(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 April 2017 (13.04.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/062456 A2

(51) International Patent Classification:
C07K 16/24 (2006.01) *G01N 33/50* (2006.01)
C07K 14/715 (2006.01)

(21) International Application Number:
PCT/US2016/055502

(22) International Filing Date:
5 October 2016 (05.10.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/237,624 6 October 2015 (06.10.2015) US

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(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, 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,

[Continued on next page]

(54) Title: BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF

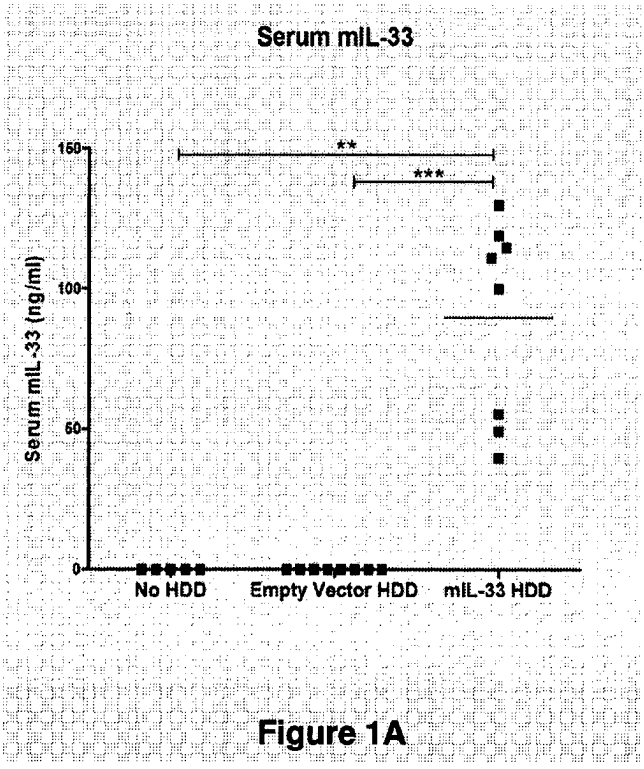

Figure 1A

(57) Abstract: The present invention relates to the identification of certain biomarkers for use in identifying patients who have, or are likely to develop an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein. Also provided are methods for decreasing the level of at least one biomarker in a subject suffering from an IL-33-mediated disease or disorder, and methods for treating such diseases or disorders according to the expression levels of one or more biomarkers. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising an interleukin-33 antagonist.

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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).

Declarations under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF

REFERENCE TO A SEQUENCE LISTING

[0001] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file 10187WO01-Sequence.txt, created on October 4, 2016 and containing 159,920 bytes.

FIELD OF THE INVENTION

[0002] The present invention relates to the identification of particular biomarkers in a mammal that correlate with expression of IL-33 and the use of these biomarkers to identify patients who have, or are likely to develop, an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein.

BACKGROUND

[0003] Interleukin-33 (IL-33), which is a member of the IL-1 superfamily of cytokines, is expressed predominantly by stromal cells, such as epithelial and endothelial cells, following pro-inflammatory stimulation. IL-33 is a ligand for ST2, a toll-like/interleukin-1 receptor super-family member that associates with an accessory protein, IL-1RAcP (for reviews, *see, e.g.*, Kakkar and Lee, *Nature Reviews – Drug Discovery 7(10)*:827-840 (2008), Schmitz et al., Immunity 23:479-490 (2005); Liew et al., *Nature Reviews – Immunology 10*:103-110 (2010); US 2010/0260770; US 2009/0041718). Upon activation of ST2/IL-1RAcP by IL-33, a signaling cascade is triggered through downstream molecules such as MyD88 (myeloid differentiation factor 88) and TRAF6 (TNF receptor associated factor 6), leading to activation of NFκB (nuclear factor-κB), among others.

[0004] IL-33 signaling has been implicated as a factor in a variety of diseases and disorders (Liew et al., *Nature Reviews – Immunology 10*:103-110 (2010)). For example, while IL-33 is protective against helminth infection in a host and also reduces atherosclerosis by promoting T_H2-type immune responses, it can also promote the pathogenesis of asthma by expanding T_H2 cells and mediate joint inflammation, atopic dermatitis and anaphylaxis by mast cell activation. As such, IL-33 may be a new target for therapeutic intervention across a range of diseases; for example, blockade of IL-33 signaling may offer the potential to ameliorate multiple pathogenic features of certain inflammatory diseases.

[0005] IL-33 antagonists are described in U.S. patent numbers 5,576,191; 7,666,622; 8,119,771; 8,187,596; 9,090,694; 9,212,227; 9,382,318; U.S. patent publication numbers 2007/0042978; 2009/0041718; 2010/0260770; 2012/0207752; 2012/0263709; 2014/0140954;

2014/0271642; 2014/0212412; EP patents or patent application numbers 1725261B1; 2069784A1; 2152740A1; 2283860A2; 2475388A1; and PCT publication numbers WO05/079844; WO08/132709; WO08/144610; WO09/053098; WO11/031600; WO14/152195 and WO14/164959.

[0006] Calcitonin (CT) is a polypeptide hormone, thought to be produced primarily by parafollicular cells of the thyroid (Foster, G. V., *et. al.,* (1964), Nature 202: 1303-1305). CT is a product of the *CALCA* gene, located on chromosome 11. The *CALCA* gene encodes the polypeptides procalcitonin (PCT) and PCT gene–related peptide α (proCGRPα), which are differentially expressed by alternative splicing (Christ-Crain, M. *et al.* (2008), Crit Care Med 36:1684-1687; Hoff, AO, *et al.* (2002), J. Clin. Invest. 110:1849-1857). Calcitonin (CT) is derived from a larger precursor, Procalcitonin (PCT, 116 amino acids), which is cleaved to immature calcitonin (33 amino acids) and then to mature calcitonin, a monomer of a 3.5-kd peptide composed of 32 amino acids. CT is predominantly produced by neuroendocrine cells such as C cells of the thyroid and pulmonary neuroendocrine cells (PNECs) by proteolytic cleavage in the secretory granules of producing cells before being released as mature CT polypeptide. Other cells that express the *calca* gene include mast cells, dorsal route ganglion cells (DRGs) and cells of the spinal cord. Multiple forms of circulating calcitonin precursors are found in the serum of healthy and diseased individuals (Becker K. *et al.* (2004), J Clin Endocrinol Metab 89:1512-1525).

[0007] Calcitonin gene related peptide (CGRP) is a member of the calcitonin family of peptides. α-CGRP is a 37-amino acid peptide formed from the alternative splicing of the calcitonin/CGRP gene located on chromosome 11 (Amara, SG, *et al.,* (1982), Nature, 298 (5871): 240-244).

[0008] CT acts to reduce blood calcium, inhibits both osteoclasts and bone resorption, opposing the activity of parathyroid hormone (PTH) (Naot, D. and J. Cornish (2008), Bone 43(5): 813-818). *In vivo* and *in vitro* experiments suggest that the major physiological function of CT is to combat hypercalcemia in states of calcium stress such as during pregnancy, growth or lactation (Hoff, A. O., *et. al.,* (2002), J Clin Invest 110(12): 1849-1857; Zaidi, M., *et. al.,* (2002), J Clin Invest 110(12): 1769-1771). Diagnostically, CT is used as biomarker for medullary thyroid carcinoma (MTC). Normal circulating levels of CT peptides are low, however under physiological conditions these levels increase either systemically or locally. High CT levels indicate the presence of MTC and are used to evaluate the efficacy of surgical extirpation and recurrences. CT is also elevated in C-cell hyperplasia, pulmonary and pancreatic tumors, kidney failures and thyroid autoimmune disease (Becker, K. L., *et.al.,* (2004). J Clin Endocrinol Metab 89(4): 1512-1525).

[0009] To date, no biomarkers for identifying patients that have, or are prone to develop an IL-33 mediated disease or disorder, or to determine a patient's likelihood to respond to therapy with an IL-33 antagonist have been identified. Although IL-33 antagonists have been identified

that show promise in the treatment of inflammatory conditions, or allergies, biomarkers that may predict the efficacy of anti-IL-33 therapy are needed for the effective identification and selection of patient sub-populations that respond favorably to anti-IL-33 therapy. Accordingly, an unmet need exists in the art for identifying and validating predictive and prognostic biomarkers in patients with inflammatory conditions, or allergies, who are administered anti-IL-33 therapy.

BRIEF SUMMARY OF THE INVENTION

[0010] According to certain aspects of the present invention, methods are provided for treating, preventing and/or reducing the severity or duration of symptoms of an interleukin-33 (IL-33)-mediated disease or disorder in a subject. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 (IL-33) antagonist.

[0011] In one embodiment of the present invention, the IL-33 antagonist is an antibody or antigen-binding fragment thereof that specifically binds IL-33.

[0012] In one embodiment of the present invention, the IL-33 antagonist is a receptor-based antagonist of IL-33, such as an IL-33 trap, as described herein.

[0013] The present invention also provides for the identification of particular biomarkers that correlate with the expression of IL-33 in a subject suffering from an IL-33 mediated disease or disorder. Exemplary biomarkers associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention include, but are not limited to, polypeptides encoded by the *CALCA* gene, including calcitonin, procalcitonin, and calcitonin gene-related peptide (CGRP). In one embodiment, the biomarker associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention is calcitonin. Other biomarkers associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention include, but are not limited to resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Kirg1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f).

[0014] In a related aspect, the invention provides for the expression of at least one biomarker in the serum of a subject suffering from an IL-33 mediated disease or disorder, which correlates with the level of expression of IL-33 in at least one tissue sample from the subject.

[0015] In one embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is calcitonin.

[0016] In a certain embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is procalcitonin.

[0017] In a related embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is calcitonin gene-related peptide (CGRP).

[0018] In another related aspect, the present invention provides a method for treating an interleukin-33 (IL-33) mediated disease or disorder in a subject, the method comprising: (a) selecting a subject who exhibits an elevated level of at least one biomarker associated with an IL-33 mediated disease or disorder prior to, or at the time of treatment, and (b) administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 antagonist.

[0019] According to a related aspect of the present invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise administering to a subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist, wherein administration of the pharmaceutical composition to the subject results in a decrease in at least one biomarker associated with an IL-33-mediated disease or disorder in the subject.

[0020] In one embodiment, the biomarker that is elevated in a subject suffering from an IL-33 mediated disease or disorder is calcitonin and the IL-33 antagonist is a monoclonal antibody that specifically binds to IL-33.

[0021] The present invention also provides methods for decreasing the level of one or more IL-33-mediated disease or disorder-associated biomarker(s) in a subject, which results in improving one or more IL-33-mediated disease or disorder-associated parameter(s) in a subject, wherein the methods comprise administering to a subject in need thereof a single initial dose of a pharmaceutical composition comprising an IL-33 antagonist. In certain embodiments, the invention provides administering one or more secondary doses of the pharmaceutical composition comprising the IL-33 antagonist, wherein the administering results in further reduction in the level of one or more of the biomarkers described herein and further improvement in one or more IL-33 mediated disease or disorder-associated parameters in a subject. In one embodiment, there is a strong correlation between the level of the biomarker present in the serum of the subject having an IL-33 mediated disease or disorder and the severity of the disease or disorder-associated parameter, e.g. infiltration of eosinophils or neutrophils into the lungs of the subject, or an increase in cytokine levels (e.g. IL-5) in the lungs of the subject. In certain embodiments, the level of IL-33 in the tissue, e.g. the lung, correlates with the severity of the disease or disorder-associated parameter. In one embodiment, the IL-33 antagonist is a human monoclonal antibody that binds specifically to IL-33. In one embodiment, the IL-33 antagonist is an IL-33 trap, as described herein.

[0022] In a related aspect, the present invention provides methods for treating an IL-33-mediated disease or disorder in a subject comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds IL-33, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder and has also been selected for treatment on the basis of the subject exhibiting an elevated level of at least one IL-33-mediated disease or

disorder-associated biomarker before treatment, as compared to a reference level of the biomarker (*e.g.*, expression of the biomarker in a subset of subjects diagnosed with an IL-33-mediated disease or disorder and/or expression of the biomarker in healthy subjects).

[0023] In another related aspect, the present invention also provides methods for treating an IL-33-mediated disease or disorder by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds IL-33, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has already been treated with the anti-IL33 antagonist for a defined period of time, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting expression of at least one biomarker (*e.g.*, calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP)) after treatment for a defined period of time, wherein the expression of the biomarker is determined based on a comparison to the level of expression of the respective biomarker in the subject prior to treatment with the anti-IL-33 antagonist.

[0024] The invention also provides a method for identifying a subject suffering from an IL-33 mediated disease or disorder who is likely to respond favorably to therapy with an IL-33 antagonist, the method comprising obtaining a sample from the subject and measuring the level of calcitonin in the sample; wherein an elevated level of calcitonin, as compared to the lower level of calcitonin in subjects not suffering from an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

[0025] In certain embodiments, the subject who is likely to respond favorably to therapy with an IL-33 antagonist may be identified by measuring the level of procalcitonin, or calcitonin gene-related peptide (CGRP) in a tissue sample, wherein an elevated level of either procalcitonin, or calcitonin gene-related peptide (CGRP), as compared to the lower level of procalcitonin, or CGRP in subjects not suffering from an IL-33 mediated disease or disorder, identifies that patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

[0026] In another related aspect, the invention provides a method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of calcitonin; wherein an elevated level of calcitonin, as compared to a reference standard, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder.

[0027] In certain embodiments, the invention provides a method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of procalcitonin, or CGRP; wherein an elevated level of procalcitonin, or CGRP, as compared to reference standards for either of the two biomarkers, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder. Methods for measuring the levels of any of the above biomarkers are known to those of skill in the art.

[0028] The sample from the subject may be a solid tissue sample, a cell sample, or a blood sample. The solid tissue sample may be selected from the group consisting of heart, kidney, liver, lung, colon and spleen. The cell sample may be a dorsal root ganglion cell sample, sputum cell sample, bronchoalveolar lavage cell sample, nasal polyps cell sample, fecal cell sample, lung, colon heart, kidney, skin biopsy, or spleen biopsy cell sample. The blood sample may be whole blood, plasma, or serum.

