



- (51) **International Patent Classification:**
C07K 14/715 (2006.01) C12N 5/00 (2006.01)
- (21) **International Application Number:**
PCT/EP2017/057944
- (22) **International Filing Date:**
4 April 2017 (04.04.2017)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
16163883.8 5 April 2016 (05.04.2016) EP
- (71) **Applicants:** VIB VZW [BE/BE]; Rijvisschestraat 120, 9052 Gent (BE). UNIVERSITEIT GENT [BE/BE]; Sint-Pietersnieuwstraat 25, 9000 Gent (BE).
- (72) **Inventors:** SAVVIDES, Savvas; Morinngoed 9, 8500 Kortrijk (BE). BEYAERT, Rudi; Gentssesteenweg 16, 9750 Zingem (BE). VERSTRAETE, Kenneth; Monterreysstraat 58, 9000 Gent (BE). BRAUN, Harald; Krijgslaan 141, 9000 Gent (BE). PEELMAN, Frank; Désiré Mercierlaan 46, 9050 Gentbrugge (BE).
- (74) **Common Representative:** VIB VZW; Rijvisschestraat 120, 9052 Gent (BE).

- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))



WO 2017/174556 A1

(54) **Title:** NOVEL TSLP INHIBITORS

(57) **Abstract:** The present invention relates to monomeric fusion proteins comprising the extracellular part of the thymic stromal lymphopoietin receptor (TSLPR) and the extracellular part of the interleukin- 7 receptor alpha (IL-7Ralpha) as inhibitors of thymic stromal lymphopoietin (TSLP) activity. The invention relates further to the use of said inhibitors as a medicament in the treatment of - but not limited to - inflammatory diseases, cancer and fibrosis.

NOVEL TSLP INHIBITORS

FIELD OF THE INVENTION

The present invention relates to monomeric fusion proteins comprising the extracellular part of the thymic stromal lymphopoietin receptor (TSLPR) and the extracellular part of the interleukin-7 receptor alpha (IL-7Ralpha) as inhibitors of thymic stromal lymphopoietin (TSLP) activity. The invention relates further to the use of said inhibitors as a medicament in the treatment of – but not limited to – inflammatory diseases, cancer and fibrosis.

BACKGROUND

10 Thymic stromal lymphopoietin (TSLP) is an interleukin-2 (IL-2) family cytokine produced by epithelial cells at barrier surfaces in the lung and gut, and by epidermal keratinocytes in the skin. It regulates immunity by driving the activation of immature dendritic cells (DC), mast cells, basophils, eosinophils and lymphocytes into a type 2 polarizing phenotype (Bell et al., 2013; Ziegler, 2010, 2012; Ziegler and Artis, 2010; Ziegler et al., 2013). Cell signaling mediated by TSLP is initiated at the cell surface via a heteromeric receptor complex with its cognate receptor, TSLPR (encoded by *CRLF2*) (Pandey et al., 2000; Park et al., 2000), and IL-7Ralpha, a receptor that also serves as the cognate receptor for IL-7 to regulate T-cell development and homeostasis (Mackall et al., 2011). Indeed, the crystal structure of mouse TSLP in complex with TSLPR and IL-7Ralpha has revealed how TSLP establishes extensive interfaces with its two receptors to evoke membrane-proximal receptor-receptor contacts poised for intracellular signaling. In addition, binding of TSLP to TSLPR is a mechanistic prerequisite for recruitment of IL-7Ralpha to the high-affinity ternary complex (Verstraete et al., 2014).

Aberrant signaling by TSLP has serious consequences for human health and imprints a massive healthcare and socioeconomic footprint. This is because type 2 helper T cell (Th2)–mediated inflammatory responses primed by activated dendritic cells (DCs), are pivotal for the onset of widespread allergic diseases of the airways, skin and gut. In fact, TSLP is now widely considered to be the master regulator for most prevalent inflammatory allergic disorders, such as the triad of atopic diseases (asthma, atopic dermatitis and atopic rhinitis), chronic obstructive pulmonary disease (COPD), and eosinophilic esophagitis (Noti et al., 2013; Redhu and Gounni, 2012; Siracusa et al., 2013; Ziegler, 2012; Ziegler et al., 2013), and has been annotated as a genetic risk factor for the development of asthma (Hunninghake et al., 2010; Liu et al., 2012; Torgerson et al., 2011) and eosinophilic esophagitis (Rothenberg et al., 2010). In addition, there is a clear pathophysiological connection among some of these diseases. For instance, 70% of patients with atopic dermatitis go on to develop asthma via what has been called “atopic march” (Spergel,

2010), while adults with active asthma are strongly predisposed for acquiring COPD when compared to non-asthmatic individuals (Guerra, 2009). Several recent developments have added new dimensions to the pathophysiological pleiotropy of TSLP. First, TSLP mediated signaling has been identified as the molecular liaison between the skin epithelium and neuronal cells to trigger itch associated with atopic dermatitis (Wilson et al., 2013). Second, TSLP has been identified as a new molecular factor contributing to the development of psoriasis, a large-scale autoimmune disease, by virtue of its effects on IL-23 production by DC (Volpe et al., 2014). Third, TSLP has been reported to have causative effects in terms of tumor progression in breast- and pancreatic cancer (De Monte et al., 2011; Pedroza-Gonzalez et al., 2011), and genetic rearrangements and mutations in the TSLPR gene (*CRLF2*) have been reported in pediatric acute lymphoblastic leukemia (Perez-Andreu et al., 2013). Fourth, TSLP has been implicated in the pathogenesis of non-allergic diseases characterized by a type 2 immune response and in promoting organ fibrosis (Ying et al., 2015).

Considering the above described pathophysiological profile and the increasing prevalence of allergic diseases worldwide, it is no surprise that TSLP has emerged as therapeutic target, in particular for the treatment of atopic diseases (Borowski et al., 2013; Romeo et al., 2013, 2014; Zhang et al., 2011). In this regard, a recent study employing a primate animal model has shown that blockade of the TSLPR reduces allergic inflammation (Cheng et al., 2013). Recently, the validity of TSLP as a therapeutic target in humans was further demonstrated in a clinical trial in which asthmatic patients were treated with an anti-TSLP monoclonal antibody (Gauvreau et al., 2014). Nevertheless, despite proof of in vitro potency of the currently available agents that target the TSLP/TSLPR/IL-7R α complex, the in vivo effects seem to be mild. Also uncertainties regarding the efficacy of monoclonal antibodies against TSLP may arise due to possible cross-reactivity with the recently described short form of human TSLP (Bjerkkan et al., 2015; Fornasa et al., 2015). In addition, EP2703414 (Amgen Inc) discloses alternative TSLP blockers consisting of soluble huIL-7R α -huTSLPR-Fc protein fusions which are dimeric and are very large fusion proteins. Intrinsic shortcomings of therapeutic monoclonal antibodies and the fusion proteins in EP2703414 are poor tissue penetration, Fc-mediated immunostimulatory effects, limitations in administration and dose indications and cost of production which necessitate the exploration of alternative strategies. Accordingly, and as there is no effective treatment available, there is an unmet need for a potent inhibitor of TSLP signaling that does not have the above mentioned limitations and which can be used for effective treatment of TSLP related diseases.

SUMMARY

It is surprisingly shown herein that a monomeric fusion protein comprising the extracellular part of the TSLPR and the extracellular part of the IL-7Ralpha (herein referred to as "TSLP-trap") can have strong TSLP neutralization activity. This is unexpected, as neither the extracellular TSLPR alone nor the extracellular IL-7Ralpha alone nor a mixture of both show a strong inhibitory activity on TSLP mediated signaling events.

It is an aspect of the present invention to provide monomeric fusion proteins comprising the extracellular part of the TSLPR and the extracellular part of the IL-7Ralpha.

In one embodiment, the invention envisages the monomeric fusion protein as described above, wherein said extracellular part of the TSLPR and said extracellular part of the IL-7Ralpha are connected by a linker, wherein said linker comprises at least 10 amino acids and/or wherein said linker is a GGS linker consisting of 5 to 20 GGS units (which corresponds with 15 to 60 amino acids).

In one embodiment, the invention envisages the monomeric fusion protein as described above, wherein said fusion protein comprises an amino acid sequence with at least 80% or at least 90% sequence identity with the amino acid sequence of SEQ ID N° 1 and an amino acid sequence with at least 80% or at least 90% sequence identity with the amino acid sequence of SEQ ID N° 2. Also envisaged is the fusion protein as described above, wherein said fusion protein comprises the amino acid sequence of SEQ ID N° 1 and the amino acid sequence of SEQ ID N° 2.

It is an aspect of the present invention to provide a nucleic acid encoding at least the fusion protein as described above.

It is an aspect of the present invention to provide a host cell comprising the nucleic acid as described above. Also envisaged is said host cell for use in the production of said fusion protein.

Also envisaged is the monomeric fusion protein as described above, for use as a medicament, for use in the treatment of an inflammatory disease (wherein said inflammatory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, allergic rhinitis, atopic dermatitis, eosinophilic esophagitis, rheumatoid arthritis and psoriasis), and for the use in the treatment of cancer and fibrosis.

It is an aspect of the present invention to provide a pharmaceutical composition comprising the monomeric fusion protein as described above in association with a pharmaceutically acceptable carrier.

Objects of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic representation of the monomeric TSLP-trap fusion proteins TSLP-trap1 (a) and TSLP-trap2 (b). (GGG)₂₀: linker consisting of twenty glycine-glycine-serine (GGG) repeats; pHLsec-SS: secretion signal of the pHLsec-vector (Aricescu et al., 2006); His-tag: polyhistidine tag. The ranges of amino acid residue numbers of the TSLPR and the IL-7Ralpha are indicated for both traps and relate to the human amino acid sequence.

Figure 2 shows the analysis of purified human TSLP-trap1 protein. (a) Size exclusion chromatography (SEC) elution profile of purified TSLP-trap1 on a Superdex 200 column. (b) SDS-PAGE gel of purified TSLP-trap1 under reducing conditions. Protein bands were visualized using the TGX stain-free technology (Biorad). The peak fractions analyzed on the SDS-PAGE gel are indicated on the SEC profile in (a). Following mixing with Laemmli-buffer, samples were either submitted to denaturation (boiled) or not (non-boiled) before loading.

Figure 3 shows the affinities to TSLP, evaluated by biolayer interferometry. The interferometric wavelength shift (nm) in function of time is plotted as a grey solid line. Fitted curves are plotted as black dashed lines. The concentrations on the plots refer to the concentrations of the ligand analyzed: (a) TSLPR, (b) IL-7Ralpha and (c) TSLP-trap1. The reported K_D -value represents the average from three replicate measurements.

Figure 4 shows the dose response curve of TSLP binding to HEK293T cells transfected with pMET7-Flag-hTSLPR, pMET7-HA-hIL-7Ralpha and pGL3- β -casein-luci, as evaluated in the STAT5-luciferase reporter assay. Cells were stimulated with increasing concentrations of TSLP and the effect on luciferase activity was determined. TSLP has an EC₅₀ value of 9 pM in this assay.

