et al) 22 September 2005 (22.09.2005) entire document	1-20
A	US 2017/0002077 A1 (PROTHENA BIOSCIENCES LIMITED et al) 05 January 2017 (05.01.2017) entire document	1-20
A	WO 2019/126594 A2 (RADIMMUNE THERAPEUTICS, INC. et al) 27 June 2019 (27.06.2019) entire document	1-20
A	WO 2016/194363 A1 (R-TECH UENO, LTD.) 08 December 2016 (08.12.2016) entire document	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

01 October 2020

Date of mailing of the international search report

19 OCT 2020

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/041642

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017/0320967 A1 (SANOFI) 09 November 2017 (09.11.2017) entire document	1-20
A	US 2016/0289332 A1 (STEMCENTRX, INC.) 06 October 2016 (06.10.2016) entire document	1-20
A	US 2016/0376371 A1 (THE ROCKEFELLER UNIVERSITY et al) 29 December 2016 (29.12.2016) entire document	1-20
A	US 2008/0064856 A1 (WARNE et al) 13 March 2008 (13.03.2008) entire document	1-20