[0029] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

[0030] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0031] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

[0032] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having

an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

[0033] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated IL-33 receptor based antagonist (*e.g.* an IL-33 trap), comprising a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein. In one embodiment, the IL-33 trap further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

[0034] In one embodiment, the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

[0035] In one embodiment, the IL-33 receptor based antagonist is an IL-33 trap comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

[0036] In one embodiment, the methods of the invention provide administration of an effective amount of a second therapeutic agent useful for diminishing at least one symptom of an IL-33 mediated disease or disorder.

[0037] In one embodiment, the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid, a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-13 antagonist, an IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-25 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33.

[0038] In one embodiment, the invention provides for treatment of a subject suffering from an IL-33 mediated disease or disorder with an IL-33 antagonist selected from an anti-IL-33 antibody or an IL-33 trap, which may be used in combination with any one or more of the second therapeutic agents noted above, wherein the effectiveness of treatment with these agents may be monitored by assessing the level of at least one biomarker selected from the group consisting of calcitonin (Calca), procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

[0039] In one embodiment, the biomarker is calcitonin and the calcitonin level increases in the serum of the subject having an IL-33 mediated disease or disorder, and the increase in serum calcitonin correlates with an increased level of calcitonin and IL-33 in the lungs of the subject.

[0040] In one embodiment, the increased level of calcitonin in the serum of the subject having an IL-33 mediated disease or disorder is reduced to a reference range level following treatment

with an anti-IL-33 antagonist.

[0041] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum calcitonin level of greater than about 5 pg/ml, greater than about 10 pg/ml, greater than about 50 pg/ml, or greater than about 100 pg/ml prior to, or at the time of treatment.

[0042] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum procalcitonin level of greater than about 0.15 ng/ml, greater than about 1.0 ng/ml, greater than about 5 ng/ml, greater than about 10 ng/ml, greater than about 50 ng/ml, or greater than about 100 ng/ml prior to, or at the time of treatment.

[0043] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum calcitonin gene-related peptide (CGRP) level of greater than about 45 pg/ml, greater than about 100 pg/ml, greater than about 250 pg/ml, or greater than about 500 pg/ml prior to, or at the time of treatment.

[0044] In certain embodiments, the methods of the invention provide for treatment of an IL-33 mediated disease or disorder, which is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

[0045] In one embodiment, the IL-33 mediated disease or disorder to be treated is an allergy and the subject selected for treatment with an IL-33 antagonist is selected on the basis of an increase in calcitonin levels that are above normal (*e.g.* based on a reference level of the biomarker as determined by established values for healthy subjects compared to subjects diagnosed with an IL-33 mediated disease or disorder), wherein the treatment with an IL-33 antagonist comprises administration of a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that specifically binds IL-33 and comprises an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282, and wherein the administration results in an improvement in at least one symptom associated with the IL-33 disease or disorder, or an improvement in at least one disease associated parameter; and wherein the calcitonin level is restored to a normal range as determined by comparison to a reference standard.

[0046] In one embodiment, the IL-33 mediated disease or disorder to be treated is an allergy and the subject selected for treatment with an IL-33 antagonist is selected on the basis of an increase in serum calcitonin levels as determined by comparison with a reference standard,

wherein the calcitonin level correlates with an increase in IL-33 level in the subject, or with an increase in at least one disease parameter in the subject, and wherein the increase in IL-33 level is determined using a binding assay comprising an antibody that binds specifically to human IL-33 and comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0047] The present invention also includes uses of the IL-33 antagonists (*e.g.*, IL-33 trap), anti-IL-33 antibodies and pharmaceutical compositions discussed above or herein for treating, or reducing a symptom of, an IL-33 mediated disease or disorder. The present invention further includes uses of the IL-33 antagonists (*e.g.*, IL-33 trap), anti-IL-33 antibodies and pharmaceutical compositions discussed above or herein in the manufacture of a medicament for treating, or reducing a symptom of, an IL-33 mediated disease or disorder.

[0048] The present invention also includes any combination of the embodiments discussed above or herein. Particular embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0049] **Figures 1A and 1B:** Show mouse serum IL-33 (Fig. 1A) and mouse Calcitonin (CT) levels (Fig. 1B) in a mouse IL-33 HDD experiment.

[0050] **Figure 2:** Shows mouse Calcitonin (Calca) gene expression in the chronic HDM model.

[0051] **Figure 3:** Shows the correlation between serum and lung Calcitonin (CT) levels in the chronic HDM model.

[0052] **Figure 4:** Shows the correlation of serum Calcitonin (CT) and lung IL-33 levels in the chronic HDM model.

[0053] **Figures 5A, 5B and 5C:** Show the parameters affected by anti-IL-33 treatment in chronic HDM model. Fig. 5A: Frequency of lung neutrophils; Fig. 5B: Frequency of lung eosinophils; Fig. 5C: lung IL-5 levels.

[0054] **Figures 6A, 6B and 6C:** Show the correlation of serum Calcitonin (CT) and the parameters affected by anti-IL-33 treatment in the chronic HDM model. Fig. 6A: correlation between serum CT levels and frequency of lung neutrophils; Fig. 6B: correlation between serum CT levels and frequency of lung eosinophils; Fig. 6C: correlation between serum CT and lung IL-5 protein levels.

[0055] **Figures 7A, 7B and 7C:** Show the correlation of lung IL-33 levels and the parameters affected by anti-IL-33 treatment in the chronic HDM model. Fig. 7A: correlation between lung IL-33 levels and frequency of lung neutrophils; Fig. 7B: correlation between lung IL-33 levels and frequency of lung eosinophils; Fig. 7C: correlation between lung IL-33 and lung IL-5 protein levels.

DETAILED DESCRIPTION

[0056] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0057] 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 this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.). As used herein, the terms "treat", "treating", or the like, mean to alleviate symptoms, eliminate the causation of symptoms either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of the named disorder or condition.

[0058] Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described.

Biomarkers Associated with an IL-33-Mediated Disease or Disorder

[0059] The present invention includes methods involving the use, quantification, and analysis of biomarkers associated with an IL-33-mediated disease or disorder.

[0060] An "IL-33 mediated disease or disorder" may be selected from any inflammatory disease or disorder such as, but not limited to, asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

[0061] The asthma may be selected from the group consisting of allergic asthma, non-allergic asthma, severe refractory asthma, asthma exacerbations, viral-induced asthma or viral-induced asthma exacerbations, steroid resistant asthma, steroid sensitive asthma, eosinophilic asthma or non-eosinophilic asthma and other related disorders characterized by airway inflammation or airway hyperresponsiveness (AHR).

[0062] The COPD may be a disease or disorder associated in part with, or caused by, cigarette smoke, air pollution, occupational chemicals, allergy or airway hyperresponsiveness.

[0063] The allergy may be associated with foods, pollen, mold, dust mites, animals, or animal dander.

[0064] The IBD may be selected from the group consisting of ulcerative colitis, Crohn's Disease, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis, and other disorders characterized by inflammation of the mucosal layer of the large intestine or colon.

[0065] The arthritis may be selected from the group consisting of osteoarthritis, rheumatoid arthritis and psoriatic arthritis.

[0066] As used herein, the term "an IL-33-mediated disease or disorder-associated biomarker", or a "biomarker associated with an IL-33-mediated disease or disorder" means any biological response, cell type, parameter, protein, polypeptide, enzyme, enzyme activity, metabolite, nucleic acid, carbohydrate, or other biomolecule which is present or detectable in a patient suffering from an IL-33-mediated disease or disorder at a level or amount that is different from (*e.g.*, greater than or less than) the level or amount of the marker present or detectable in a non-IL-33-mediated disease or disorder patient. Exemplary IL-33-mediated disease or disorder-associated biomarkers include, but are not limited to, *e.g.*, polypeptides encoded by the *CALCA* gene, including calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP). Other biomarkers associated with an IL-33-mediated disease or disorder include, but are not limited to resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Kirg1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f).

[0067] Methods for detecting and/or quantifying such biomarkers are known in the art; kits for measuring such biomarkers are available from various commercial sources; and various commercial diagnostic laboratories offer services, which provide measurements of such biomarkers as well.

[0068] In one embodiment, the biomarker is calcitonin and the level of calcitonin is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. In one embodiment, the biomarker is procalcitonin and the level of procalcitonin is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. In a related embodiment, the biomarker is calcitonin gene-related peptide (CGRP) and the level of CGRP is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. The level of calcitonin, procalcitonin, or CGRP may be assessed using any method known to those skilled in the art. Methods of detection include an immunoassay using any label for detection, such as a radioisotopic label, an enzymatic label, or a chemiluminescent label. The interpretation of the results must take into account the method used, the subject's age, gender and weight (See d'Herbomez, M. *et. al.*, (2007), Eur. J. Endocrinology 157:749-755). Generally, a "normal" reference value of calcitonin in males is less than about 16 pg/mL

and less than about 8 pg/mL for females. More recently, Camacho *et.al.* developed a new immunofluorometric assay for serum calcitonin and have validated it in samples from 794 patients. The results obtained using this assay showed that the normal cut-off range for males was less than 11.1 pg/mL and less than 5.5 pg/mL for females. (See Camacho, *et. al.*, (2014), Eur. Thyroid J., Jun;3(2):117-24. A "reference" range of procalcitonin in adults and children older than 72 hours is about 0.15 ng/mL or less. In healthy adults, the reference range of procalcitonin is below the level of detection (See Dandona, P. *et al.* (1994), J. Clin. Endocrinol. Metab. Dec. 79(6):1605-8.). A normal range of CGRP in healthy individuals is generally below about 45 pg/ml. As such, a value above that may be considered indicative of the presence of a disease or disorder.

[0069] An "IL-33 associated disease parameter" may include, but not be limited to, an increase in infiltration of neutrophils, eosinophils or ST2+ CD4 T cells to a tissue (*e.g.* lung), goblet cells metaplasia, an increase in ST2 levels in the tissue or an increase in the level of a cytokine, such as IL-1β, IL-4, IL-5, IL-6, IL-9, IL-13, IL-33, MCP-1, TNFα in the tissue.

[0070] According to certain aspects of the invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise: (a) selecting a subject who exhibits a level of at least one biomarker prior to or at the time of treatment which signifies the disease state, and (b) administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist. In one embodiment of this aspect of the invention, the subject is selected on the basis of an elevated level of calcitonin. In one embodiment, the IL-33 antagonist is an isolated monoclonal antibody the specifically binds to IL-33, or a receptor based IL-33 antagonist (an IL-33 trap as described herein).

[0071] According to other aspects of the invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise administering to a subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist, wherein administration of the pharmaceutical composition to the subject results in a decrease in at least one biomarker (*e.g.*, calcitonin (Calca), procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f), etc.) at a time after administration of the pharmaceutical composition, as compared to the level of the biomarker in the subject prior to the administration.

[0072] As will be appreciated by a person of ordinary skill in the art, an increase or decrease in a biomarker associated with an IL-33 mediated disease or disorder can be determined by comparing (i) the level of the biomarker measured in a subject at a defined time point after administration of the pharmaceutical composition comprising an IL-33 antagonist to (ii) the level of the biomarker measured in the patient prior to the administration of the pharmaceutical composition comprising an IL-33 antagonist (*i.e.*, the "baseline measurement"). The defined

time point at which the biomarker is measured can be, *e.g.*, at about 4 hours, 8 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 15 days, 20 days, 35 days, 40 days, 50 days, 55 days, 60 days, 65 days, 70 days, 75 days, 80 days, 84 days, 85 days, or more after administration of the of the pharmaceutical composition comprising an IL-33 antagonist.

[0073] According to certain particular embodiments of the present invention, a subject may exhibit a decrease in the level of one or more of calcitonin, procalcitonin, CGRP, resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f) following administration of a pharmaceutical composition comprising an IL-33 antagonist (*e.g.*, an anti-IL-33 antibody, or an IL-33 receptor based antagonist (an IL-33 trap). For example, at about day 4, day 8, day 14, day 15, day 22, day 25, day 29, day 36, day 43, day 50, day 57, day 64, day 71, day 84, or day 85, following administration of a first, second, third or fourth dose of a pharmaceutical composition comprising about an anti-hIL-33 antagonist. The dose of an anti-IL-33 antagonist administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When an IL-33 antagonist is used for treating a condition or disease associated with IL-33 activity in an adult patient, it may be advantageous to intravenously administer the anti-IL-33 antagonist normally at a dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight.

[0074] The subject, according to the present invention, may exhibit a decrease in at least one of the following biomarkers: a polypeptide encoded by the *CALCA* gene, selected from the group consisting of calcitonin, procalcitonin and CGRP, or may exhibit a decrease in at least one of the following biomarkers: resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Kirg1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f), of about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from baseline (wherein "baseline" is defined as the level of biomarker in the subject just prior to the first administration). Similarly, at about day 4, day 8, day 14, day 15, day 22, day 25, day 29, day 36, day 43, day 50, day 57, day 64, day 71, day 84 or day 85, following administration of a first, second, third or fourth dose of a pharmaceutical composition of an anti-hIL-33 antagonist, the subject, according to the present invention, may exhibit a decrease in at least one of the biomarkers described above of about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from baseline (wherein "baseline" is defined as the level of biomarker in the subject just prior to the first administration).