Figure 5 shows the inhibitory effect of the TSLP-traps on the TSLP induced STAT5-luciferase reporter assay in HEK293T cells. Cells were co-incubated with increasing concentrations of either TSLP-trap1 or TSLP-trap2 and 10 pM TSLP. In this assay, the TSLP-trap1 has an IC₅₀ value of 67 pM and the TSLP-trap2 has an IC₅₀ value of 44 pM.

Figure 6 shows the inhibitory effect of an anti-TSLP monoclonal antibody (Mab, described in WO2009035577) and its derived Fab fragment on the TSLP induced STAT5-luciferase reporter assay in HEK293T cells. Cells were co-incubated with increasing concentrations of either Mab or Fab fragment and 10 pM TSLP. In this assay, the antibody has an IC₅₀ of 1.4 nM and the Fab fragment has an IC₅₀ value of 1.7 nM.

Figure 7 shows the effect of the TSLPR extracellular part (TSLPRecto) and/or the IL-7Ralpha extracellular part (IL-7Recto) on the TSLP induced STAT5-luciferase reporter assay in HEK293T cells. Cells were co-incubated with 10 pM TSLP combined with different concentrations of the extracellular part of the TSLPR or of the IL-7Ralpha or with the extracellular parts of both receptors (as separate protein chains, not fused together via a linker). The extracellular parts show slight inhibition only at the highest tested concentration in this assay.

Figure 8 A) WT mice or TSLPR^{-/-} mice were shaved and epicutaneously (e.c.) exposed to PBS (negative control) or 10x tape stripped and subsequently e.c. sensitized to house dust mite (HDM). Seven days later, all mice received daily HDM challenge in the lungs during 5 consecutive days and analysis was performed 3 days after the last challenge. One group was treated with TSLP-trap; 50 µg subcutaneously during e.c. sensitization and 25 µg intranasally (i.n.) during each HDM challenge. B) Cell infiltrations in broncho-alveolar lavage fluid (BALF) and C) cytokine secretion by mediastinal lymph node (MLN) cells, restimulated with HDM for 3 days *ex vivo*. Data represent means ± SEM, n = 3-5 (p <0,05 calculated by Mann-Whitney U test; * compared to PBS group, # compared to other).

Figure 9 Mice were treated every day for 7 days with PBS or TSLP-TRAP (5µg or 25µg), followed by topical application of MC903 (1nmol) or ethanol on the ear. Ear thickness was measured daily using a dial thickness gauge. Data are represented as ± SEM (n=5)

Figure 10 Determination of the cellularity of the ear draining lymph node (EDLN) under the different conditions

Figure 11 Lymphocytes of the ear draining lymph node were isolated and counted. 180.000 lymphocytes were restimulated *in vitro* with CD3/CD28. After 72 hours of stimulation, the medium was collected and assayed for IL-13 production by ELISA. Data are represented as ± SEM (n=5).

DETAILED DESCRIPTION

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term "comprising" is used in the present description and claims, it does not exclude other elements

or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

The following terms or definitions are provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Press, Plainsview, New York (2012); and Ausubel et al., *Current Protocols in Molecular Biology* (Supplement 47), John Wiley & Sons, New York (1999), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

A "fusion protein" or chimeric protein is a protein created through joining of two or more proteins or protein parts that originally were separate proteins or parts of separate proteins.

A "monomeric fusion protein" is a monomeric chimeric protein created through joining of two or more proteins or protein parts that originally were separate proteins or parts of separate proteins.

Hence, a monomeric fusion protein of the invention is different from a dimeric fusion protein described in EP2703414. Indeed, fusion proteins described in EP2703414 are Fc – fusion proteins which upon expression in a cell lead to dimers formed by sulfur (S-S) bridges between the Fc regions. As such the "monomeric fusion protein" of the invention is a single chain fusion protein which is different from a two chain fusion protein formed in EP2703414.

In the present invention with the wording "fusion protein" or "fusion protein of the invention" it is meant a "monomeric fusion protein".

"Protein", "polypeptide" or "peptide" refers to a polymer of amino acids and does not refer to a specific length of the molecule. This term also includes post-translational modifications, such as – but not limited to – glycosylation, phosphorylation and acetylation.

The term "extracellular part" (or ectodomain) of a protein, as used herein, refers to the portion of an integral membrane protein that is exposed to the extracellular space, wherein said integral membrane protein contains one or more transmembrane domain/s made up predominantly of hydrophobic amino acids. Such an extracellular part mainly comprises hydrophilic amino acids, which are typically positioned at the surface of a folded structure of a protein, and thus is soluble

in an aqueous environment. For most cell surface receptor proteins, extracellular parts or extracellular domains serve to bind specific ligands, while intracellular domains play an important role in signal transduction.

5 A "linker", as used herein, is any linker or spacer that connects two or more proteins or two or more protein parts. This definition includes peptide-based linkers as well as non-peptide linkers.

The term "nucleic acid", as used herein, refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Nucleic acids may have any three-dimensional structure, and may perform any function, known or unknown. Non-limiting examples of nucleic acids include a gene, a gene fragment, exons, introns, messenger RNA
10 (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, control regions, isolated RNA of any sequence, nucleic acid probes, and primers. The nucleic acid molecule may be linear or circular.

"Treatment" and "treating," as used herein refer to both therapeutic treatment and prophylactic
15 or preventative measures, wherein the objective is to inhibit or slow down (lessen) the targeted disorder (e.g. cancer) or symptom of the disorder, or to improve a symptom, even if the treatment is partial or ultimately unsuccessful. Those in need of treatment include those already diagnosed with the disorder as well as those prone or predisposed to contract the disorder or those in whom the disorder is to be prevented. For example, in tumor (e.g. cancer) treatment, a therapeutic
20 agent can directly decrease the pathology of tumor cells, or render the tumor cells more susceptible to treatment by other therapeutic agents or by the subject's own immune system.

As used herein, "cancer" refers to any uncontrolled growth of cells that may interfere with the normal functioning of the bodily organs and systems, and includes both primary and metastatic tumors. Primary tumors or cancers that migrate from their original location and seed vital organs
25 can eventually lead to the death of the subject through the functional deterioration of the affected organs. A metastasis is a cancer cell or group of cancer cells, distinct from the primary tumor location, resulting from the dissemination of cancer cells from the primary tumor to other parts of the body. Metastases may eventually result in death of a subject. For example, cancers can include benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or
30 micrometastases.

A first aspect of the present invention relates to a monomeric fusion protein that comprises the extracellular part (ectodomain) of the TSLPR and the extracellular part (ectodomain) of the IL-7Ralpha. In yet another embodiment the invention provides a monomeric fusion protein that comprises the functional extracellular part (functional ectodomain) of the TSLPR and the

functional extracellular part (functional ectodomain) of the IL-7Ralpha. The TSLPR and the IL-7Ralpha are known to the person skilled in the art (Uniprot ID: Q9HC73 for human TSLPR, Uniprot ID: P16871 for human IL-7Ralpha). Functional assays to measure the binding of TSLP with the TSLPR or to measure the binding of the IL-7Ralpha to IL7 or to TSLP are known to the person skilled in the art. It is known that the N-terminal and carboxy-terminal ends of extracellular receptors tolerate some flexibility in order to maintain their functionality. Based on rational design we calculated that SEQ ID NO: 1 (corresponding with TSLPR) and SEQ ID NO: 2 (corresponding with IL-7Ralpha) are functional extracellular parts of TSLPR and IL-7Ralpha. In another embodiment, the functional TSLPR ectodomain is derived from a mammal and the functional IL-7Ralpha ectodomain is derived from a mammal. Mammal refers to any member of the class Mammalia, including, without limitations, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats, lamas and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term. In one specific embodiment, the TSLPR extracellular part is derived from the human TSLPR and the IL-7Ralpha extracellular part is derived from the human IL-7Ralpha. Alternatively, homologues of those proteins may be used. Homologues encompass proteins having amino acid substitutions (such as mutations), deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived. In various embodiments, the fusion protein comprises an amino acid sequence that is at least 80% or at least 90% identical to SEQ ID N° 1 and an amino acid sequence that is at least 80% or at least 90% identical to SEQ ID N° 2. For example, the fusion protein may comprise a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID N° 1 and a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID N° 2. In a specific embodiment, the fusion protein comprises the sequence of SEQ ID N° 1 and the sequence of SEQ ID N° 2. The fusion protein of the present application may be expressed with a secretion signal, such as – but not limited to – the secretion signal of the human TSLPR (SEQ ID N° 5) or the secretion signal of the pHlsec-vector (SEQ ID N° 6). Typically, said secretion signals are cleaved during the process of protein maturation and are not present in the mature, secreted protein. The fusion of the above described fusion protein may be at the amino-terminal or at the carboxy-terminal

end of the extracellular part of the TSLPR. This allows flexibility of the combination of the described extracellular parts. In one embodiment, the fusion is at the amino-terminal end of the extracellular part of the TSLPR. In one embodiment, the fusion is at the carboxy-terminal end of the extracellular part of the TSLPR. In one specific embodiment, the fusion protein is the human
5 TSLP-trap1 of SEQ ID N° 3. In one specific embodiment, the fusion protein is the human TSLP-trap2 of SEQ ID N° 4.

According to particular embodiments, the extracellular part of the TSLPR and the extracellular part of the IL-7Ralpha are connected by a linker. In one embodiment, there is no particular limitation on the length or the flexibility of the linker used in the fusion protein of the invention. In
10 one embodiment, the linker may be a peptide-based linker, such as a suitable amino acid sequence. In particular, the linker may be an amino acid sequence of 1 to 60, 1 to 50, 1 to 40, 1 to 30, 1 to 20, 1 to 10 or 1 to 5 amino acid residues. In one embodiment, said linker comprises at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids or at least 30 amino acids. In one embodiment, said linker is a glycine-glycine-serine (GGS)
15 linker, i.e. said linker consists of GGS units. In a specific embodiment, said GGS linker consists of 5 to 20 GGS units (which corresponds with 15 to 60 amino acids). In one embodiment, the linker is a non-peptide linker. In one particular embodiment, said non-peptide linker is not based on polyethylene glycol (PEG). In one particular embodiment, said non-peptide linker is a PEG based spacer. In one particular embodiment, said PEG linker is activated with sulfhydryl-reactive
20 chemical groups, such as – but not limited to – haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols, BMH (bismaleimidoethane), BMB (1,4-bismaleimidobutane), bismaleimidoethane, dithiobismaleimidoethane, tris(2-maleimidoethyl)amine and disulfide reducing agents. In one particular embodiment, said PEG linker is activated with chemical groups reactive to amino- and/or carboxyl-groups in proteins,
25 such as – but not limited to – N-hydroxysuccinimide, primary amine groups and tert-butylloxycarbonyl protected-amino groups. In one particular embodiment, said PEG linker is activated with sulfhydryl-reactive chemical groups and chemical groups reactive to amino- and/or carboxyl-groups in proteins. Also envisaged are homobifunctional cross-linkers allowing linkage of the same type of functional group. Also envisaged are heterobifunctional cross-linkers
30 allowing linkage of two distinct functional groups. In one embodiment, the linker can be conjugated to the extracellular part of the TSLPR and to the extracellular part of the IL-7Ralpha via – but not limited to – naturally occurring cysteine residues in the sequences of the TSLPR and/or the IL-7Ralpha or via engineered cysteines.

The monomeric fusion protein as described herein may contain a protein tag, such as – but not limited to – a histidine (His) tag. In a different embodiment, the monomeric fusion protein as described herein contains no protein tag.

5 Said monomeric fusion protein may be any construct comprising two or more proteins or protein parts comprising the TSLPR ectodomain and the IL-7Ralpha ectodomain. That is to say, said monomeric fusion protein may comprise further moieties. Said further moieties may bind to TSLP or not. As a non-limiting example, said monomeric fusion protein may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as – but not limited to – PEG), so as to provide a derivative of a fusion protein of the invention with increased half-
10 life.

In various embodiments, the binding affinity of the monomeric fusion protein of the invention for the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or monomeric and/or multimeric forms and/or any other naturally occurring or synthetic analogs, variants, or mutants (including monomeric and/or multimeric forms) of TSLP may be
15 described by the equilibrium dissociation constant (K_D). In various embodiments, the monomeric fusion protein binds to the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or any other naturally occurring or synthetic analogs, variants, or mutants (including monomeric and/or multimeric forms) of TSLP with a K_D of less than 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2.5 nM, 1 nM, 900 pM, 800
20 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM, 100 pM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 40 pM, 30 pM, 20 pM or 10 pM.

In some embodiments, the monomeric fusion protein described herein includes derivatives that are modified, i.e. by the covalent attachment of any type of molecule to the fusion protein such that covalent attachment does not prevent the activity of the protein. For example, but not by
25 way of limitation, derivatives include fusion proteins that have been modified by, inter alia, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a ligand or other protein etc. Any of numerous chemical modifications can be carried out by techniques known to the skilled person.

30 “Inhibition”, as used herein, refers to the fact that the monomeric fusion protein interferes with/inhibits/prevents/reverses or slows the ability of TSLP to induce signaling events as measured in an in vitro potency assay, such as – but not limited to – a TSLP induced STAT5 reporter assay. Inhibition, also referred to as neutralization, can mean full neutralization (no TSLP induced signal is observable) or may mean partial neutralization. The neutralization
35 activity typically will be evaluated against a suitable control (e.g. treatment with an irrelevant

protein), as will be readily chosen by the skilled person. For fusion proteins with known concentration, the inhibitory activity can be expressed as 50% inhibitory concentration (IC₅₀). The IC₅₀ is the fusion protein concentration at which 50% inhibition (or neutralization) is achieved. It is a measure of the inhibitory potential, also referred to as potency, of a protein. In various embodiments, the fusion protein as described herein inhibits TSLP induced signaling with a potency of less than 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2.5 nM, 1 nM, 900 pM, 800 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM, 100 pM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 40 pM, 30 pM, 20 pM or 10 pM.

According to a further aspect, the fusion proteins are not provided as such, but are provided as nucleic acid, i.e. nucleic acid molecules encoding fusion proteins as herein described. According to a further aspect, also provided are recombinant expression vectors comprising such nucleic acids or nucleic acid molecules. In another aspect, a host cell comprising the above described nucleic acid or vector is envisaged. A host cell, as used herein, may be any cell that is suitable for production of said fusion protein. Typically, the nucleic acids will have been introduced in the host cell by transfection, transformation or transduction techniques, although the way in which the nucleic acid is introduced in the host cell is not limiting the invention. The host cells comprising the nucleic acids (or vectors) described herein are particularly suited for production of the fusion proteins. Thus, such use for production is explicitly envisaged herein. Illustrative host cells are E.coli cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, monkey kidney cells (COS) and yeast cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the fusion protein of the invention. Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in E. coli, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, e.g. Trp or Tac, and a prokaryotic signal sequence. In another example, if the engineered gene is to be expressed in eukaryotic host cells, e.g. CHO cells, it is first inserted into an expression vector containing for example, a suitable eukaryotic promoter, a secretion signal, enhancers, and various introns. The gene construct can be introduced into the host cells using transfection, transformation, or transduction techniques. The fusion protein of the invention can be produced by growing a host cell transfected with an expression vector encoding the fusion protein under conditions that permit expression of the protein. Following expression, the protein can be harvested and purified using techniques well known in the art, e.g. affinity tags such as glutathione-S-transferase (GST) and histidine (His) tags or by chromatography. In an embodiment, the fusion protein comprises a His-tag.

According to an embodiment, the monomeric fusion proteins are provided herein for use in medicine. That is to say, the fusion proteins are provided for use as a medicament. The same goes for the nucleic acid molecules or recombinant expression vectors encoding the fusion proteins, i.e. it is envisaged that nucleic acid molecules or vectors encoding the fusion proteins
5 are provided for use as a medicament. According to particular embodiments, the fusion proteins (or nucleic acids or vectors encoding them) are provided for use in the treatment of inflammatory diseases or conditions, such as inflammation, acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic rhinitis, atopic dermatitis, psoriasis, chronic obstructive pulmonary disease (COPD), eosinophilic esophagitis, septic
10 shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, glycerol kinase deficiency, familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease, tuberculosis, chronic cholecystitis, bronchiectasis, silicosis and other pneumoconioses. In a specific embodiment, the fusion proteins described herein are provided for use in the treatment
15 of asthma, COPD, allergic rhinitis, atopic dermatitis, eosinophilic esophagitis, rheumatoid arthritis and psoriasis.

According to particular embodiments, the monomeric fusion proteins (or nucleic acids or vectors encoding them) are provided for use in the treatment of cancer. Illustrative cancers that may be treated include, but are not limited to, basal cell carcinoma, biliary tract cancer, bladder cancer,
20 bone cancer, brain and central nervous system cancer, breast cancer, cancer of the peritoneum, cervical cancer, choriocarcinoma, colon and rectum cancer, connective tissue cancer, cancer of the digestive system, endometrial cancer, esophageal cancer, eye cancer, cancer of the head and neck, gastric cancer (including gastrointestinal cancer), glioblastoma, hepatic carcinoma, hepatoma, intra-epithelial neoplasm, kidney or renal cancer, larynx cancer, leukemia, liver
25 cancer, lung cancer (e.g. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), melanoma, myeloma, neuroblastoma, oral cavity cancer (lip, tongue, mouth, and pharynx), ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, rhabdomyosarcoma, rectal cancer, cancer of the respiratory system, salivary gland carcinoma, sarcoma, skin cancer, squamous cell cancer, stomach cancer,
30 testicular cancer, thyroid cancer, uterine or endometrial cancer, cancer of the urinary system, vulval cancer, lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL,
35 bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL),

Hairy cell leukemia, chronic myeloblastic leukemia, as well as other carcinomas and sarcomas, and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (e.g. associated with brain tumors), and Meigs' syndrome.

- 5 According to particular embodiments, the monomeric fusion proteins (or nucleic acids or vectors encoding them) are provided for use in the treatment of fibrosis. Illustrative examples include, but are not limited to organ fibrosis, pulmonary fibrosis, airway remodeling, myofibroblast hyperplasia, asthmatic airway remodeling and fibrosis, idiopathic pulmonary fibrosis and cutaneous systematic sclerosis.
- 10 This is equivalent as saying that methods are provided for the treatment of inflammatory diseases, cancer or fibrosis for a subject in need thereof, comprising administering a therapeutically effective amount of the fusion protein to said subject. Such methods typically will result in improvement of symptoms of the disease in said subject. Here also, the monomeric fusion protein may be provided as a protein, as a pharmaceutical composition or may be
- 15 administered as a nucleic acid molecule encoding the monomeric fusion protein or as a vector comprising such nucleic acid molecule. If the monomeric fusion protein is administered as a protein, different routes of administration can be envisaged. For example – but not by way of limitation – routes of administration include: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral,
- 20 intravaginal, transdermal, rectally, by inhalation, or topically. Administration can be local or systemic. The mode of administration can be left to the discretion of the practitioner, and depends in-part upon the site of the medical condition. In most instances, administration results in the release of the fusion protein described herein into the bloodstream. In case the fusion protein is provided as a nucleic acid or vector, it is particularly envisaged that the monomeric
- 25 fusion protein is administered through gene therapy.

In a further aspect, the disclosure contemplates a pharmaceutical composition comprising the monomeric fusion protein as described above, in association with a pharmaceutically acceptable carrier. Typically, such pharmaceutical compositions comprise at least the fusion protein. Said pharmaceutical compositions may comprise further moieties. Said further moieties may bind to

30 TSLP or not. Pharmaceutically acceptable carriers are known to the person skilled in the art, and are inherently non-toxic and nontherapeutic. Carriers may be, as a non-limiting example, Ringer's solution, dextrose solution or Hank's solution. Non aqueous solutions such as fixed oils and ethyl oleate may also be used. A preferred carrier is 5% dextrose in saline. The pharmaceutically acceptable carrier may contain minor amounts of additives such as substances

35 that enhance isotonicity and chemical stability, including buffers and preservatives. It is

envisaged herein that the pharmaceutical compositions are provided for use as a medicament. Particularly, they are provided for use in the treatment of inflammatory diseases, cancer or fibrosis. This is equivalent as stating that methods are provided for the treatment of inflammatory diseases, cancer or fibrosis for a subject in need thereof, comprising administering a pharmaceutical composition as described herein to said subject. Here also, different routes of administration of the pharmaceutical composition can be envisaged. For example – but not by way of limitation – routes of administration include: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically. Administration can be local or systemic. The mode of administration can be left to the discretion of the practitioner, and depends in-part upon the site of the medical condition. In most instances, administration results in the release of the pharmaceutical composition described herein into the bloodstream. In various embodiments, the pharmaceutical composition of the present invention is co-administered in conjunction with additional therapeutic agent(s). Co-administration can be simultaneous or sequential.

It is to be understood that although particular embodiments, specific configurations as well as materials and/or molecules, have been discussed herein for cells and methods according to the present invention, various changes or modifications in form and detail may be made without departing from the scope and spirit of this invention. The following examples are provided to better illustrate particular embodiments, and they should not be considered limiting the application. The application is limited only by the claims.

EXAMPLES

Materials and methods to the examples

25 Design of the TSLP-traps

Human TSLP-trap1 consists of the human TSLPR ectodomain (residues 25 to 231 from Uniprot ID: Q9HC73, SEQ ID N° 7) interconnected to the human IL-7Ralpha ectodomain (residues 21 to 239 from Uniprot ID: P16871, SEQ ID N° 8) through a (GGG)₂₀-linker region that connects the C-terminus of the TSLPR ectodomain and the N-terminus of the IL-7Ralpha ectodomain.

30 Human TSLP-trap2 consists of the human IL-7Ralpha ectodomain (Uniprot ID: P16871, residues 21 to 239) interconnected to the human TSLPR ectodomain (Uniprot ID: Q9HC73, residues 23 to 231) through a (GGG)₂₀-linker region that connects the C-terminus of the IL-7Ralpha ectodomain and the N-terminus of the TSLPR ectodomain.