[0075] The present invention also includes methods for determining whether a subject is a suitable subject (*e.g.,* a subject who is likely to respond favorably to therapy with an IL-33 antagonist) for whom administration of a pharmaceutical composition comprising an IL-33 antagonist would be beneficial. For example, if an individual, prior to receiving a pharmaceutical composition comprising an IL-33 antagonist, exhibits a level of a biomarker associated with an IL-33-mediated disease or disorder, which signifies the disease state, the individual is therefore identified as a suitable patient for whom administration of a pharmaceutical composition of the invention (a composition comprising an anti-IL-33 antagonist) would be beneficial. According to certain exemplary embodiments, an individual may be identified as a good candidate for anti-IL-33 therapy if the individual exhibits one or more of the following: (i) a calcitonin level greater than about 5 pg/mL, greater than about 10 pg/mL, greater than about 20 pg/mL, greater than about 30 pg/mL, greater than about 40 pg/mL, greater than about 50 pg/mL, greater than about 60 pg/mL, greater than about 70 pg/mL, greater than about 80 pg/mL, greater than about 90 pg/mL, greater than about 100 pg/mL, or greater than about 200 pg/mL. Additional criteria, such as other clinical indicators of an IL-33-mediated disease or disorder may be used in combination with any of the biomarkers described herein to identify an individual as a suitable candidate for anti-IL-33 therapy as described elsewhere herein (*e.g.*, increase in infiltration of neutrophils, eosinophils or ST2+ CD4 T cells to a tissue (*e.g.* lung), goblet cells metaplasia, an increase in ST2 levels in the tissue or an increase in the level of a cytokine, such as IL-1β, IL-4, IL-5, IL-6, IL-9, IL-13, IL-33, MCP-1, TNFα in the tissue, as well as interferon gamma)..

[0076] In other aspects of the invention, biomarker levels and/or changes in biomarker levels with treatment may have predictive value for efficacy of anti-IL33 therapy with particular anti-IL33 agents.

[0077] An additional aspect of the invention provides methods of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, wherein the method comprises obtaining a biological sample from the subject and measuring the level of at least one of the biomarkers described herein; wherein the elevated level of at least one biomarker as compared to the level of the biomarker from a subject not having an IL-33 mediated disease or disorder, or not likely to develop an IL-33 mediated disease or disorder, identifies the subject as a subject who is likely to have, or develop an IL-33 mediated disease or disorder.

[0078] An additional aspect of the invention features methods of treating an IL-33-mediated disease or disorder in a subject comprising administration of an anti-IL33 antagonist, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has been treated with the anti-IL33 antagonist, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting reduced expression of a biomarker (*e.g.* calcitonin), wherein the reduced expression of the biomarker is determined based on a comparison to the level of expression of the respective biomarker in the subject prior to treatment with the anti-IL-

33 antagonist. A particular aspect of the invention features methods of treating an IL-33-mediated disease or disorder in a subject comprising administration of an anti-IL33 antagonist, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has been treated with the anti-IL33 antagonist, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting reduced expression of the biomarker, wherein the reduced expression of the biomarker in comparison to pre-treatment expression levels is equal to or greater than about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in biomarker expression.

Interleukin-33 Antagonists

[0079] As disclosed in detail above, the present invention includes methods, which comprise administering to a subject in need thereof a therapeutic composition comprising an IL-33 antagonist. As used herein, an "IL-33 antagonist" is any agent, which binds to or interacts with IL-33, or with its receptor and inhibits the normal biological signaling function of IL-33 when IL-33 is expressed on a cell *in vitro* or *in vivo*. Non-limiting examples of categories of IL-33 antagonists include small molecule IL-33 antagonists, anti-IL-33 aptamers, peptide-based IL-33 antagonists (*e.g.*, "peptibody" molecules), antibodies or antigen-binding fragments of antibodies that specifically bind human IL-33, or receptor based IL-33 antagonists (*e.g.* an IL-3 trap), such as those described herein.

[0080] In one embodiment, the present invention includes methods that comprise administering to a patient a human antibody, or an antigen-binding fragment thereof, that binds specifically to hIL-33. As used herein, the term "hIL-33" means a human cytokine that specifically binds to its receptor, ST2. The amino acid sequence of human ST2 is shown in SEQ ID NO: 334. The amino acid sequence of human IL-33 is shown in SEQ ID NO: 306. The nucleic acid encoding human IL-33 is shown in SEQ ID NO: 305. The anti-IL-33 antibodies may be selected from any one or more of those having the amino acid sequences described in Table 1.

[0081] In one embodiment, the present invention includes methods that comprise administering to a patient a receptor based IL-33 antagonist, for example, an IL-33 trap, such as those described herein. Five different exemplary IL-33 traps of the invention were constructed using standard molecular biological techniques. Table 2a sets forth a summary description of the different IL-33 traps and their component parts. Table 2b sets forth the amino acid sequences of the IL-33 traps and their component parts.

[0082] The term "antibody," as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3.

Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-IL-33 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0083] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0084] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.*, monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0085] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For

example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0086] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0087] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0088] The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0089] The term "human antibody", as used herein, is intended to include non-naturally occurring human antibodies. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[0090] The term "recombinant human antibody," as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor et al. (1992) Nucl. Acids

Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0091] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0092] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0093] An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0094] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" IL-33, as used in the context of

the present invention, includes antibodies that bind IL-33 or portion thereof with a K_D of less than about 1000 nM, less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. An isolated antibody that specifically binds human IL-33 may, however, have cross-reactivity to other antigens, such as IL-33 molecules from other (non-human) species.

[0095] The anti-IL-33 antibodies useful for the methods of the present invention may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes methods involving the use of antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one

or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. The use of antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0096] The present invention also includes methods involving the use of anti-IL-33 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes the use of anti-IL-33 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0097] The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0098] The term "K_D," as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0099] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

Preparation of Human Antibodies

[0100] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human IL-33.

[0101] Using VELOCIMMUNE™ technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to IL-33 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The

DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0102] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0103] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc, using standard procedures known to those skilled in the art. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0104] In general, the antibodies that can be used in the methods of the present invention possess high affinities, as described above, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0105] Specific examples of human antibodies or antigen-binding fragments of antibodies that specifically bind IL-33 which can be used in the context of the methods of the present invention include any antibody or antigen-binding fragment which comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308. The antibody or antigen-binding fragment may comprise the three light chain CDRs (LCVR1, LCVR2, LCVR3) contained within a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein.

Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0106] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises the six CDRs (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3) from the heavy and light chain variable region amino acid sequence pairs (HCVR/LCVR) selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0107] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises HCVR/LCVR amino acid sequence pairs selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0108] The study summarized in the attached Examples utilized an anti-hIL-33 antibody referred to as H4H9675P. This antibody comprises an HCVR/LCVR amino acid sequence pair having SEQ ID NOs: 274/282, and HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 domains represented by SEQ ID NOs: 276-278-280 / SEQ ID NOs: 284-286-288. However, the methods of the present invention can be practiced using any anti-IL-33 antibody disclosed herein, as well as variants and antigen-binding fragments of such antibody.

Pharmaceutical Compositions

[0109] The present invention includes methods, which comprise administering an IL-33 antagonist to a patient, wherein the IL-33 antagonist is contained within a pharmaceutical composition. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral

formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0110] The dose of antibody administered to a patient according to the methods of the present invention may vary depending upon the age and the size of the patient, symptoms, conditions, route of administration, and the like. The dose is typically calculated according to body weight or body surface area. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering pharmaceutical compositions comprising anti-IL-33 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351). Specific exemplary doses of anti-IL33 antibodies, and administration regimens involving the same, that can be used in the context of the present invention are disclosed elsewhere herein.

[0111] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0112] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0113] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN),

NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0114] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0115] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule.

[0116] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

[0117] Exemplary pharmaceutical compositions comprising an anti-IL-33 antibody that can be used in the context of the present invention are disclosed, *e.g.*, in US Patent Application Publication No. 2014/0271658.

Dosage

[0118] The amount of IL-33 antagonist (*e.g.*, anti-IL-33 antibody) administered to a subject according to the methods of the present invention is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means an amount of IL-33 antagonist that results in a detectable improvement in one or more symptoms or indicia of an IL-33 mediated disease or disorder. A "therapeutically effective amount" also includes an amount of IL-33 antagonist that inhibits, prevents, lessens, or delays the progression of such disease or disorder in a subject.

[0119] In the case of an anti-IL-33 antibody, a therapeutically effective amount can be from about 0.05 mg to about 600 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the anti-IL-33 antibody. In certain embodiments, 75 mg, 150 mg, 200 mg, or 300 mg of an anti-IL-33 antibody is administered to a subject.

[0120] The amount of IL-33 antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the IL-33 antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

Combination Therapies

[0121] The methods of the present invention, according to certain embodiments, comprise administering to the subject one or more additional therapeutic agents in combination with the IL-33 antagonist. As used herein, the expression "in combination with" means that the additional therapeutic agents are administered before, after, or concurrent with the pharmaceutical composition comprising the IL-33 antagonist.

[0122] For example, when administered "before" the pharmaceutical composition comprising the IL-33 antagonist, the additional therapeutic agent may be administered about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes or about 10 minutes prior to the administration of the pharmaceutical

composition comprising the IL-33 antagonist. When administered "after" the pharmaceutical composition comprising the IL-33 antagonist, the additional therapeutic agent may be administered about 10 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours or about 72 hours after the administration of the pharmaceutical composition comprising the IL-33 antagonist.

[0123] Administration "concurrent" with the pharmaceutical composition comprising the IL-33 antagonist means that the additional therapeutic agent is administered to the subject in a separate dosage form within less than 5 minutes (before, after, or at the same time) of Administration of the pharmaceutical composition comprising the IL-33 antagonist, or administered to the subject as a single combined dosage formulation comprising both the additional therapeutic agent and the IL-33 antagonist.

[0124] The present invention includes methods of treating an IL-33 mediated disease or disorder, which comprise administering to a patient in need of such treatment an anti-hIL-33 antibody, or an IL-33 trap in combination with at least one additional therapeutic agent. Examples of additional therapeutic agents which can be administered in combination with an anti-hIL-33 antibody in the practice of the methods of the present invention include, but are not limited to a non-steroidal anti-inflammatory (NSAID), a steroid, a corticosteroid (inhaled or topical), an immunosuppressant (*e.g.* cyclophosphamide), an anticholinergic agent (*e.g.* tiotropium), a muscarinic agent (*e.g.* glycopyrronium), a phosphodiesterase inhibitor (e.g. theophylline, roflumilast, cilomilast), a beta blocker, cyclosporine, tacrolimus, pimecrolimus, azathioprine, methotrexate, cromolyn sodium, a proteinase inhibitor, a bronchial dilator, a beta-2-agonist, an antihistamine, epinephrine, a decongestant, a leukotriene inhibitor, a mast cell inhibitor, a thymic stromal lymphopoietin (TSLP) antagonist, a TNF antagonist, an IgE antagonist, an IL-1 antagonist, an IL-4 or IL-4R antagonist, an IL-13 or IL-13R antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 or IL-6R antagonist, an antagonist of IL-8, an IL-9 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor, an IL-25 antagonist and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33, and any other compound known to treat, prevent, or ameliorate an IL-33 mediated disease or disorder in a human subject. For example, for concurrent administration, a pharmaceutical formulation can be made which contains both an anti-hIL-33 antibody and at least one additional therapeutic agent. The amount of the additional therapeutic agent that is administered in combination with the anti-hIL-33 antibody in the practice of the methods of the present invention can be easily determined using routine methods known and readily available in the art.

Administration Regimens

[0125] The present invention includes methods comprising administering to a subject a

pharmaceutical composition comprising an IL-33 antagonist at a dosing frequency of about four times a week, twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every eight weeks, once every twelve weeks, or less frequently so long as a therapeutic response is achieved. In certain embodiments involving the administration of a pharmaceutical composition comprising an anti-IL-33 antibody, once a week dosing at an amount of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight.

[0126] According to certain embodiments of the present invention, multiple doses of an IL-33 antagonist may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an IL-33 antagonist. As used herein, "sequentially administering" means that each dose of IL-33 antagonist is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an IL-33 antagonist, followed by one or more secondary doses of the IL-33 antagonist, and optionally followed by one or more tertiary doses of the IL-33 antagonist.

[0127] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the IL-33 antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of IL-33 antagonist, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of IL-33 antagonist contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses area at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0128] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 14 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of IL-33 antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0129] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an IL-33 antagonist. For example, in

certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0130] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[0131] The present invention includes administration regimens comprising an up-titration option (also referred to herein as "dose modification"). As used herein, an "up-titration option" means that, after receiving a particular number of doses of an IL-33 inhibitor, if a patient has not achieved a specified improvement in one or more defined therapeutic parameters (e.g., at least a 20% improvement), or otherwise exhibits a clear lack of efficacy in the opinion of a physician or health care provider, the dose of the IL-33 inhibitor is thereafter increased. For example, in the case of a therapeutic regimen comprising administration of 150 mg doses of an anti-IL-33 antibody to a patient at a frequency of once every two weeks, if after 8, 10, 12, 14, 16 or more weeks, the patient has not achieved at least a 20% improvement in one parameter, or if the patient exhibits a clear lack of efficacy in the opinion of a physician or other health care provider, then the dose of anti-IL-33 antibody is increased to e.g., 200 mg, 300 mg, or more, administered once every two weeks thereafter (e.g., starting at week 10, 12, 14, 16, 18, or later).

Treatment Populations

[0132] The present invention includes methods, which comprise administering to a subject in need thereof a therapeutic composition comprising an IL-33 antagonist. As used herein, the expression "a subject in need thereof" means a human or non-human animal that exhibits one or more symptoms or indicia of an IL-33 mediated disease or disorder, such as those described herein (*e.g.*, inflammation, eosinophilia in the lungs, neutrophil infiltration into the lungs, elevated levels of certain cytokines in the lungs, such as, but not limited to IL-5, etc.) and/or who has been diagnosed with any of the IL-33 mediated disease or disorders described herein.

EXAMPLES

[0133] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to human IL-33

[0134] An immunogen comprising human IL-33 was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by an IL-33-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce IL-33-specific antibodies. Using this technique several anti-IL-33 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1M9559N, H1M9566N, H1M9568N and H1M9565N. The human variable domains from the chimeric antibodies were subsequently cloned onto human constant domains to make fully human anti-IL-33 antibodies as described herein.

[0135] Anti-IL-33 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1. Using this method, several fully human anti-IL-33 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H9629P, H4H9633P, H4H6940P, H4H9659P, H4H9660P, H4H9662P, H4H9663P, H4H9664P, H4H9665P, H4H9666P, H4H9667P, H4H9670P, H4H9671P, H4H9672P, H4H9675P, and H4H9676P.

[0136] Certain biological properties of the exemplary anti-IL-33 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0137] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs, and CDR sequences, of selected anti-IL-33 antibodies and their corresponding antibody identifiers.