To enable secretion of TSLP-traps from mammalian cells a secretion signal is included in the open reading frame encoding the TSLP-trap proteins. This secretion signal is proteolytically removed from the mature protein. For TSLP-trap1, the native secretion signal of human TSLPR is used (residues 1 to 24 from Uniprot ID: Q9HC73; SEQ ID N° 5), while for TSLP-trap2 the signal peptide from the pHLsec-vector is used (Aricescu et al., 2006; SEQ ID N° 6). Both TSLP-trap1 and TSLP-trap2 carry a His-tag at their C-terminus. The human TSLP-trap1 and TSLP-trap2 fusion proteins are shown schematically in Fig 1.

Cloning and recombinant production of TSLP, TSLPR, IL-7Ralpha and TSLP-traps

cDNA fragments encoding full-length human TSLP_R127A_R130S (NP_149024.1; residue 1-159), the extracellular fragments of human TSLPR (NP_071431.2; residue 1 - 221) and human IL-7Ralpha (NP_002176.2; residue 1- 239) and human TSLP-trap1 were cloned into the multicloning site of the pcDNA4/TO-expression vector (ThermoFisher) in frame with a C-terminal His-tag. Human TSLP carries the R127A and R130S mutations (TSLP_R127A_R130S) to remove a potential furin cleavage site (Lyman et al., 2013). Human TSLP-trap2 was cloned into a modified version of the pcDNA4/TO vector which carried the signal peptide from the pHLsec-vector (SEQ ID N° 6) at its multicloning site.

Stable, tetracycline-inducible cell lines for TSLP_R127A_R130S and the TSLPR and IL-7Ralpha ectodomains were generated in HEK293S MGAT1^{-/-} cells (Reeves et al., 2002). Stable, tetracycline-inducible cell lines for TSLP-trap1 and TSLP-trap2 were generated in T-RExTM-293 cells (Thermo Fisher Scientific). HEK293S MGAT1^{-/-} and T-RExTM-293 cells were grown in high-glucose DMEM medium supplemented with 10% fetal calf serum, 106 units.L-1 penicillin G and 1 gL-1 streptomycin in a 5% CO₂ atmosphere at 37°C. For the growth of T-RExTM-293 cells, the medium was supplemented with 5 µg/mL blasticidin.

Stable cell lines were generated by selection with 200 µg/mL zeocine as described in Verstraete et al., 2011. To induce expression, the medium of confluent grown cells was replaced with serum-free medium supplemented with 2 µg/mL tetracycline. Recombinant protein expression from pools of stable transfected cells was confirmed by Western blot analysis using an HRP-coupled antibody directed against the C-terminal His-tag (Invitrogen, catalogue No. R931-25) or small-scale immobilized metal affinity chromatography (IMAC) purifications in batch mode using 2 mL of conditioned medium (using Ni Sepharose beads from GE Healthcare). For large-scale expression experiments, pools of stable transfected cells were expanded to thirty 175 cm² tissue-culture flasks. Four to five days following induction with tetracycline the conditioned medium was harvested and cleared by centrifugation and filtration through a 0.22 µm bottle top filter. Recombinant proteins were captured from the cleared conditioned medium by IMAC

purification using a cOmplete His-Tag purification column (Roche) and further purified by size-exclusion chromatography (SEC) using a Superdex 200 or Superdex 75 column (GE Healthcare) with HBS pH 7.4 as running buffer. As an example, the SEC elution profile for TSLP-trap1 and an SDS-PAGE gel (under reducing conditions) demonstrate the sample homogeneity and purity
5 of the TSLP-trap1 preparation (Fig. 2).

Production of a neutralizing anti-TSLP antibody

A neutralizing human anti-TSLP IgG2 monoclonal antibody (Mab) with a lambda light chain, and its derived Fab-fragment were recombinantly produced by transient transfection in HEK293T cells. cDNA fragments encoding the light chain and heavy chain for antibody A5 described in
10 patent WO2009035577 were ordered from Gen9. To generate a Fab fragment, the V_H-C_H1 heavy fragment was generated by PCR. All cDNAs were cloned between the AgeI and KpnI sites of the pHLsec expression plasmid, in frame with the signal peptide of the vector (Aricescu et al. 2006). The heavy chain and the V_H-C_H1 heavy chain fragment were cloned in frame with the C-terminal hexahistidine tag, while the light chain carried a stop codon. The full-length Mab or Fab-
15 fragment were produced by co-transfecting HEK293T cells with expression plasmids for the light chain and heavy chain or V_H-C_H1-fragment in a 1:1 ratio. The Mab or Fab-fragment were purified from the conditioned medium by IMAC and SEC.

Biolayer interferometry

The binding affinity and binding kinetics for the interaction between TSLP_R127A_R130S and
20 the TSLPR ectodomain, the IL-7Ralpha ectodomain and TSLP-trap1 were investigated by biolayer interferometry (BLI). Streptavidin-coated biosensors were functionalized with biotinylated TSLP_R127A_R130S and quenched with a 10 µg/ml biotin solution and then exposed to different concentrations of ligand.

To produce biotinylated TSLP, a cDNA fragment encoding TSLP_R127A_R130S was cloned
25 between the EcoRI and KpnI sites of the pHL-AVITAG vector (Aricescu et al., 2006). Prior the transfection in HEK293T cells, the culture medium was changed to serum-free DMEM medium to which 100 µM D-biotin was added. To allow specific C-terminal in vivo biotinylation the pHL-TSLP_R127A_R130S-AVITAG construct was co-transfected with the pDisplay-BirA-ER plasmid (Howarth et al., 2008) in a 5:1 ratio. Five days post-transfection conditioned medium was
30 harvested and loaded onto a Ni sepharose column (GE Healthcare). Recombinant proteins were eluted with imidazole and loaded onto a Superdex 75 column with HBS as running buffer.

All BLI experiments were performed in PBS buffer supplemented with 0.01% (w/v) BSA and 0.002% (v/v) Tween 20, with an Octet RED96 instrument (ForteBio), operating at 25°C.

To verify that no aspecific binding was present during the interaction assay, non-functionalized biosensors were used as a control. All data were fitted with the FortéBio Data Analysis 9.0.0.4 software using a 1:1 ligand model. The reported values for the fitted parameters represent the average (and standard deviation) from three replicate measurements.

5 Constructs for the TSLP induced STAT5 reporter assay

pMET7-Flag-hTSLPR and pMET7-HA-hIL-7Ralpha allow the expression of a full-length FLAG-tagged human TSLPR and full-length HA-tagged human IL-7Ralpha. pMET7-Flag-hTSLPR was created by ligating a codon optimized hTSLPR cDNA sequence into the ClaI/XbaI opened pMet7-FLAG-mTSLP vector (Verstraete et al., 2014). pMET7-HA-hIL-7Ralpha was created by
10 ligating a codon optimized hIL-7Ralpha cDNA sequence into the BspEI/XbaI opened pMet7-HA-mIL-7Ralpha vector (Verstraete et al., 2014).

TSLP induced STAT5 reporter assay

For measuring TSLP signaling in HEK293T cells, HEK293T cells were co-transfected with 1.5 ng pMET7-Flag-hTSLPR, 1.5 ng pMET7-HA-hIL-7Ralpha, 893 ng empty pMET7 vector and 23.5
15 ng pGL3- β -casein-luciferase reporter plasmid per well of a 6-well plate. For transfection, we incubated the DNA with linear PEI 25 kDa (Polysciences) at a 2.17:1 PEI:DNA ratio for 12 minutes in serum-free Optimem I medium (Life technologies), before addition of this DNA:PEI mix to the cells. The pGL3- β -casein-luciferase reporter contains 5 repeated STAT5-responsive motifs of the β -casein promoter (Verstraete et al., 2014). The day after transfection, the cells were detached
20 with cell dissociation buffer (Life Technologies), and resuspended in DMEM + 10% foetal bovine serum. 3% of the cells were seeded in 50 μ l medium per well in 96 well plates and stimulated 50 μ l medium with increasing concentrations of mammalian-cell derived recombinant TSLP (TSLP_R127A_R130S). On day two after transfection, the luciferase activity in the 96 well plates was determined on an Envision chemiluminescence counter as described previously (Peelman et al., 2004). Fold induction of luciferase activity was calculated by dividing the luminescence
25 signal (cps) of the TSLP stimulated cells by the luminescence signal of the unstimulated cells. The data were fitted to a log agonist versus response curve in GraphPad Prism.

Inhibition in the TSLP induced STAT5 reporter assay

To study the effect of different inhibitors (traps, receptor ectodomains, anti-TSLP antibody or
30 derived Fab fragment) on TSLP induced STAT5 reporter assays, HEK293T cells were seeded and transfected as described above. The day after transfection, the cells were detached with cell dissociation buffer (Life Technologies), and resuspended in DMEM + 10% foetal bovine serum. 3% of the cells were seeded in 50 μ l medium per well in 96 well plates. In a separate

plate, mammalian cell derived recombinant TSLP (TSLP_R127A_R130S) in medium was incubated with increasing concentrations of the inhibitors for 30 minutes at room temperature. After this pre-incubation, 50 μ l of this TSLP-inhibitor mix was added to the seeded cells. The reported concentrations for inhibitor and TSLP (10 pM) are their final concentrations in this 100 μ l volume. Cells were incubated overnight with this mixture and STAT5 reporter luciferase activity was measured 24 hours after the start of the stimulation, as described above. Fold induction of luciferase activity was calculated by dividing the luminescence signal (cps) of the TSLP stimulated cells by the luminescence signal of the unstimulated cells. The data were fitted to a log inhibitor versus response curve in GraphPad Prism.

10 Mouse strains

C57Bl/6J (WT) mice were purchased at Janvier labs and TSLPR^{-/-} (Cr1f2^{-/-}) mice were provided by M. Comeau. Mice were maintained in the animal facility of the VIB-UGent Center of Inflammation Research under specific pathogen-free conditions. Experiments were approved by the animal ethical committees of the Ghent University and the VIB-UGent Center for Inflammation Research.

Example 1: Generation of the TSLP-traps

The engineered human TSLP-traps encode monomeric fusion proteins consisting of the extracellular regions of human TSLPR and human IL-7Ralpha, separated by an amino acid linker, here exemplified by a flexible Gly-Gly-Ser (GGG) linker. In one example of a human TSLP-trap, the GGS-linker region connects the C-terminus of the TSLPR ectodomain to the N-terminus of the IL-7Ralpha ectodomain, (denoted as TSLP-trap1, Fig. 1a). In another example, the GGS-linker region connects the C-terminus of the IL-7Ralpha ectodomain to the N-terminus of the TSLPR ectodomain (denoted as TSLP-trap2, Fig. 1b). TSLP-traps contain a signal peptide leading to the secretion of the recombinant protein into the medium fraction when expressed in mammalian cells. As a signal peptide, either the native secretion signal of TSLPR or IL-7Ralpha can be used, as for example in TSLP-trap1 which carries the native secretion signal of TSLPR. Alternatively, TSLP-trap fusion proteins may carry another secretion signal, as for example in TSLP-trap2 which carries the secretion signal of the pHlsec-vector (Aricescu et al., 2006). The C-terminal His-tag allows easy detection and purification of TSLP-traps from conditioned medium of transfected cells. TSLP-trap1 and TSLP-trap2 were purified by a combination of IMAC and SEC to a purity of ~95%, judged by SDS-PAGE analysis. As an example, the final SEC-profile and SDS-PAGE analysis for purified TSLP-trap1 are shown in Fig. 2.