Table 1

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1M9559N	2	4	6	8	10	12	14	16
H1M9566N	18	20	22	24	26	28	30	32
H1M9568N	34	36	38	40	42	44	46	48
H4H9629P	50	52	54	56	58	60	62	64
H4H9633P	66	68	70	72	74	76	78	80
H4H9640P	82	84	86	88	90	92	94	96
H4H9659P	98	100	102	104	106	108	110	112
H4H9660P	114	116	118	120	122	124	126	128
H4H9662P	130	132	134	136	138	140	142	144
H4H9663P	146	148	150	152	154	156	158	160
H4H9664P	162	164	166	168	170	172	174	176
H4H9665P	178	180	182	184	186	188	190	192
H4H9666P	194	196	198	200	202	204	206	208
H4H9667P	210	212	214	216	218	220	222	224
H4H9670P	226	228	230	232	234	236	238	240
H4H9671P	242	244	246	248	250	252	254	256
H4H9672P	258	260	262	264	266	268	270	272
H4H9675P	274	276	278	280	282	284	286	288
H4H9676P	290	292	294	296	298	300	302	304
H1M9565N	308	310	312	314	316	318	320	322

[0138] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1M," or "H4H"), followed by a numerical identifier (*e.g.* "9559," "9566," or "9629" as shown in Table 1), followed by a "P," or "N" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1M9559N," "H1M9566N," "H4H9629P," etc. The H1M and H4H prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H1M" antibody has a mouse IgG1 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

Example 3. Construction of IL-33 Traps

[0139] Five different exemplary IL-33 antagonists (IL-33 traps) of the invention were constructed using standard molecular biological techniques. The first IL-33 antagonist (hST2-hFc, SEQ ID NO:323) consists of the soluble extracellular region of human ST2 (SEQ ID NO:327) fused at its C-terminus to the N-terminus of a human IgG1 Fc region (SEQ ID

NO:331). The second IL-33 antagonist (hST2-mFc, SEQ ID NO:324) consists of the soluble extracellular region of human ST2 (SEQ ID NO:327) fused at its C-terminus to the N-terminus of a mouse IgG2a Fc region (SEQ ID NO:332). The third IL-33 antagonist (hST2-hIL1RAcP-mFc, SEQ ID NO: 325) consists of an in-line fusion having human ST2 (SEQ ID NO:327) at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO:329), followed by a mouse IgG2a Fc (SEQ ID NO:332) at its C-terminus. The fourth IL-33 antagonist (mST2-mIL1RAcP-mFc, SEQ ID NO: 326) consists of an in-line fusion having mouse ST2 (SEQ ID NO:328) at its N-terminus, followed by the extracellular region of mouse IL-1RAcP (SEQ ID NO:330), followed by a mouse IgG2a Fc (SEQ ID NO:332) at its C-terminus. The fifth IL-33 antagonist (hST2-hIL1RAcP-hFc, SEQ ID NO:335) consists of an in line fusion having human ST2 of SEQ ID NO: 327 at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO: 329) followed by a human IgG1 Fc (SEQ ID NO: 331) at its C terminus. Table 2a sets forth a summary description of the different IL-33 antagonists and their component parts. Table 2b sets forth the amino acid sequences of the IL-33 antagonists and their component parts.

Table 2a: Summary of IL-33 Antagonists (IL-33 traps)

IL-33 Antagonist	Amino Acid Sequence of Full Antagonist Molecule	D1 Component	D2 Component	M Component
hST2-hFc	SEQ ID NO:323	human ST2 extracellular (SEQ ID NO:327)	Absent	human IgG1 Fc (SEQ ID NO:331)
hST2-mFc	SEQ ID NO:324	human ST2 extracellular (SEQ ID NO:327)	Absent	mouse IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-mFc	SEQ ID NO:325	human ST2 extracellular (SEQ ID NO:327)	human IL-1RAcP extracellular (SEQ ID NO:329)	mouse IgG2a Fc (SEQ ID NO:332)
mST2-mIL1RAcP-mFc	SEQ ID NO:326	mouse ST2 extracellular (SEQ ID NO:328)	mouse IL-1RAcP extracellular (SEQ ID NO:330)	mouse IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-hFc	SEQ ID NO: 335	human ST2 extracellular (SEQ ID NO:327)	human IL-1RAcP extracellular (SEQ ID NO:329)	human IgG1 Fc (SEQ ID NO:331)

Table 2b: Amino Acid Sequences

Identifier	Sequence
SEQ ID NO:323 (hST2-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:324 (hST2-mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSEPRGPTIKPCPPCKCP APNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHT AQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPK GSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKN TEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG K
SEQ ID NO:325 (hST2-hIL1RAcP-mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQI QVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPINFRLPEN RISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKL PVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFL IALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHSPNDHVVYE KEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDET RTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPAPRYTVE**SG**EPRG PTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQI SWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKD LPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHN HHTTKSFSRTPGK
SEQ ID NO:326 (mST2-mIL1RAcP-mFc)	SKSSWGLENEALIVRCPQRGRSTYPVEWYYSDTNESIPTQKRNRIFVSRDRLKF LPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIPDYLMYSTVRGSDKNF KITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQF THAENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIACSACF GKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHRSERCDDWGLDTMRQIQVFED EPARIKCPLFEHFLKYNYSTAHSSGLTLIWYWTRQDRDLEEPINFRLPENRISKEK DVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSAMRFPVHKM YIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTEIVDFHNVLPEGMNLSFFIPLVS NNGNYTCVVTYPENGRLFHLTRTVTVKVVGSPKDALPPQIYSPNDRVVYEKEPG EELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDVTVDITINESVSYSSTEDETRTQI LSIKKVTPEDLRRNYVCHARNTKGEAEQAAKVKQKVIPPRYTVE**SG**EPRGPTIKP CPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFV NNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPI ERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGK

Identifier	Sequence
	TELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTK SFSRTPGK
SEQ ID NO:327 (human ST2 extracellular domain)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHS
SEQ ID NO:328 (mouse ST2 extracellular domain)	SKSSWGLENEALIVRCPQRGRSTYPVEWYYSDTNESIPTQKRNRIFVSRDRLKF LPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIPDYLMYSTVRGSDKNF KITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQF THAENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIACSACF GKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHR
SEQ ID NO:329 (human IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKI QNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKN AVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITI DVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQK VPAPRYTVE
SEQ ID NO:330 (mouse IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKYNYSTAHSSGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVVQKDSCFNSAMRFPVHKMYIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTE IVDFHNVLPEGMNLSFFIPLVSNNGNYTCVVTYPENGRLFHLTRTVTVKVVGSPK DALPPQIYSPNDRVVYEKEPGEELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDV TVDITINESVSYSSTEDETRTQILSIKKVTPEDLRRNYVCHARNTKGEAEQAAKVK QKVIPPRYTVE
SEQ ID NO:331 (human IgG1 Fc)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
SEQ ID NO:332 (mouse IgG2a Fc)	EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDD PDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKV NNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIY VEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHE GLHNHHTTKSFSRTPGK
SEQ ID NO:333 (*M. fascicularis* IL-33-6His)	SITGISPITESLASLSTYNDQSITFALEDESYEIYVEDLKKDKKKDKVLLSYYESQH PSSESGDGVDGKMLMVTLSPTKDFWLQANNKEHSVELHKCEKPLPDQAFFVLH NRSFNCVSFECKTDPGVFIGVKDNHLALIKVDYSENLGSENILFKLSEILEHHHHH H
SEQ ID NO:335 (hST2-hIL1RAcP-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQI QVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPINFRLPEN RISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKL PVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFL IALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHSPNDHVVYE KEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDET

Identifier	Sequence
	RTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPAPRYTVEDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK

Example 4: Biomarkers for IL-33 Activity

[0140] Studies were done to determine which genes were elevated in mice that overexpressed IL-33. The studies described below summarize the methods and animal models used to determine which genes correlated with IL-33 expression *in vivo* and which genes were modulated following treatment of the animals with an IL-33 antagonist.

Hydrodynamic DNA delivery

[0141] Mouse IL-33 was overexpressed in wild type (WT) mice by hydrodynamic DNA delivery (HDD). For the HDD experiment, WT mice were injected with either 50 µg or 25ug of a plasmid expressing full-length mouse IL-33 (See GenBank accession number NM_001164724; mIL-33) or with 50 µg or 25 ug of the same plasmid devoid of coding sequence (empty vector). Mice were sacrificed 7 days after the HDD injection, and blood, dorsal root ganglia (DRG), heart, kidney, liver, lung and spleen were collected. The top ten genes expressed in these tissues are shown in table 4 below.

Microarray analysis of collected tissue

[0142] Cy3-CTP was incorporated into amplified cRNA from 500 ng of total RNA using QuickAmp RNA Amplification Kit (Agilent). Cy3 labeled cRNA from each sample was then hybridized to a custom Agilent array comprising of 43538 60mer oligos covering mouse transcriptome. The hybridization and wash of the arrays were performed according to the Agilent protocol and arrays were scanned on an Agilent Microarray scanner. The data was extracted from scanned array images using Agilent Feature Extraction Software 9.5.

Serum collection

[0143] Whole blood was collected into Microtainer® tubes by cardiac puncture at the end of the study. Blood was allowed to clot by leaving it undisturbed at room temperature for at least 30 minutes. Clotted blood and cells were pelleted by centrifuging at 18,000 x g for 10 minutes at 4°C in a refrigerated centrifuge. The resulting supernatant, designated serum, was transferred into clean polypropylene plates and processed or stored appropriately.

Determination of IL-33 protein concentrations in the serum or cell lysate extracts

[0144] ELISA kits from R&D systems were used to determine IL-33 concentrations for human

(DY3625) and mouse (DY3626) IL-33 according to the manufacturers instructions.

Determination of Mouse calcitonin (CT) concentrations in the serum or cell lysate extracts

[0145] Mouse calcitonin was measured in the serum of mice using a mouse Calcitonin (CT) ELISA kit from CusaBio (Through ARP) Cat# CSB-E05133m. The assay procedure was carried out according to the manufacturers instructions provided with the kits.

Determination of Mouse IL-5 concentrations in cell lysate extracts

[0146] Cytokine concentrations in the lung protein extracts were measured using a V-Plex custom Mouse multiplex immunoassay kit (MesoScale Discovery, # K152A41), according to the manufacturer's instructions.

House dust mite induced chronic lung inflammation model

[0147] IL-33 HumIn mice were intranasally administered either 50µg house dust mite extract (HDM; Greer, #XPB70D3A2.5) diluted in 20µL of 1X phosphate buffered saline (PBS) or 20µL of 1X PBS for 3 days per week for 15 weeks. A second control group of IL33 HumIn mice were administered 50µg HDM extract diluted in 20µL of 1X PBS for 3 days per week for either 4 or 11 weeks, to assess the severity of the disease at the onset of antibody treatment. Two groups of HDM challenged mice were injected subcutaneously with 25 mg/kg of either an anti-IL-33 antibody, H4H9675P, or an isotype control antibody starting after either 4 or 11 weeks of HDM challenge and then twice per week until the end of the HDM challenge (8 or 4 weeks of antibody treatment). On day 85 of the study, all mice were sacrificed and their lungs were harvested. Experimental dosing and treatment protocol for groups of mice are shown in Table 3.

[0148] IL-33 HumIn mice were intranasally administered either 50µg house dust mite extract (HDM; Greer, #XPB70D3A2.5) diluted in 20µL of 1X phosphate buffered saline (PBS) or 20µL of 1X PBS for 3 days per week for 15 weeks. A second control group of IL33 HumIn mice were administered 50µg HDM extract diluted in 20µL of 1X PBS for 3 days per week for 11 weeks, to assess the severity of the disease at the onset of antibody treatment. Two groups of HDM challenged mice were injected subcutaneously with 25 mg/kg of either an anti-IL-33 antibody, H4H9675P, or an isotype control antibody starting after 11 weeks of HDM challenge and then twice per week until the end of the HDM challenge (8 weeks of antibody treatment). On day 85 of the study, all mice were sacrificed and their lungs were harvested. Experimental dosing and treatment protocol for groups of mice are shown in Table 3.

Table 3: Experimental dosing and treatment protocol for groups of mice

Group	Mice	Intranasal challenge	Length of intranasal challenge	Antibody

1	IL-33 HumIn mice	1X PBS	15 weeks	None
2	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	4 or 11 weeks	None
3	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	None
4	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Isotype control antibody
5	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Anti-IL-33 antibody (H4H9675P)

Tissue harvest for Gene Expression Analysis

[0149] After exsanguination, lung was removed, placed into tubes containing 400µL of RNA Later (Ambion, Cat# AM720) and stored at -20°C until processing. Tissues were homogenized in TRIzol and chloroform was used for phase separation. The aqueous phase, containing total RNA, was purified using MagMAX™-96 for Microarrays Total RNA Isolation Kit according to manufacturer's specifications. Genomic DNA was removed using MagMAX™Turbo™DNase Buffer and TURBO DNase from the MagMAX kit listed above. mRNA (up to 2.5µg) was reverse-transcribed into cDNA using SuperScript® VILO™ Master Mix. cDNA was diluted to 2ng/µL and 10ng cDNA was amplified with the TaqMan® Gene Expression Master Mix and the relevant probes (mouse B2m, mouse Calca, human IL33) using the ABI 7900HT Sequence Detection System (Applied Biosystems). The B2m probe was used to amplify the beta-2 microblobulin (B2m) gene as an internal control in order to normalize cDNA input differences. Data analysis was performed using Microsoft Excel and GraphPad Prism™ software. Expression of each gene was normalized to B2m expression within the same sample.

Lung harvest for cytokine analysis

[0150] After exsanguination, the cranial and middle lobes of the right lung from each mouse were removed and placed into tubes containing a solution of tissue protein extraction reagent (1X T-PER reagent; Pierce, #78510) supplemented with 1X Halt Protease inhibitor cocktail (Pierce, #78430). All further steps were performed on ice. The volume of T-PER Reagent (containing the protease inhibitor cocktail) was adjusted for each sample to match a 1:8 (w/v) tissue to T-PER ratio. Lung samples were homogenized in the tubes, using the Tissue Lyser II (Qiagen, Cat#85300). The resulting lysates were centrifuged to pellet debris. The supernatants containing the soluble protein extracts were transferred to fresh tubes and stored at 4°C until further analysis.