Example 2: The TSLP-trap has high affinity for TSLP

To characterize the interaction between TSLP and the TSLPR ectodomain, the IL-7Ralpha ectodomain and TSLP-trap1 real time kinetic bio-layer interferometry (BLI) experiments were performed. As ligand C-terminally biotinylated human TSLP was immobilized on streptavidin-coated biosensor tips. Firstly, it was determined that the TSLPR ectodomain binds to TSLP with an affinity (K_D) of $3,2E-08$ M and an association rate (k_{on}) of $1,7E+05$ $M^{-1}s^{-1}$ and a dissociation rate (k_{off}) of $5,2E-03$ s^{-1} (Fig. 3a), while for IL-7Ralpha no interaction was observed up to a concentration of 100 nM (Fig. 3b). Using higher concentrations of IL-7Ralpha to probe the possible low affinity interaction between TSLP and IL-7Ralpha resulted in non-specific binding of IL-7Ralpha under the experimental conditions used.

Surprisingly, it is shown herein that TSLP-trap1 binds very strongly to immobilized TSLP with an affinity (K_D) of $1,2E-10$ M and an association rate (k_{on}) of $1,4E+05$ $M^{-1}s^{-1}$ and a dissociation rate (k_{off}) of $1,7E-05$ s^{-1} (Fig. 3c). The kinetic profile of the TSLP-trap reveals that this strong binding affinity arises from a markedly lower dissociation rate constant ($k_{off} = 1,72E-05$ s^{-1}) as compared with the dissociation rate of the unfused ectodomains.

Example 3: The TSLP-trap is a potent inhibitor of TSLP

The potential of the engineered TSLP-traps to block cytokine activity of TSLP was evaluated by an *in vitro* bioassay. The inhibitory potency of the TSLP-traps was compared to the TSLPR and the IL-7Ralpha ectodomain alone, to a combination of both ectodomains, and to a neutralizing anti-TSLP monoclonal antibody (Mab, described in WO2009035577) and its derived Fab fragment. Their ability to block was evaluated in a TSLP induced STAT5 reporter assay in HEK293T cells transfected with full-length TSLPR and IL-7Ralpha. Stimulation of transfected cells with TSLP strongly induces STAT5-dependent luciferase activity with an EC50 value of 9 pM (Fig 4.). Pre-incubation of a fixed concentration of TSLP (10 pM) with increasing concentrations of either TSLP-trap1 or TSLP-trap2 effectively inhibits TSLP dependent STAT5-activation, with IC50 values of 67 pM and 44 pM for TSLP-trap1 and TSLP-trap2, respectively (Fig. 5). Pre-incubation of 10 pM TSLP with a neutralizing human anti-TSLP IgG2 monoclonal antibody (Mab) described in patent WO2009035577, or its derived Fab-fragment resulted in IC50 values of 1.4 nM and 1.7 nM, respectively (Fig .6). On the other hand, pre-incubation of 10 pM TSLP with the TSLPR or the IL-7Ralpha ectodomain alone, or with a combination of both ectodomains did not result in significant inhibition (Fig 6.). This surprisingly demonstrates that linking the TSLPR and IL-7Ralpha ectodomains creates an enormous advantage when aiming to inhibit TSLP signaling. Moreover, the here described TSLP-traps show a 20- to 40-fold lower

IC50 value as compared to a neutralizing anti-TSLP antibody, indicating the inhibitory potency of the TSLP-traps.

Example 4: Effect of TSLP-trap in a mouse model of HDM-induced allergic airway inflammation

5 WT and TSLPR^{-/-} mice were shaved on the skin of the back, 10x tape-stripped and exposed to 10 µg HDM under an occlusive patch on the skin. As a negative control, WT mice were exposed to PBS under a patch on shaved skin. One group (TSLP-trap) received an injection of 50 µg TSLP-trap subcutaneously (s.c.) at the time of HDM exposure and this treatment was repeated 8 hours later. Seven days later, all mice were challenged with HDM via the lungs during 5
10 consecutive days and the TSLP-trap group was simultaneously treated with intranasal administration (i.n.) of 25 µg TSLP-trap (see Figure 8A). Features of allergic airway inflammation were assessed 3 days after the last challenge.

The lack of Th2 response in TSLPR-deficient mice indicates that TSLP signaling contributed to the development of lung eosinophilia in a model of allergic airway inflammation, induced via
15 epicutaneous (e.c.) sensitization to HDM. Therapeutic removal of TSLP by injection of TSLP-trap was also able to significantly reduce the infiltration of eosinophils in the BALF (see Figure 8B). Production of Th2 cytokines (IL-5 and IL-13) by Mediastinal Lymph Node (MLN) cells, which were restimulated with HDM during 3 days *ex vivo*, was significantly reduced when mice were previously treated with TSLP-trap. These experiments indicate that inactivation of TSLP
20 signaling by TSLP-trap results in a reduction of Th2-mediated allergic airway inflammation in a model of e.c. HDM sensitization (see Figure 8C). We conclude that the TSLP-Trap is able to suppress lung eosinophilia in a mouse model of HDM-induced allergic airway inflammation.

Example 5: Effect of TSLP-trap in an atopic dermatitis mouse model

To study the potential of the TSLP-trap as a therapeutic against atopic dermatitis, we tested the
25 ability of the TSLP-trap to inhibit atopic dermatitis induced by the vitamin D3 analogue MC903. In this mouse model, atopic dermatitis is induced by MC903 in a TSLP-dependent manner (Li M. *et al* (2009) *J. Invest. Dermatol.* 129: 498-502; Li M. *et al.* (2006) *Proc. Natl. Acad. Sci USA* 103: 11736-41). During 7 days, mice were injected intraperitoneally every day with TSLP-trap (5µg or 25µg) or PBS followed by topical application of MC903 (1nmol) or ethanol onto the ear.
30 To assess atopic dermatitis-like inflammation, the thickening of the ear was measured daily using a dial thickness gauge. Mice treated with a dose of 25µg TSLP-trap showed less thickening of the ears upon MC903 application compared to PBS-treated controls (Figure 9). After 7 days, mice were sacrificed and lymphocytes from the ear draining lymph node (EDLN) were isolated. The cellularity of the EDLN was increased upon MC903 treatment but did not differ between the

groups treated with PBS and 5 µg and 25 µg TSLP-trap (Figure 10). Next, IL-13 cytokine production by the lymphocytes was assayed in response to *in vitro* CD3/CD28 re-stimulation. Lymphocytes derived from mice treated with MC903 showed strong induction of IL-13 cytokine production which was less pronounced in mice that received 25 µg TSLP-trap (Figure 11).

5 Together these data point to a protective role for TSLP-trap in a mouse model for atopic dermatitis.

Sequences

10 SEQ ID N° 1: amino acid sequence of part of the extracellular region of the human TSLPR (residues 29 - 211)

GVQIQIIFYNLETVQVTWNASKYSRTNLTFFHYRFNGDEAYDQCTNYLLQEGHTSGCLLDAEQR
DDILYFSIRNGTHPVFTASRWMVYYLKPSSPKHVRFSWHQDAVTVTCSDSLSDLLYEYQYR
SPFDTEWQSKQENTCNVTIEGLDAEKCYSFVVRVKAMEDVYGPDTYPSDWSEVTCWQR

15 SEQ ID N° 2: amino acid sequence of part of the extracellular region of the human IL-7Ralpha (residues 38 -229)

YSFSCYSQLEVNGSQHSLTCAFEDPDVNTTNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLI
GKSNICVKVGEKSLTCKKIDLTIVKPEAPFDLSVIYREGANDFVVTFNNTSHLQKKYVKVLMHD
VAYRQEKDENKWTHVNLSSSTKLTLQRKLQPAAMYEIKVRSIPDHYFKGFWSEWSPSYFFRT
20 P

SEQ ID N° 3: human TSLP-trap1 amino acid sequence (without secretion signal)

GAAEGVQIQIIFYNLETVQVTWNASKYSRTNLTFFHYRFNGDEAYDQCTNYLLQEGHTSGCLLDAEQR
25 AEQRDDILYFSIRNGTHPVFTASRWMVYYLKPSSPKHVRFSWHQDAVTVTCSDSLSDLLYEYQYR
VQYRSPFDTEWQSKQENTCNVTIEGLDAEKCYSFVVRVKAMEDVYGPDTYPSDWSEVTCWQR
QRGEIRDACAETPTPPKPKLSKSRGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG
SGGSGGSGGSGGSGGSGGSGGSGGSLQESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQ
HSLTCAFEDPDVNTTNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCK
KIDLTIVKPEAPFDLSVIYREGANDFVVTFNNTSHLQKKYVKVLMHDVAYRQEKDENKWTHV
30 NLSSTKLTLQRKLQPAAMYEIKVRSIPDHYFKGFWSEWSPSYFFRTPEINNSSGEMDGTKHH
HHHH

SEQ ID N° 4: human TSLP-trap2 amino acid sequence (without secretion signal)

ETGESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNTTNLEFEICGALVE
35 VKCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTIVKPEAPFDLSVIYREGANDF
VVTFNNTSHLQKKYVKVLMHDVAYRQEKDENKWTHVNLSSSTKLTLQRKLQPAAMYEIKVRSIP
DHYFKGFWSEWSPSYFFRTPEINNSSGEMDSRGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG
SGGSGGSGGSGGSGGSGGSGGSGGSLQQGGAAEGVQIQIIFYNLETVQVT
WNASKYSRTNLTFFHYRFNGDEAYDQCTNYLLQEGHTSGCLLDAEQRDDILYFSIRNGTHPVF
40 TASRWMVYYLKPSSPKHVRFSWHQDAVTVTCSDSLSDLLYEYQYRSPFDTEWQSKQENT
CNVTIEGLDAEKCYSFVVRVKAMEDVYGPDTYPSDWSEVTCWQRGEIRDACAETPTPPKPK
LSKGTKHHHHHHH

SEQ ID N° 5: secretion signal of human TSLPR

45 MGRLLVLLWGAAVFLLGGWMALGQG

SEQ ID N° 6: secretion signal of the pHLsec-vector (Aricescu et al., 2006)

MGILPSPGMPALLSLVSLLSVLLMGCA

- 5 SEQ ID N° 7: amino acid sequence of the extracellular part of human TSLPR (residues 25 - 231) as depicted in Figure 1

GAAEGVQIQIIFYFNLETVQVTWNASKYSRTNLTFHYRFNGDEAYDQCTNYLLQEGHTSGCLLD
AEQRDDILYFSIRNGTHPVFTASRWMVYYLKPSSPKHVRFWSHQDAVTVTCSDLSYGDLLYE
VQYRSPFDTEWQSKQENTCNVTIEGLDAEKCYFWVRVKAMEDVYGPDTYPSDWSEVTCW
10 QRGEIRDACAETPTPPKPKLSK

SEQ ID N° 8: amino acid sequence of the extracellular part of human IL-7Ralpha (residues 21 - 239) as depicted in Figure 1

ESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNTTNLEFEICGALVEVKC
LNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTIVKPEAPFDLSVIYREGANDFVVT
15 FNTSHLQKKYVKVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMYEIKVRSIPDHY
FKGFWSEWSPSYFRTPEINNSSGEMD

REFERENCES

- Aricescu, A.R., Lu, W., and Jones, E.Y. (2006). A time- and cost-efficient system for high-level protein production in mammalian cells. *Acta Crystallogr D Biol Crystallogr* 62, 1243-1250.
- 5 Bell, B.D., Kitajima, M., Larson, R.P., Stoklasek, T.A., Dang, K., Sakamoto, K., Wagner, K.U., Reizis, B., Hennighausen, L., and Ziegler, S.F. (2013). The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses. *Nat Immunol* 14, 364-371.
- Bjerkkan, L., Schreurs, O., Engen, S.A., Jahnsen, F.L., Baekkevold, E.S., Blix, I.J., and Schenck, K. (2015). The short form of TSLP is constitutively translated in human keratinocytes and has
10 characteristics of an antimicrobial peptide. *Mucosal immunology* 8, 49-56.
- Borowski, A., Vetter, T., Kuepper, M., Wohlmann, A., Krause, S., Lorenzen, T., Virchow, J.C., Luttmann, W., and Friedrich, K. (2013). Expression analysis and specific blockade of the receptor for human thymic stromal lymphopoietin (TSLP) by novel antibodies to the human TSLP α receptor chain. *Cytokine* 61, 546-555.
- 15 Cheng, D.T., Ma, C., Niewoehner, J., Dahl, M., Tsai, A., Zhang, J., Gonsiorek, W., Apparsundaram, S., Pashine, A., Ravindran, P., et al. (2013). Thymic stromal lymphopoietin receptor blockade reduces allergic inflammation in a cynomolgus monkey model of asthma. *J Allergy Clin Immunol* 132, 455-462.
- De Monte, L., Reni, M., Tassi, E., Clavenna, D., Papa, I., Recalde, H., Braga, M., Di Carlo, V., Doglioni, C., and Protti, M.P. (2011). Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in
20 pancreatic cancer. *J Exp Med* 208, 469-478.
- Fornasa, G., Tsilingiri, K., Caprioli, F., Botti, F., Mapelli, M., Meller, S., Kislak, A., Homey, B., Di Sabatino, A., Sonzogni, A., et al. (2015). Dichotomy of short and long thymic stromal
25 lymphopoietin isoforms in inflammatory disorders of the bowel and skin. *J Allergy Clin Immunol* 136, 413-422.
- Gauvreau, G.M., O'Byrne, P.M., Boulet, L.P., Wang, Y., Cockcroft, D., Bigler, J., FitzGerald, J.M., Boedigheimer, M., Davis, B.E., Dias, C., et al. (2014). Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *The New England journal of medicine* 370, 2102-2110.
- 30 Guerra, S. (2009). Asthma and chronic obstructive pulmonary disease. *Current opinion in allergy and clinical immunology* 9, 409-416.
- Hunninghake, G.M., Soto-Quiros, M.E., Avila, L., Kim, H.P., Lasky-Su, J., Rafaels, N., Ruczinski, I., Beaty, T.H., Mathias, R.A., Barnes, K.C., et al. (2010). TSLP polymorphisms are associated with asthma in a sex-specific fashion. *Allergy* 65, 1566-1575.
- 35 Howarth, M., & Ting, A. Y. (2008). Imaging proteins in live mammalian cells with biotin ligase and monovalent streptavidin. *Nature protocols*, 3, 534-545. Liu, W., Xu, L.S., Liu, Q.J., Dong, F.Z., Qiu, R.F., Wen, M.C., Han, Y.L., Tang, N.B., Kang, L.J., Wu, J.X., et al. (2012). Two single nucleotide polymorphisms in TSLP gene are associated with asthma susceptibility in Chinese Han population. *Experimental lung research* 38, 375-382.

- Lyman, S. D., Van Ness K. P. and Paxton R. J. (2013). Modified human thymic stromal lymphopoietin. Patent US20130023647.
- Mackall, C.L., Fry, T.J., and Gress, R.E. (2011). Harnessing the biology of IL-7 for therapeutic application. *Nat Rev Immunol* 11, 330-342.
- 5 Noti, M., Wojno, E.D., Kim, B.S., Siracusa, M.C., Giacomini, P.R., Nair, M.G., Benitez, A.J., Ruymann, K.R., Muir, A.B., Hill, D.A., et al. (2013). Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nature medicine* 19, 1005-1013.
- Pandey, A., Ozaki, K., Baumann, H., Levin, S.D., Puel, A., Farr, A.G., Ziegler, S.F., Leonard, W.J., and Lodish, H.F. (2000). Cloning of a receptor subunit required for signaling by thymic
10 stromal lymphopoietin. *Nat Immunol* 1, 59-64.
- Park, L.S., Martin, U., Garka, K., Gliniak, B., Di Santo, J.P., Muller, W., Largaespada, D.A., Copeland, N.G., Jenkins, N.A., Farr, A.G., et al. (2000). Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor. *J Exp Med* 192, 659-670.
- 15 Pedroza-Gonzalez, A., Xu, K., Wu, T.C., Asford, C., Tindle, S., Marches, F., Gallegos, M., Burton, E.C., Savino, D., Hori, T., et al. (2011). Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. *J Exp Med* 208, 479-490.
- Peelman, F. et al. (2004). Mapping of the leptin binding sites and design of a leptin antagonist. *Journal of Biological Chemistry*, 279, 41038-41046.
- 20 Perez-Andreu, V., Roberts, K.G., Harvey, R.C., Yang, W., Cheng, C., Pei, D., Xu, H., Gastier-Foster, J., E, S., Lim, J.Y., et al. (2013). Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. *Nature genetics* 45, 1494-1498.
- Redhu, N.S., and Gounni, A.S. (2012). Function and mechanisms of TSLP/TSLPR complex in asthma and COPD. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 42, 994-1005.
- 25 Reeves, P. J., Callewaert, N., Contreras, R., & Khorana, H. G. (2002). Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line. *Proceedings of the National Academy of Sciences*, 99, 13419-13424.
- 30 Romeo, M.J., Agrawal, R., Pomes, A., and Woodfolk, J.A. (2013). A molecular perspective on T2-promoting cytokine receptors in patients with allergic disease. *J Allergy Clin Immunol*.
- Romeo, M.J., Agrawal, R., Pomes, A., and Woodfolk, J.A. (2014). A molecular perspective on TH2-promoting cytokine receptors in patients with allergic disease. *J Allergy Clin Immunol* 133, 952-960.
- 35 Rothenberg, M.E., Spergel, J.M., Sherrill, J.D., Annaiah, K., Martin, L.J., Cianferoni, A., Gober, L., Kim, C., Glessner, J., Frackelton, E., et al. (2010). Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nature genetics* 42, 289-291.
- Siracusa, M.C., Saenz, S.A., Tait Wojno, E.D., Kim, B.S., Osborne, L.C., Ziegler, C.G., Benitez, A.J., Ruymann, K.R., Farber, D.L., Sleiman, P.M., et al. (2013). Thymic stromal lymphopoietin-

mediated extramedullary hematopoiesis promotes allergic inflammation. *Immunity* 39, 1158-1170.

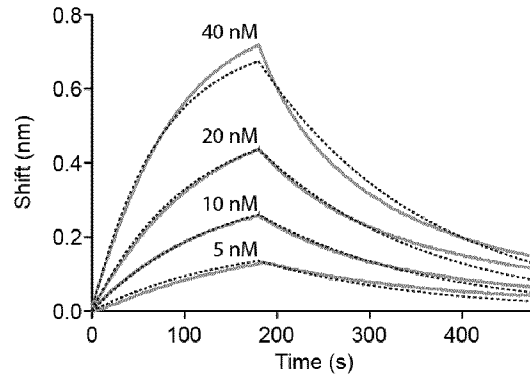
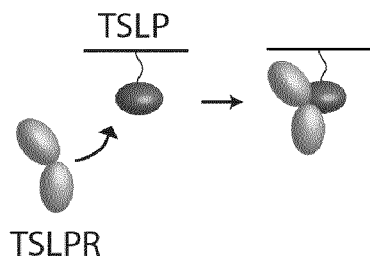
- 5 Spergel, J.M. (2010). From atopic dermatitis to asthma: the atopic march. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* 105, 99-106; quiz 107-109, 117.
- Torgerson, D.G., Ampleford, E.J., Chiu, G.Y., Gauderman, W.J., Gignoux, C.R., Graves, P.E., Himes, B.E., Levin, A.M., Mathias, R.A., Hancock, D.B., et al. (2011). Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nature genetics* 43, 887-892.
- 10 Verstraete, K. et al. (2011). Inducible production of recombinant human Flt3 ectodomain variants in mammalian cells and preliminary crystallographic analysis of Flt3 ligand–receptor complexes. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, 325-331.
- 15 Verstraete, K., van Schie, L., Vyncke, L., Bloch, Y., Tavernier, J., Pauwels, E., Peelman, F., and Savvides, S.N. (2014). Structural basis of the proinflammatory signaling complex mediated by TSLP. *Nature structural & molecular biology* 21, 375-382.
- Volpe, E., Pattarini, L., Martinez-Cingolani, C., Meller, S., Donnadieu, M.H., Bogiatzi, S.I., Fernandez, M.I., Touzot, M., Bichet, J.C., Reyat, F., et al. (2014). Thymic stromal lymphopoietin links keratinocytes and dendritic cell-derived IL-23 in patients with psoriasis. *J Allergy Clin Immunol* 134, 373-381.
- 20 Wilson, S.R., The, L., Batia, L.M., Beattie, K., Katibah, G.E., McClain, S.P., Pellegrino, M., Estandian, D.M., and Bautista, D.M. (2013). The Epithelial Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch. *Cell* 155, 285-295.
- Ying, G., Zhang, Y., Tang, G., and Chen, S. (2015). Functions of thymic stromal lymphopoietin in non-allergic diseases. *Cellular immunology* 295, 144-149.
- 25 Zhang, F., Huang, G., Hu, B., Song, Y., and Shi, Y. (2011). A soluble thymic stromal lymphopoietin (TSLP) antagonist, TSLPR-immunoglobulin, reduces the severity of allergic disease by regulating pulmonary dendritic cells. *Clin Exp Immunol* 164, 256-264.
- Ziegler, S.F. (2010). The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Current opinion in immunology* 22, 795-799.
- 30 Ziegler, S.F. (2012). Thymic stromal lymphopoietin and allergic disease. *J Allergy Clin Immunol* 130, 845-852.
- Ziegler, S.F., and Artis, D. (2010). Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 11, 289-293.
- 35 Ziegler, S.F., Roan, F., Bell, B.D., Stoklasek, T.A., Kitajima, M., and Han, H. (2013). The biology of thymic stromal lymphopoietin (TSLP). *Advances in pharmacology* 66, 129-155.