[0151] Total protein content in the lung protein extracts was measured using a Bradford

assay. For the assay, 10µL of diluted extract samples were plated into 96 well plates in duplicates and mixed with 200µL of 1X Dye Reagent (Biorad, #500-0006). Serial dilutions of bovine serum albumin (BSA; Sigma, #A7979), starting at 700µg/mL in 1X T-Per reagent were used as a standard to determine the protein concentration of the extracts. After a 5-minute incubation at room temperature, absorbance at 595nm was measured on a Molecular Devices SpectraMax M5 plate reader. Data analysis to determine total lung extract protein content based on the BSA standard was performed using GraphPad Prism™ software.

[0152] Each cytokine concentration in lung total protein extracts from all mice in each group was normalized to the total protein content of the extracts measured by the Bradford assay, and expressed for each group as average pg of cytokine per mg of total lung proteins (pg/mg lung protein, ± SD).

Table 4: Top IL-33 Perturbed Genes

DrgIL33	Heart IL33	Kidney IL33	Liver IL33	Lung IL33	Spleen IL33	Gene Symbol	Description
10.2	415.9	33.5	72.7	5.0	86.3	Calca	Calcitonin/calcitonin-gene related peptide (CGRP)
36.2	114.8	51.7	572.8	24.9	13.8	Retnla	Resistin like alpha
6.5	194.0	19.5	152.2	4.5	19.4	Ccl8	Chemokine (C-C motif) ligand 8
6.5	17.8	251.0	14.9	8.0	45.9	Saa3	Serum amyloid A 3
38.3	28.2	8.3	66.6	53.2	29.3	BC117090	Gm1975
4.9	32.8	61.2	40.4	15.3	3.6	Klrg1	Killer cell lectin-like receptor
15.4	11.7	6.8	86.7	27.1	13.8	Csta	Stefin A1
5.7	46.1	18.2	38.4	1.8	4.5	Ms4a8a	Membrane-spanning 4-domain
1.7	19.0	23.3	81.6	9.4	6.6	Ccl11	Chemokine (C-C motif) ligand 11
3.7	17.9	49.0	18.0	25.8	10.5	Serpina3f	Serine (or cysteine) peptides

Lung harvest for pulmonary cell infiltrate analysis

[0153] After exsanguination, the caudal lobe of the right lung from each mouse was removed, placed into a tube containing a solution of 20µg/mL DNase and 0.7U/mL Liberase TH diluted in Hank's Balanced Salt Solution (HBSS) and chopped into pieces that were approximately 2 to 3mm in size. The tubes with the chopped lungs were then incubated in a 37°C water bath for 20 minutes. The reaction was stopped by adding ethylenediaminetetraacetic acid (EDTA) at a final concentration of 10mM. The samples were then transferred to gentleMACS C Tubes. The volume in the C Tubes was brought up to 3mL with MACS buffer and the samples were subsequently dissociated to form single cell suspensions using a gentleMACS dissociator® (Miltenyi Biotec). The tubes were then centrifuged and the resulting pellet was resuspended in 4mL of 1X Red Blood Cell Lysing Buffer to lyse red blood cells. After incubation for 3 minutes at

room temperature, 10mL of 1X DPBS was added to deactivate the red blood cell lysing buffer. The cell suspensions were then centrifuged, and the resulting cell pellets were resuspended in 1mL of 1X DPBS. The resuspended samples were centrifuge-filtered (1 minute at 400 x g) through a 100µm filter plate and approximately $1.5x10^6$ cells per well were plated in a 96-well U-bottom plate. Cells were then centrifuged and the cell pellets resuspended in 100µL of Near-IR LIVE/DEAD® Fixable Dead Cell Stain (Invitrogen Cat#: L34976, Lot#: 1647137) diluted at 1:500 in 1X DPBS to determine cell viability. Cells were incubated with the viability dye for 20 minutes at room temperature while protected from light. After one wash in 1X DPBS, cells were incubated in 50µL of MACS buffer containing 10µg/mL of purified rat anti-mouse CD16/CD32 Fc Block, for 10 minutes at 4°C The cells were then incubated in the appropriate 2x antibody mixture diluted in Brilliant Stain Buffer (described in Table 5) for 30 minutes at 4°C while protected from light. After antibody incubation, the cells were washed twice in MACS buffer, resuspended in BD CytoFix that had been diluted 1:4 in 1X DPBS and then incubated for 15 minutes at 4°C while protected from light. The cells were subsequently washed and resuspended in MACS buffer. Cell suspensions were then filtered into a new U-Bottom plate through an AcroPrep Advance 96 Filter Plate 30-40µm. Sample data were acquired on a LSR Fortessa X-20 cell analyzer using the HTS attachment (BD Biosciences). Data analysis was performed using FlowJo X Software (Tree Star, OR) and statistical analysis was performed using GraphPad Prism™ (GraphPad Software, CA). Eosinophils were defined as live (cell viability dye negative), singlets, $CD45^+$, $F4/80^+$, $Ly6G^-$, $CD11c^{lo-Int}$, $SiglecF^{hi}$. Neutrophils were defined as live, singlets, $CD45^+$, $F4/80^-$, $Ly6G^+$. Data for eosinophils, and neutrophils were expressed as frequency of live cells.

Table 5: Antibodies Used for Flow Cytometry Analysis

Antibody	Fluorochrome	Manufacturer	Catalog #, Lot #	Final dilution
CD45	Alexa Fluor 700	BioLegend	103128, B191240	1/200
Siglec-F	BV 421	BD	562681, 4234913	1/200
F4/80	PE	BD	56410, 5054900	1/500
Ly6G	BUV395	BD	563978, 4178621	1/200
CD11c	APC	BD	550261, 5016523	1/200

Statistical Analysis

[0154] Statistical analyses were performed using GraphPad Prism™ version 6.0 (GraphPad Software, CA).

[0155] Normality of the data was evaluated using the Kolmogorov-Smirnov test. If data passed the normality test, and standard deviations of the different groups were not statistically different from each other as assessed by the Brown-Forsythe test, results were interpreted by one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test for multiple

comparisons. If data failed to pass the normality test, or standard deviations were significantly different, results were interpreted using the Kruskal-Wallis test followed by the Dunn's *post hoc* test for multiple comparisons. Differences were considered to be statistically significant when P value < 0.05.

[0156] Correlation coefficient and significance were computed using two-tailed Spearman non-parametric correlation. Correlations were considered to be statistically significant when P value < 0.05.

Summary of Results:

[0157] The results demonstrated that the top ten genes that were perturbed in animals that overexpressed IL-33 were calcitonin (Calca), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f) (See Table 4). Moreover, the data also showed that both serum IL-33 and serum calcitonin were significantly elevated in this mouse model (See Figure 1A for serum IL-33 levels and Figure 1B for serum calcitonin levels). The increase in serum IL-33 correlated with the increase in serum levels of calcitonin.

[0158] In addition, the chronic house dust mite (HDM) challenge model, which was used as a model of severe, progressive lung inflammation following exposure to an allergen, showed that calcitonin gene expression is upregulated in the lungs of mice upon chronic HDM challenge, but the expression of calcitonin is reduced significantly in the lungs of mice that have been treated with an IL-33 antagonist (H4H9675P anti-IL-33 antibody). Treatment with an isotype matched negative control antibody had no effect on the level of calcitonin gene expression (Figure 2).

[0159] In addition, serum calcitonin was also elevated in this HDM mouse model and the level of serum calcitonin correlated with the level of calcitonin seen in the lungs of these mice (See Figure 3). Moreover, the level of serum calcitonin correlated with the elevated levels of IL-33 observed in this HDM mouse model (See Figure 4), suggesting that calcitonin could be used as a potential biomarker for IL-33 in an IL-33 mediated disease process (*e.g.* an allergic response).

[0160] In terms of IL-33 mediated disease parameters, the frequency of lung neutrophils, lung eosinophils and IL5 levels were studied in this HDM model. By 15 weeks after challenge with house dust mite allergen, all three parameters were found to be elevated (See figures 5A (neutrophils), 5B (eosinophils) and 5C (IL-5)). However, mice that received the IL-33 antibody H4H9675P showed significantly fewer neutrophils, eosinophils and IL-5 in their lungs compared to the mice challenged with allergen and left untreated, or administered a negative isotype control antibody (See Figures 5A, 5B and 5C).

[0161] Furthermore, there was a significant correlation between serum calcitonin levels and the three disease parameters studied. Figure 6A shows the correlation between serum calcitonin and lung neutrophils. Figure 6B shows the correlation between serum calcitonin and

lung eosinophils and Figure 6C shows the correlation between serum calcitonin and lung IL-5 levels.

[0162] In addition, there was a significant correlation between lung IL-33 levels and the frequency of lung neutrophils (Figure 7A), lung eosinophils (Figure 7B) and lung IL-5 levels (Figure 7C).

[0163] In summary, the data obtained from the house dust mite model indicate that there is a significant correlation between the level of IL-33, the frequency of at least three disease parameters associated with this model of chronic allergy and the expression of calcitonin. As such, this information suggests that calcitonin may be one biomarker of disease severity and/or progression in a mammal and furthermore, the data suggest that calcitonin may be used to assess the effectiveness of therapy with an IL-33 antagonist as described herein.

[0164] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. Use of a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 antagonist for treating an interleukin-33 (IL-33) mediated disease or disorder in a subject who exhibits an elevated level of at least one biomarker associated with an IL-33 mediated disease or disorder.

2. The use of claim 1, wherein the biomarker is a polypeptide encoded by the *CALCA* gene and is selected from the group consisting of calcitonin, procalcitonin and calcitonin gene-related peptide (CGRP).

3. The use of claim 2, wherein the biomarker is calcitonin.

4. The use of claim 1, wherein the IL-33 antagonist comprises an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

5. The use of claim 4, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

6. The use of either claim 4 or 5, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

7. The use of any of claims 4-6, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

8. The use of any of claims 4-7, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

9. The use of claim 4, wherein the IL-33 receptor based antagonist comprises a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

10. The use of claim 9, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

11. The use of claim 4, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

12. The use of claim 4, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

13. The use of any of claims 1-12, wherein the pharmaceutical composition is for use in combination with an effective amount of a second therapeutic agent for reducing at least one symptom of an IL-33 mediated disease or disorder.

14. The use of claim 13, wherein the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid, a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-13 antagonist, an IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-25 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33.

15. The use of claim 1, wherein the biomarker associated with an IL-33 mediated disease or disorder in a subject is selected from the group consisting of calcitonin, procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif)

ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

16. The use of claim 1, wherein the biomarker is calcitonin, and wherein the calcitonin level is increased in the serum of the subject having an IL-33 mediated disease or disorder, and wherein the increase in serum calcitonin correlates with an increased level of calcitonin and IL-33 in the lungs of the subject.

17. The use of claim 16, wherein treating the IL-33 mediated disease or disorder comprises reducing the level of calcitonin in the serum of the subject to a normal level.

18. The use of claim 1, wherein the elevated level of the at least one biomarker corresponds to a mean serum calcitonin level of greater than about 5 pg/mL for females and greater than about 10 pg/mL for males.

19. The use of claim 1, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

20. A method for identifying a subject suffering from an IL-33 mediated disease or disorder who is likely to respond favorably to therapy with an IL-33 antagonist, the method comprising obtaining a sample from the subject and measuring the level of calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP) in the sample; wherein an elevated level of calcitonin, procalcitonin, or CGRP as compared to the lower level of calcitonin, procalcitonin, or CGRP in subjects not suffering from an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

21. The method of claim 20, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis,

lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

22. The method of claim 20, wherein the IL-33 antagonist is an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

23. The method of claim 22, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

24. The method of either claim 22 or 23, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

25. The method of any of claims 22-24, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

26. The method of any of claims 22-25, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

27. The method of claim 22, wherein the IL-33 receptor based antagonist comprises a

first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

28. The method of claim 27, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

29. The method of claim 22, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

30. The method of claim 22, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

31. A method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of calcitonin, procalcitonin, or CGRP; wherein an elevated level of calcitonin, procalcitonin, or CGRP, as compared to the lower level of calcitonin, procalcitonin, or CGRP in subjects not likely to have, or not likely to develop an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder.

32. The method of claim 31, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

33. The method of claim 31, wherein the sample from the subject is a solid tissue sample, a cell sample, or a blood sample.

34. The method of claim 33, wherein the solid tissue sample is selected from the group consisting of heart, kidney, liver, lung and spleen.

35. The method of claim 33, wherein the cell sample is a dorsal root ganglion cell

sample, sputum cell sample, bronchoalveolar lavage cell sample, nasal polyps cell sample, fecal cell sample, lung, colon heart, kidney, skin biopsy, or spleen biopsy cell sample.

36. The method of claim 33, wherein the blood sample is whole blood, plasma, or serum.

37. Use of a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist for treating an IL-33 mediated disease or disorder in a subject that has been diagnosed with an IL-33 mediated disease or disorder and has been selected for treatment with the IL-33 antagonist on the basis of exhibiting enhanced expression of at least one biomarker pre-treatment, wherein the enhanced expression of the biomarker is determined based on a comparison to a reference level of expression of the respective biomarker.

38. The use of claim 37, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

39. The use of claim 37, wherein the biomarker associated with an IL-33 mediated disease or disorder in a subject is selected from the group consisting of calcitonin, procalcitonin, CGRP, resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

40. The use of claim 37, wherein the IL-33 antagonist comprises an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

41. The use of claim 40, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group

consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

42. The use of either claim 40 or 41, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

43. The use of any of claims 40-42, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

44. The use of any of claims 40-43, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

45. The use of claim 40, wherein the IL-33 receptor based antagonist comprises a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

46. The use of claim 45, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

47. The use of claim 40, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

48. The use of claim 40, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

49. The use of claim 40, wherein the pharmaceutical composition is for use in combination with a second therapeutic agent for reducing at least one symptom of an IL-33 mediated disease or disorder.