CLAIMS

1. A monomeric fusion protein comprising the functional extracellular part of the thymic stromal lymphopoietin receptor (TSLPR) and the functional extracellular part of the interleukin-7 receptor alpha (IL-7Ralpha).
5
2. The monomeric fusion protein according to claim 1, wherein said extracellular part of the TSLPR and said extracellular part of the IL-7Ralpha are connected by a linker.
3. The monomeric fusion protein according to claim 1 or 2, wherein said linker comprises at least 10 amino acids.
- 10 4. The monomeric fusion protein according to anyone of claims 1 to 3, wherein said linker is a GGS linker consisting of 5 to 20 GGS units.
5. The monomeric fusion protein according to anyone of claims 1 to 4, wherein said fusion protein comprises an amino acid sequence with at least 80% sequence identity with the amino acid sequence of SEQ ID N° 1 and an amino acid sequence with at least 80%
15 sequence identity with the amino acid sequence of SEQ ID N° 2.
6. The monomeric fusion protein according to anyone of claims 1 to 5, wherein said fusion protein comprises the amino acid sequence of SEQ ID N° 1 and the amino acid sequence of SEQ ID N° 2.
7. A nucleic acid encoding at least the fusion protein according to anyone of claims 1 to 6.
- 20 8. A host cell comprising the nucleic acid of claim 7.
9. The host cell according to claim 8, for use in the production of said fusion protein.
10. The monomeric fusion protein according to anyone of claims 1 to 6, for use as a medicament.
11. The monomeric fusion protein according to anyone of claims 1 to 6, for use in the treatment of an inflammatory disease.
- 25 12. The monomeric fusion protein according to claim 11, wherein said inflammatory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, allergic rhinitis, atopic dermatitis, eosinophilic esophagitis, rheumatoid arthritis and psoriasis.
13. The monomeric fusion protein according to anyone of claims 1 to 6, for use in the treatment of cancer.
- 30 14. The monomeric fusion protein according to anyone of claims 1 to 6, for use in the treatment of fibrosis.
15. A pharmaceutical composition comprising the monomeric fusion protein according to anyone of claims 1 to 6 in association with a pharmaceutically acceptable carrier.

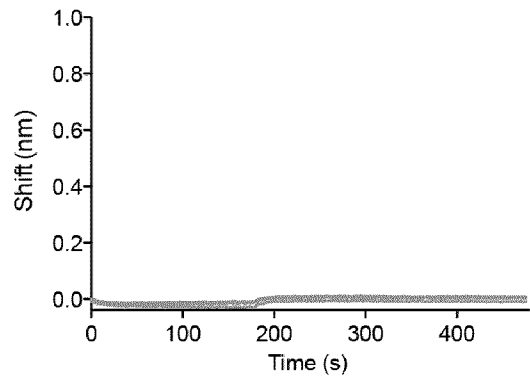
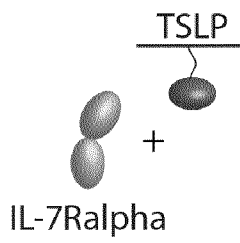
Figure 3

(a)



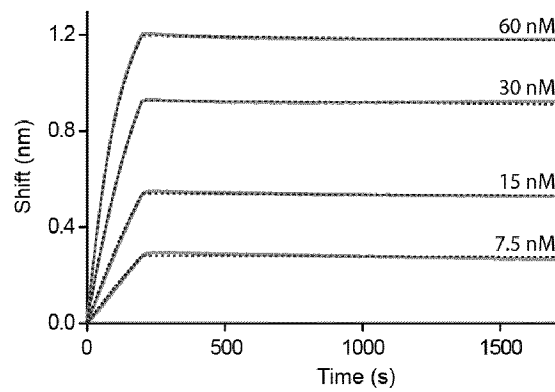
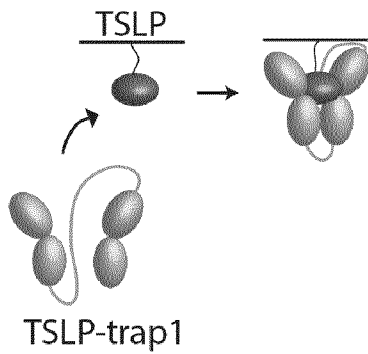
$K_D = 3,2E-08$ M

(b)



No interaction up to 100 nM of IL-7Ralpha

(c)