50. The use of claim 49, wherein the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a steroid, a corticosteroid (inhaled or topical), an immunosuppressant (*e.g.* cyclophosphamide), an anticholinergic agent (*e.g.* tiotropium), a muscarinic agent (*e.g.* glycopyrronium), a phosphodiesterase inhibitor (e.g. theophylline, roflumilast, cilomilast), a beta blocker, cyclosporine, tacrolimus, pimecrolimus, azathioprine, methotrexate, cromolyn sodium, a proteinase inhibitor, a bronchial dilator, a beta-2-agonist, an antihistamine, epinephrine, a decongestant, a leukotriene inhibitor, a mast cell inhibitor, a thymic stromal lymphopoietin (TSLP) antagonist, a TNF antagonist, an IgE antagonist, an IL-1 antagonist, an IL-4 or IL-4R antagonist, an IL-13 or IL-13R antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 or IL-6R antagonist, an antagonist of IL-8, an IL-9 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-17 antagonist, an IL-31 antagonist, an IL-33 antagonist, an oral PDE4 inhibitor, and a different antibody to IL-25.

51. A serum biomarker for measuring the presence of an IL-33 mediated disease or disorder in a subject, wherein a level of the serum biomarker correlates with enhanced IL-33 levels *in vivo*, and wherein the serum biomarker is a polypeptide encoded by the *CALCA* gene and is selected from the group consisting of calcitonin, procalcitonin, and CGRP.

52. The serum biomarker of claim 51, for measuring the presence of an IL-33 mediated disease or disorder in a subject, wherein the level of the serum biomarker correlates with enhanced IL-33 levels *in vivo*, and wherein the biomarker is calcitonin.

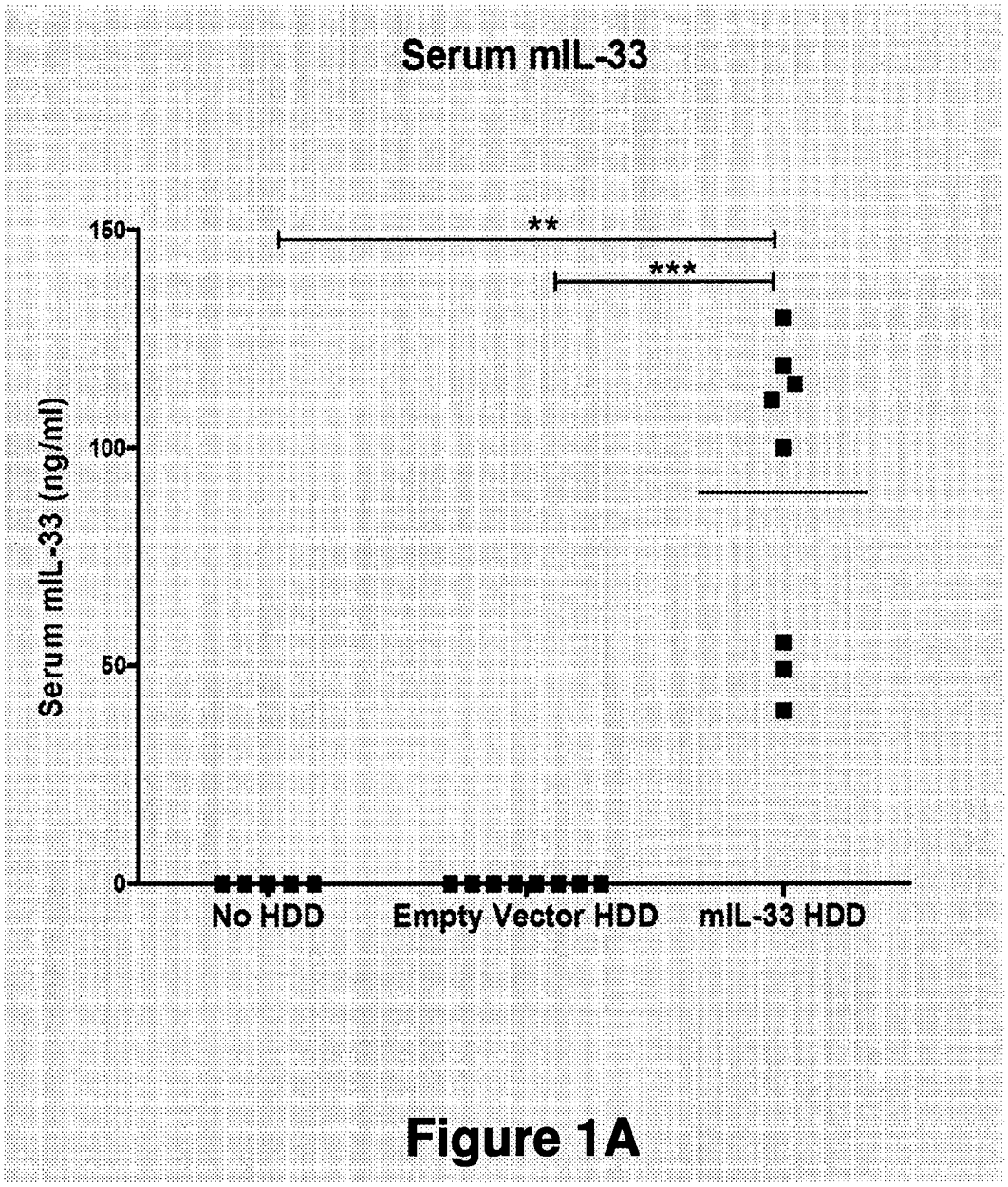

Serum mIL-33
Serum mIL-33 (ng/ml)
150
100
50
0
**
***
No HDD
Empty Vector HDD
mIL-33 HDD

Figure 1A

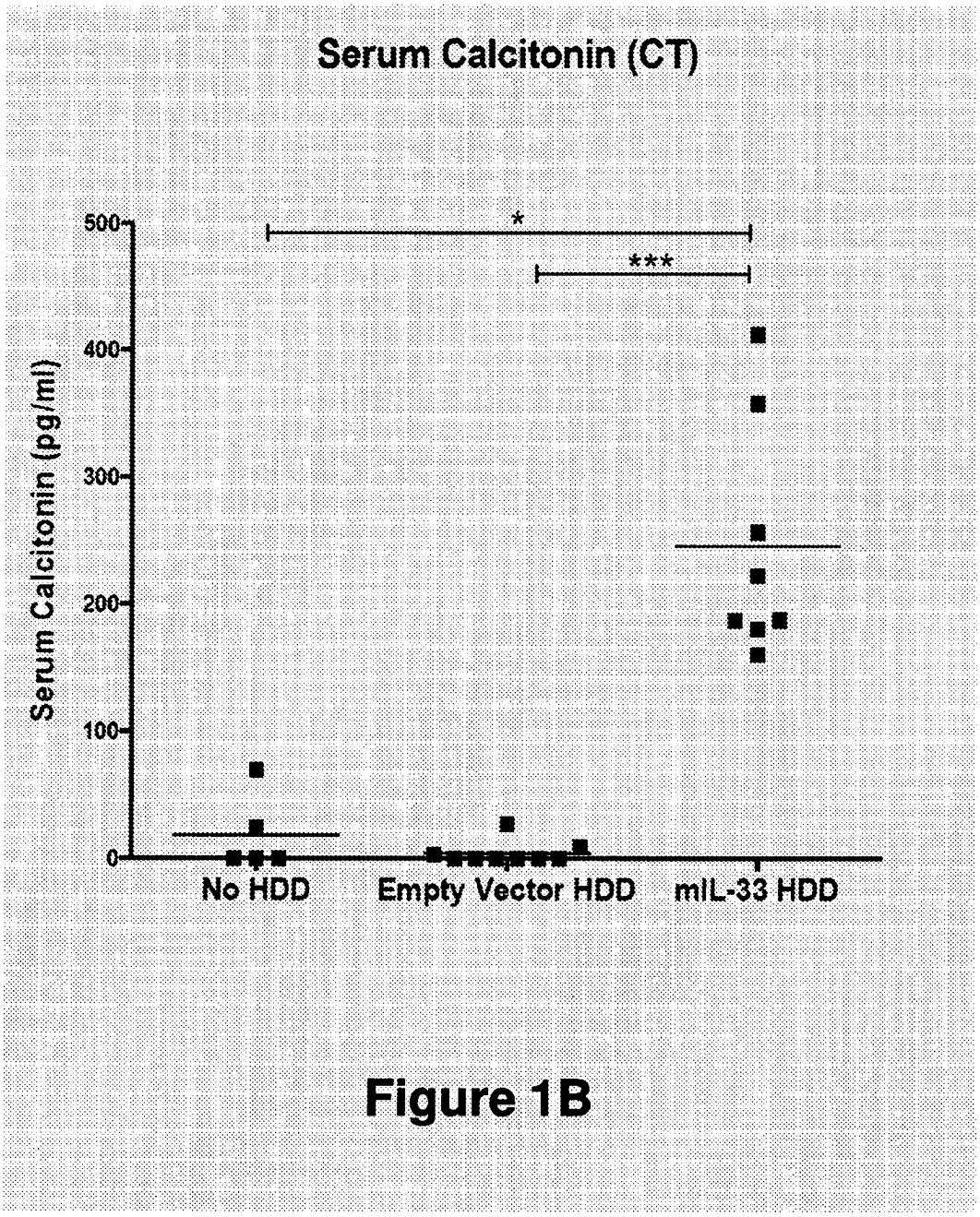

Serum Calcitonin (CT)
Serum Calcitonin (pg/ml)
500
400
300
200
100
0
*
***
No HDD
Empty Vector HDD
mIL-33 HDD

Figure 1B

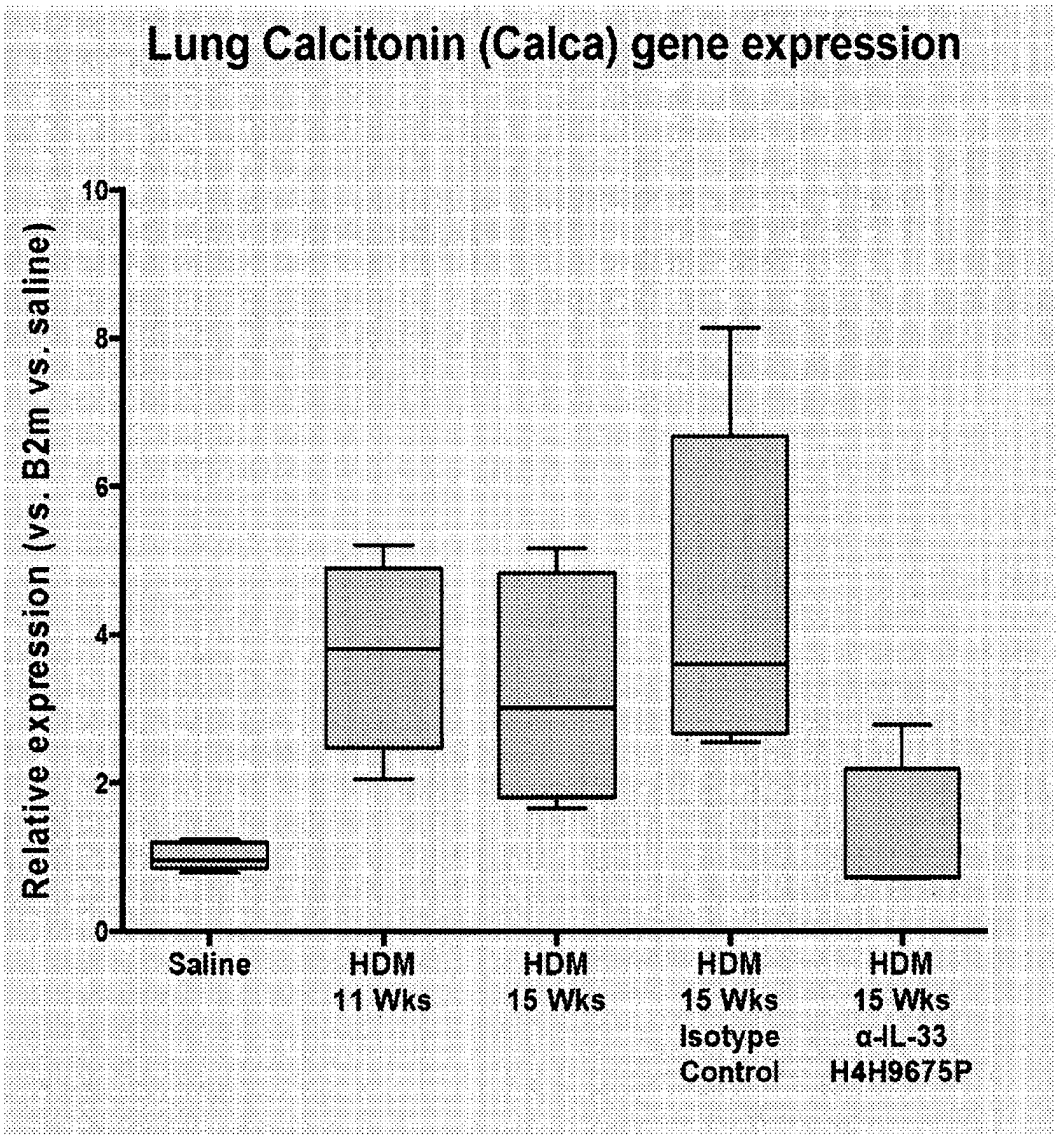

Lung Calcitonin (Calca) gene expression
Relative expression (vs. B2m vs. saline)
10
8
6
4
2
0
Saline
HDM 11 Wks
HDM 15 Wks
HDM 15 Wks Isotype Control
HDM 15 Wks α-IL-33 H4H9675P

Figure 2

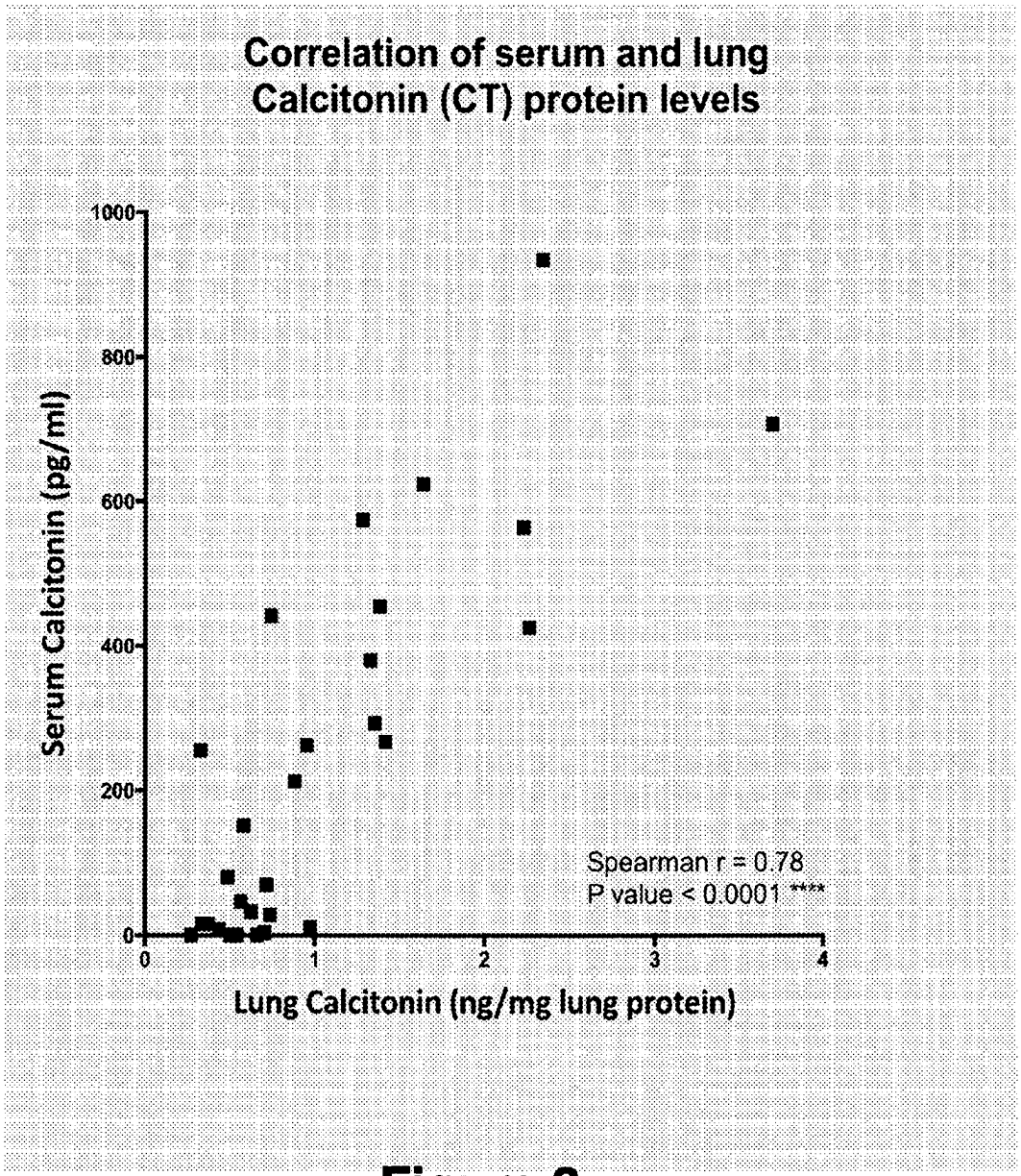

Correlation of serum and lung Calcitonin (CT) protein levels
Serum Calcitonin (pg/ml)
1000
800
600
400
200
0
0
1
2
3
4
Lung Calcitonin (ng/mg lung protein)
Spearman r = 0.78
P value < 0.0001 ****

Figure 3

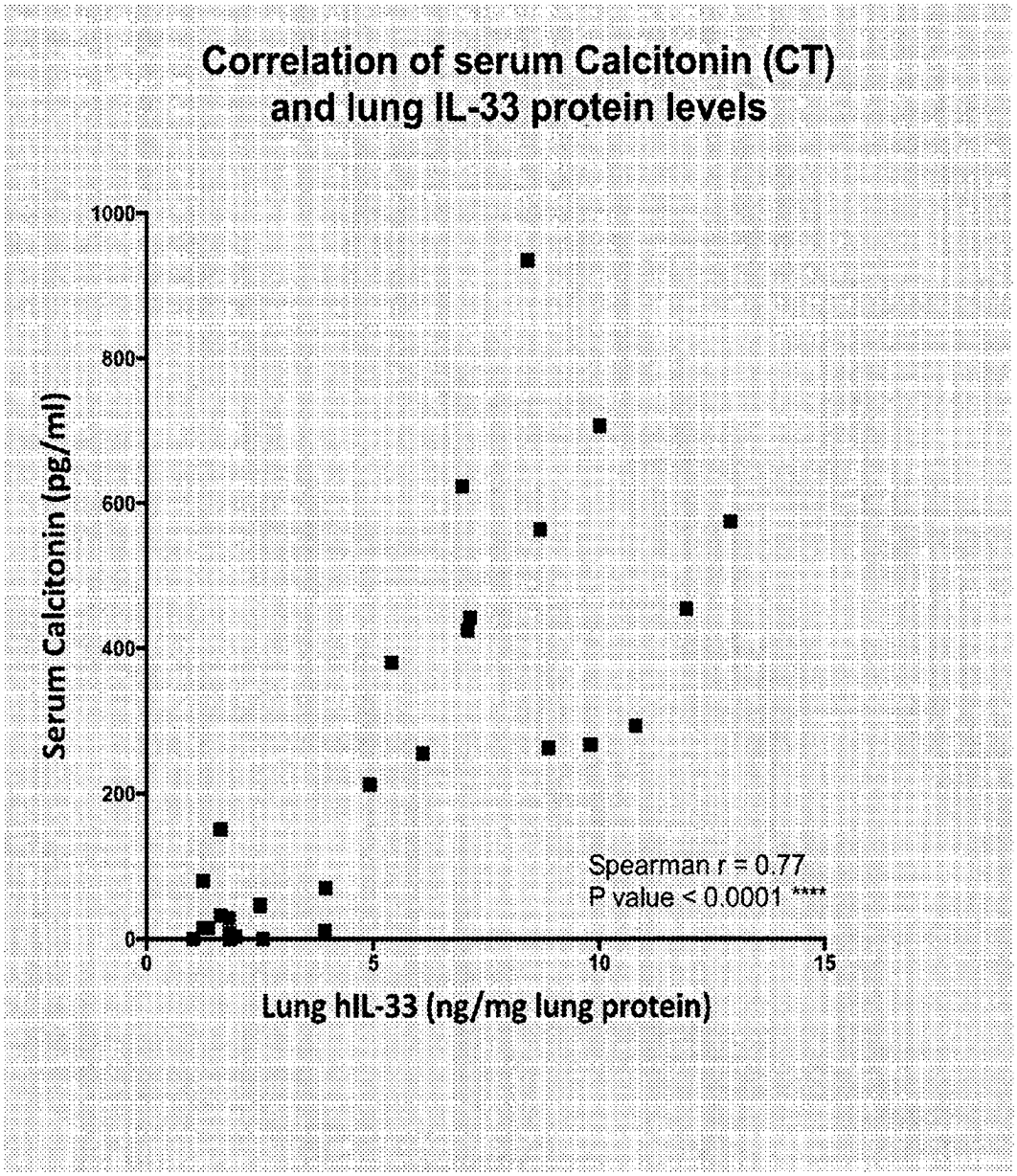

Correlation of serum Calcitonin (CT) and lung IL-33 protein levels
Serum Calcitonin (pg/ml)
1000
800
600
400
200
0
0
5
10
15
Lung hIL-33 (ng/mg lung protein)
Spearman r = 0.77
P value < 0.0001 ****

Figure 4

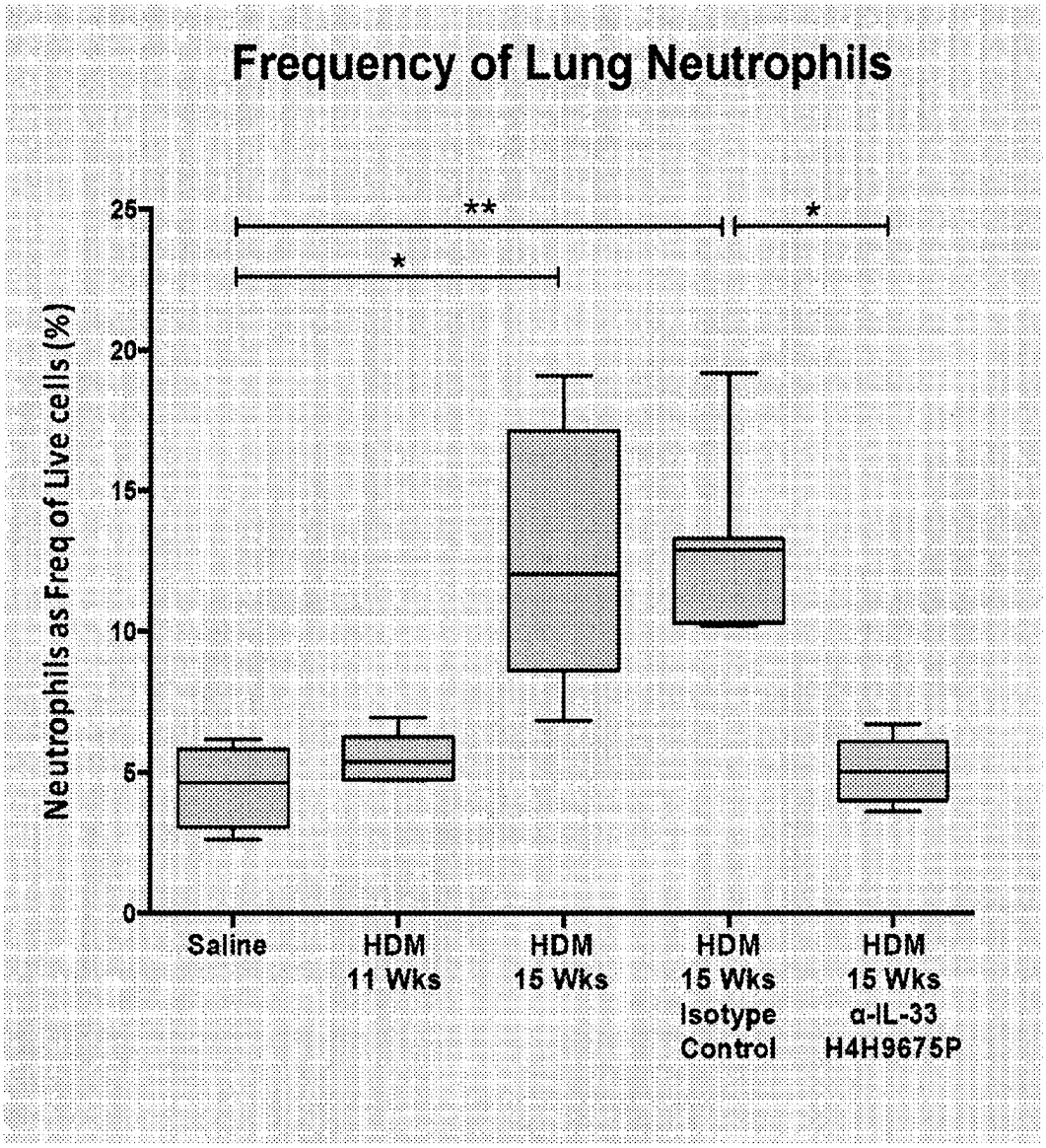

Frequency of Lung Neutrophils
Neutrophils as Freq of Live cells (%)
25
20
15
10
5
0
**
*
*
Saline
HDM 11 Wks
HDM 15 Wks
HDM 15 Wks Isotype Control
HDM 15 Wks α-IL-33 H4H9675P

Figure 5A

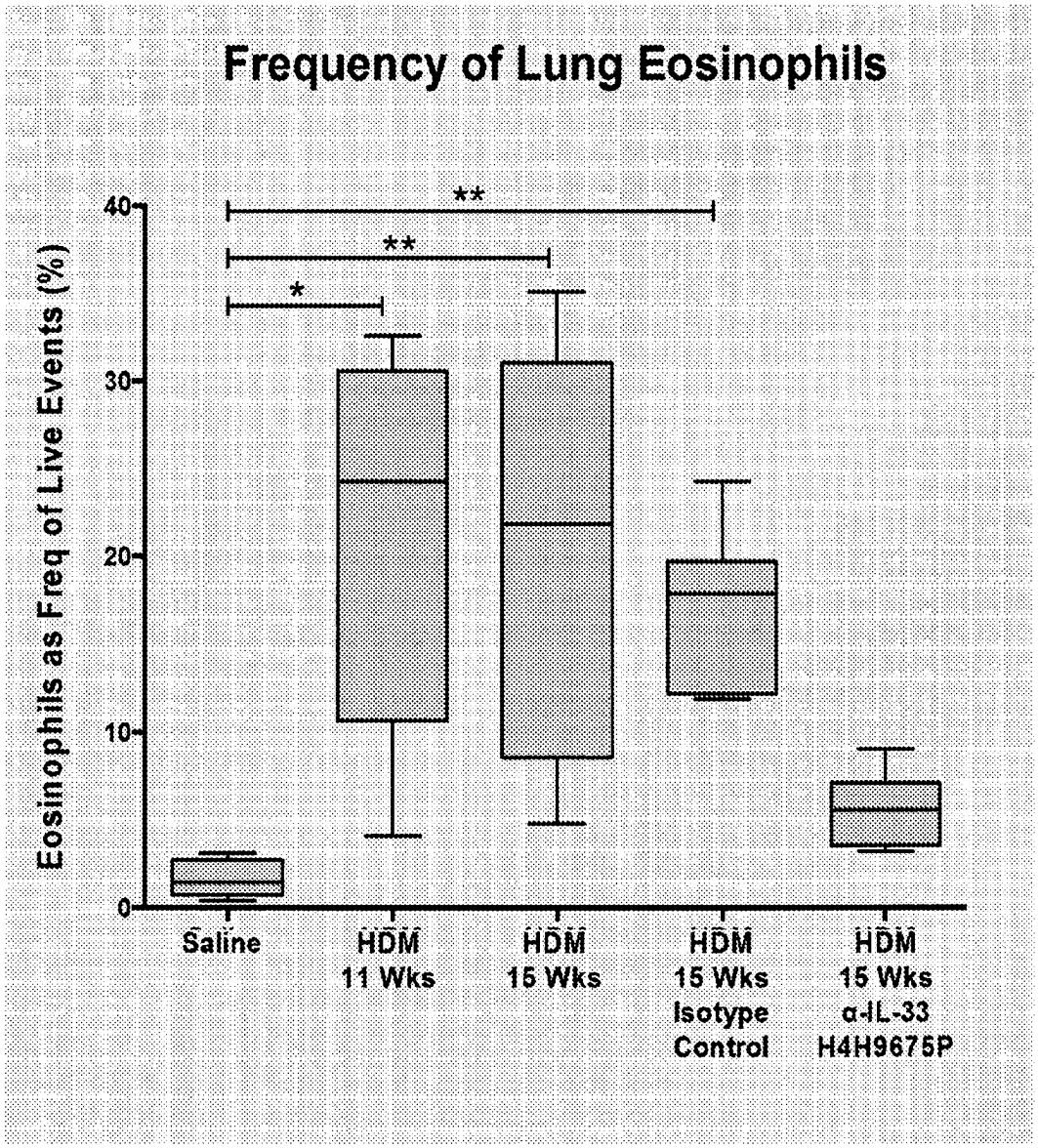

Frequency of Lung Eosinophils
Eosinophils as Freq of Live Events (%)
40
30
20
10
0
**
**
*
Saline
HDM 11 Wks
HDM 15 Wks
HDM 15 Wks Isotype Control
HDM 15 Wks α-IL-33 H4H9675P