$K_D = 1,2E-10$ M

Figure 4

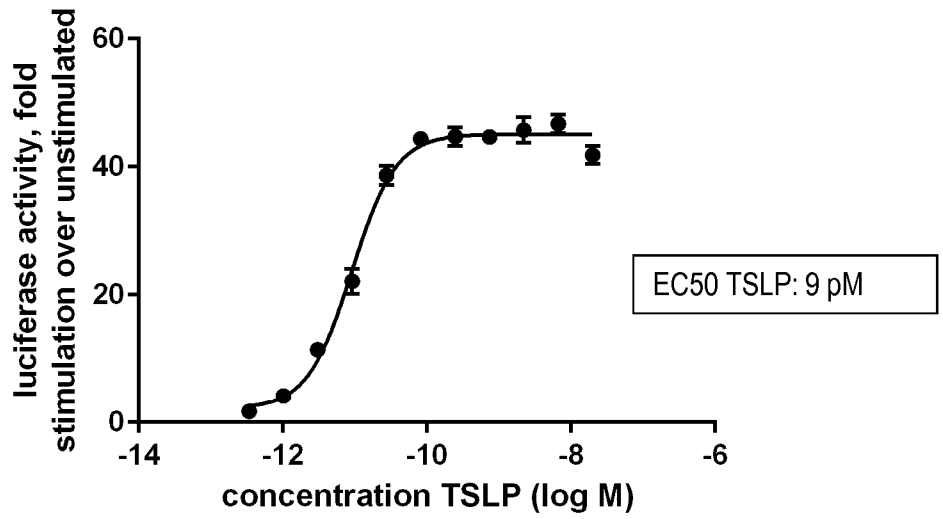


Figure 5

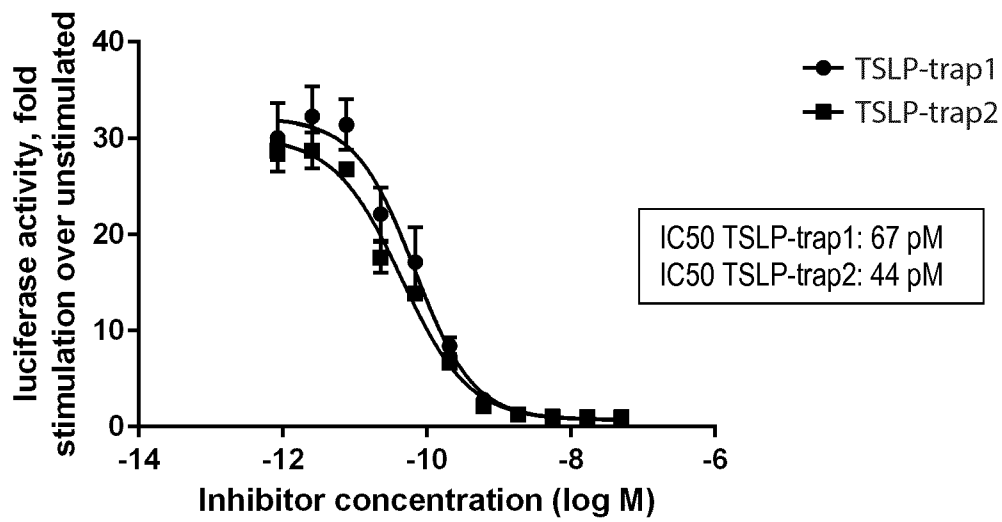


Figure 6

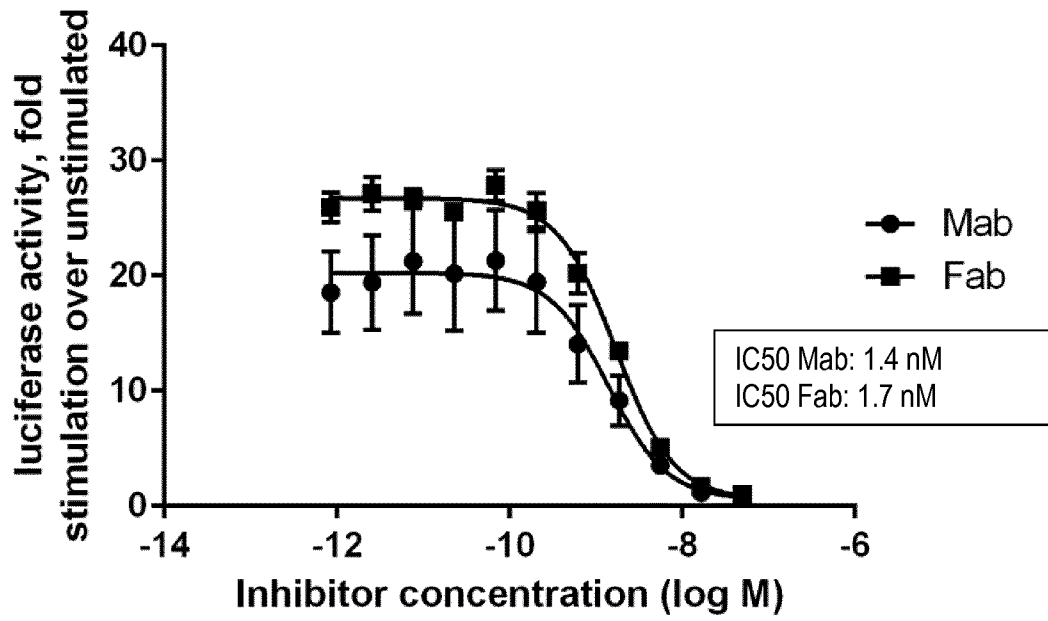


Figure 7

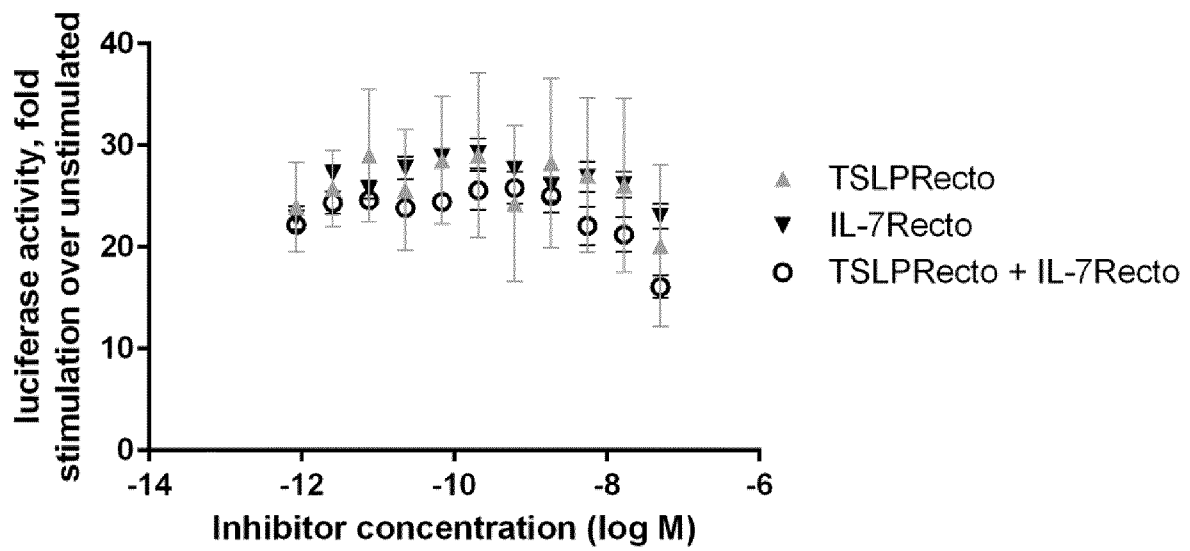


Figure 8

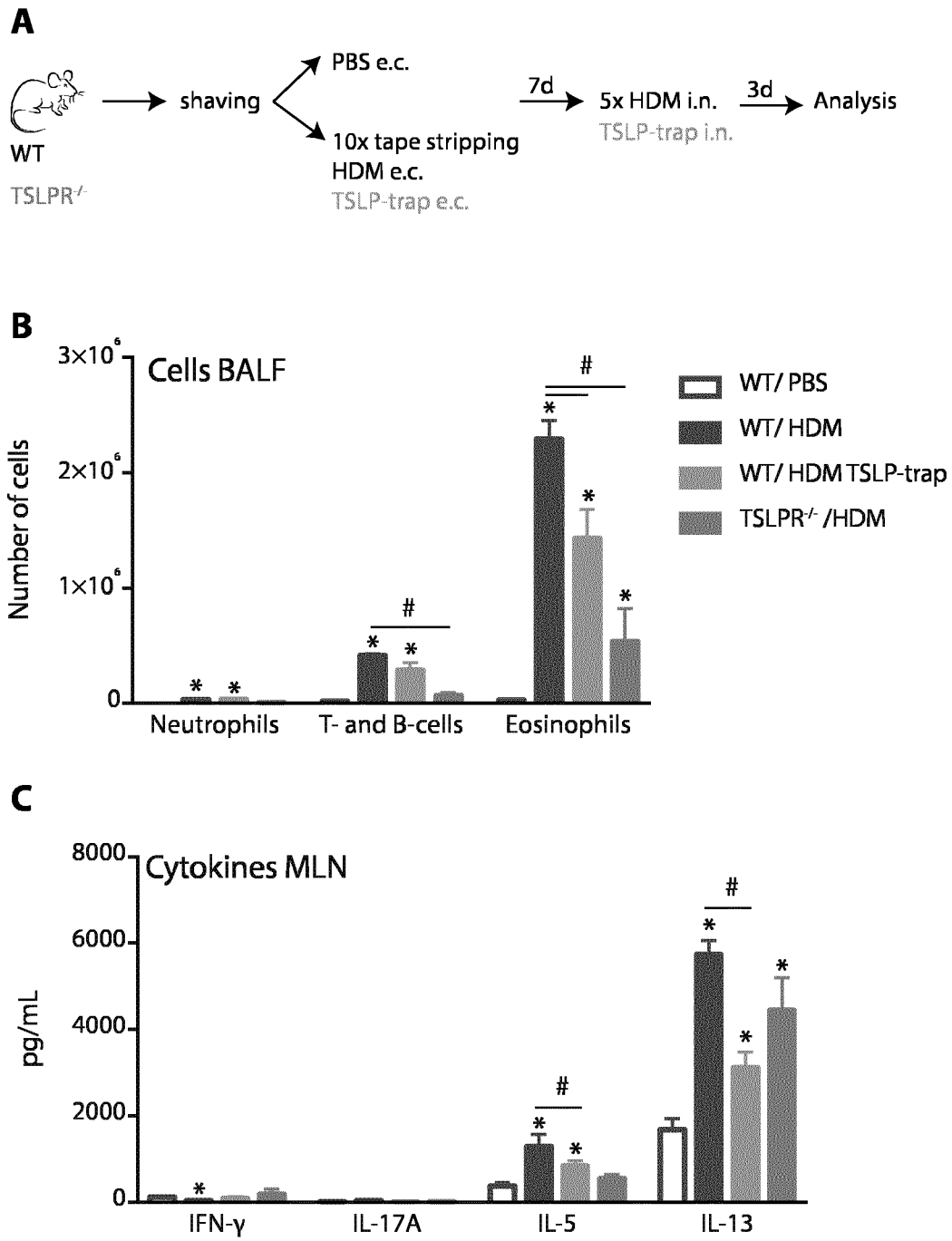


Figure 9

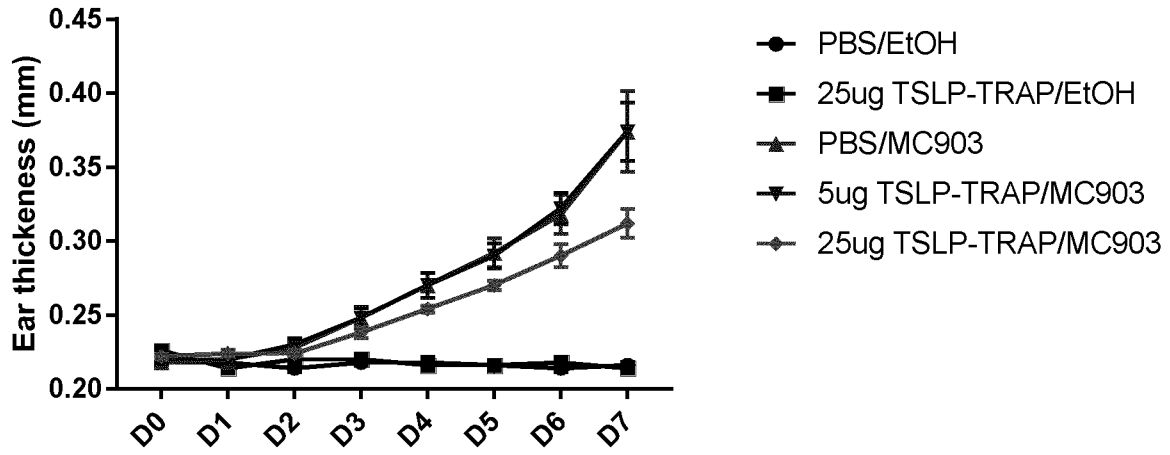


Figure 10

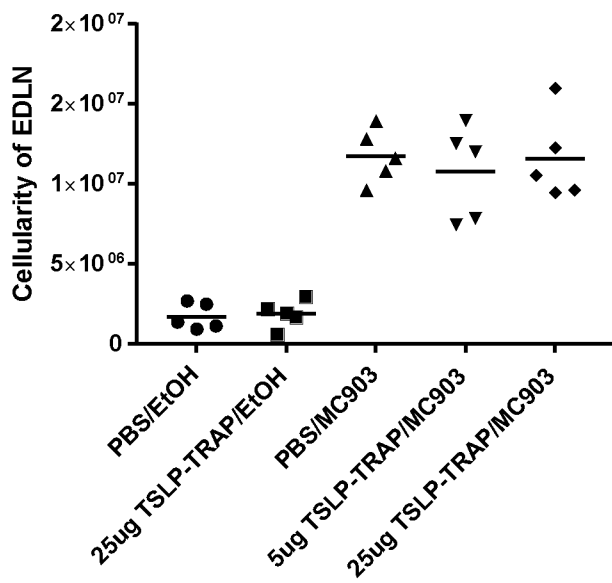
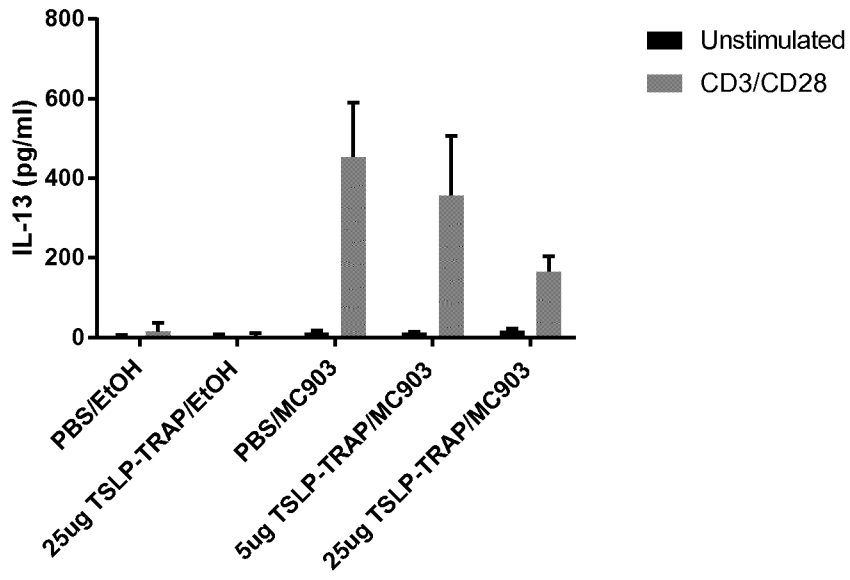


Figure 11



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/057944

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/715
ADD. C12N5/00

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)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 703 414 A1 (AMGEN INC [US]) 5 March 2014 (2014-03-05)	1-4,7-15
A	example 4	5,6
A	----- ALLAKHVERDI ZOULFIA ET AL: "Thymic stfomal lymphopietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 204, no. 2, 1 February 2007 (2007-02-01), pages 253-258, XP002511407, ISSN: 0022-1007, DOI: 10.1084/JEM.20062211 the whole document -----	1-15

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
- "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 15 May 2017	Date of mailing of the international search report 23/05/2017
--	--

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Seranski, Peter
--	---

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2017/057944

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2703414	A1	05-03-2014	AR 068508 A1 18-11-2009
			AU 2008300028 A1 19-03-2009
			CA 2698382 A1 19-03-2009
			CL 2012003245 A1 22-03-2013
			CN 101809035 A 18-08-2010
			CN 104231081 A 24-12-2014
			CR 11319 A 21-04-2010
			CR 20150095 A 06-04-2015
			DK 2205635 T3 18-07-2016
			EA 201000471 A1 29-10-2010
			EA 201590631 A1 31-03-2016
			EP 2205635 A1 14-07-2010
			EP 2703414 A1 05-03-2014
			ES 2581229 T3 02-09-2016
			HK 1145843 A1 07-04-2017
			HR P20160638 T1 01-07-2016
			HU E028361 T2 28-12-2016
			JP 5956716 B2 27-07-2016
			JP 2010538608 A 16-12-2010
			JP 2015091820 A 14-05-2015
			KR 20100067660 A 21-06-2010
			KR 20140141666 A 10-12-2014
			LT 2205635 T 26-09-2016
			MY 150242 A 31-12-2013
			NZ 583933 A 27-07-2012
			PE 02322014 A1 28-02-2014
			PE 06682011 A1 01-10-2011
			PL 2205635 T3 30-12-2016
			PT 2205635 T 13-07-2016
			SG 178781 A1 29-03-2012
			SG 10201506251Y A 27-11-2015
			SI 2205635 T1 30-09-2016
			TW 200918554 A 01-05-2009
			TW 201412774 A 01-04-2014
			US 2009238823 A1 24-09-2009
			US 2011274687 A1 10-11-2011
			US 2012190829 A1 26-07-2012
			US 2016152700 A1 02-06-2016
			WO 2009035577 A1 19-03-2009