Figure 5B

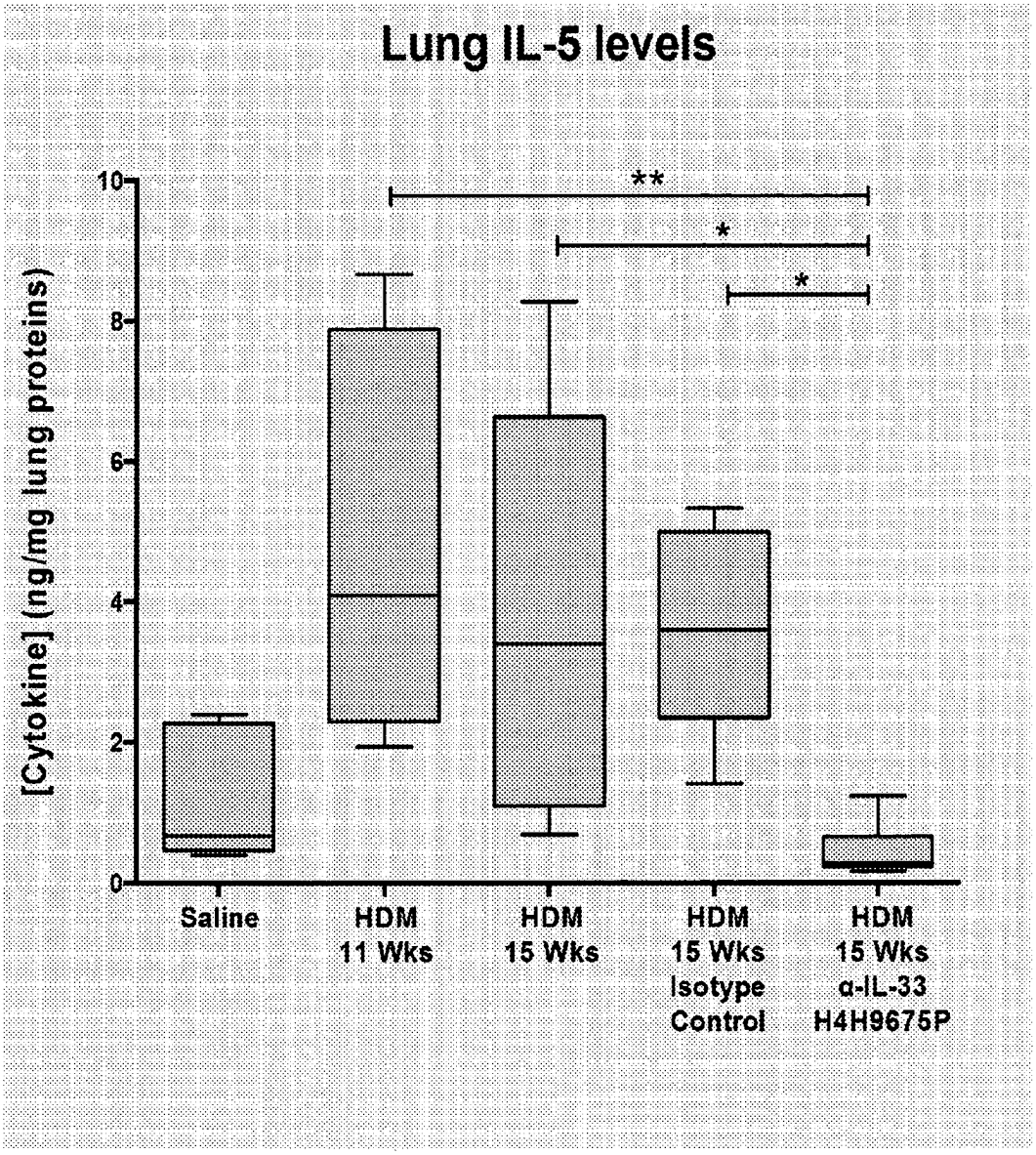

Lung IL-5 levels
[Cytokine] (ng/mg lung proteins)
10
8
6
4
2
0
**
*
*
Saline
HDM 11 Wks
HDM 15 Wks
HDM 15 Wks Isotype Control
HDM 15 Wks α-IL-33 H4H9675P

Figure 5C

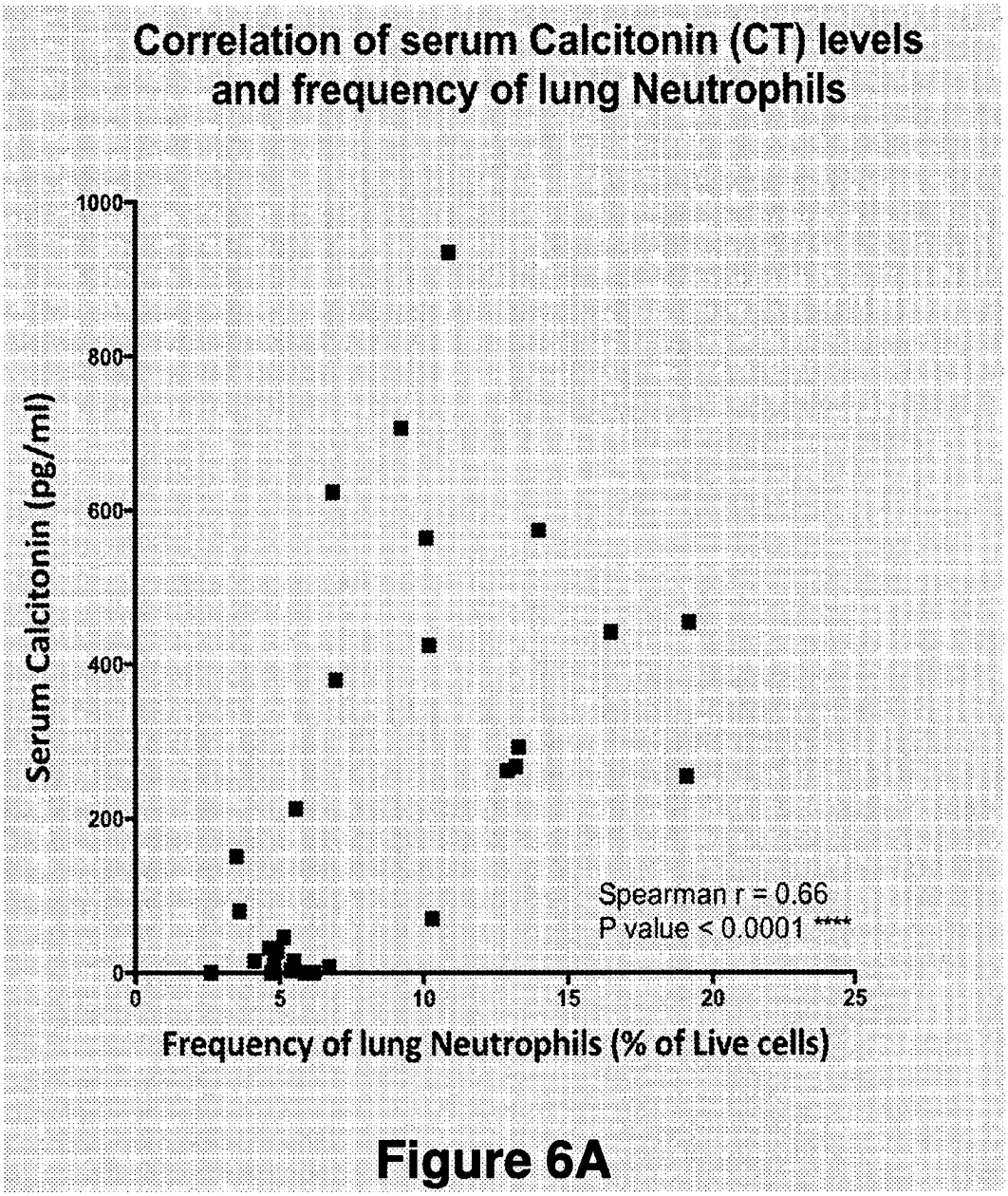

Correlation of serum Calcitonin (CT) levels and frequency of lung Neutrophils
Serum Calcitonin (pg/ml)
1000
800
600
400
200
0
0
5
10
15
20
25
Frequency of lung Neutrophils (% of Live cells)
Spearman r = 0.66
P value < 0.0001 ****

Figure 6A

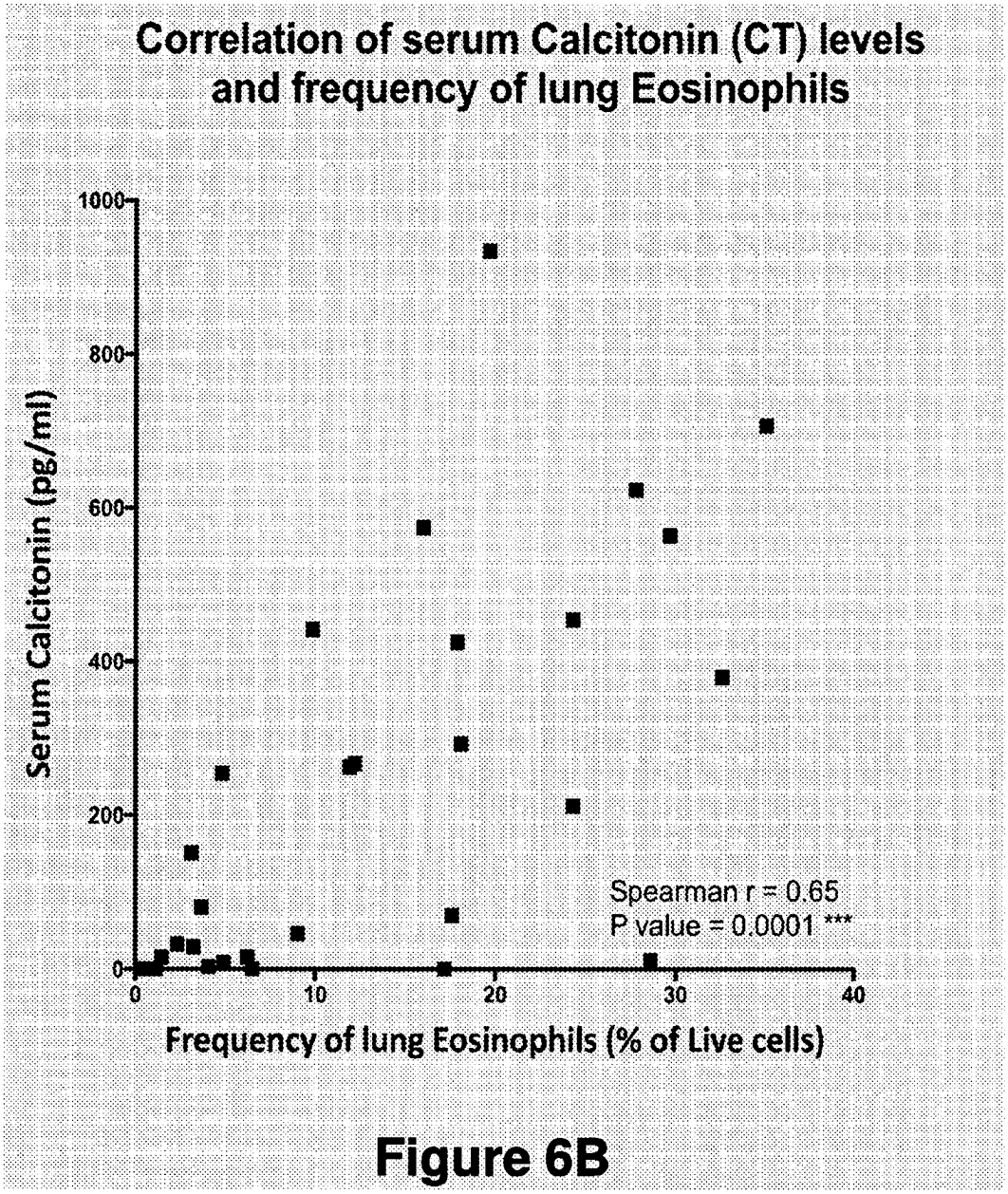

Correlation of serum Calcitonin (CT) levels and frequency of lung Eosinophils
Serum Calcitonin (pg/ml)
1000
800
600
400
200
0
0
10
20
30
40
Frequency of lung Eosinophils (% of Live cells)
Spearman r = 0.65
P value = 0.0001 ***

Figure 6B

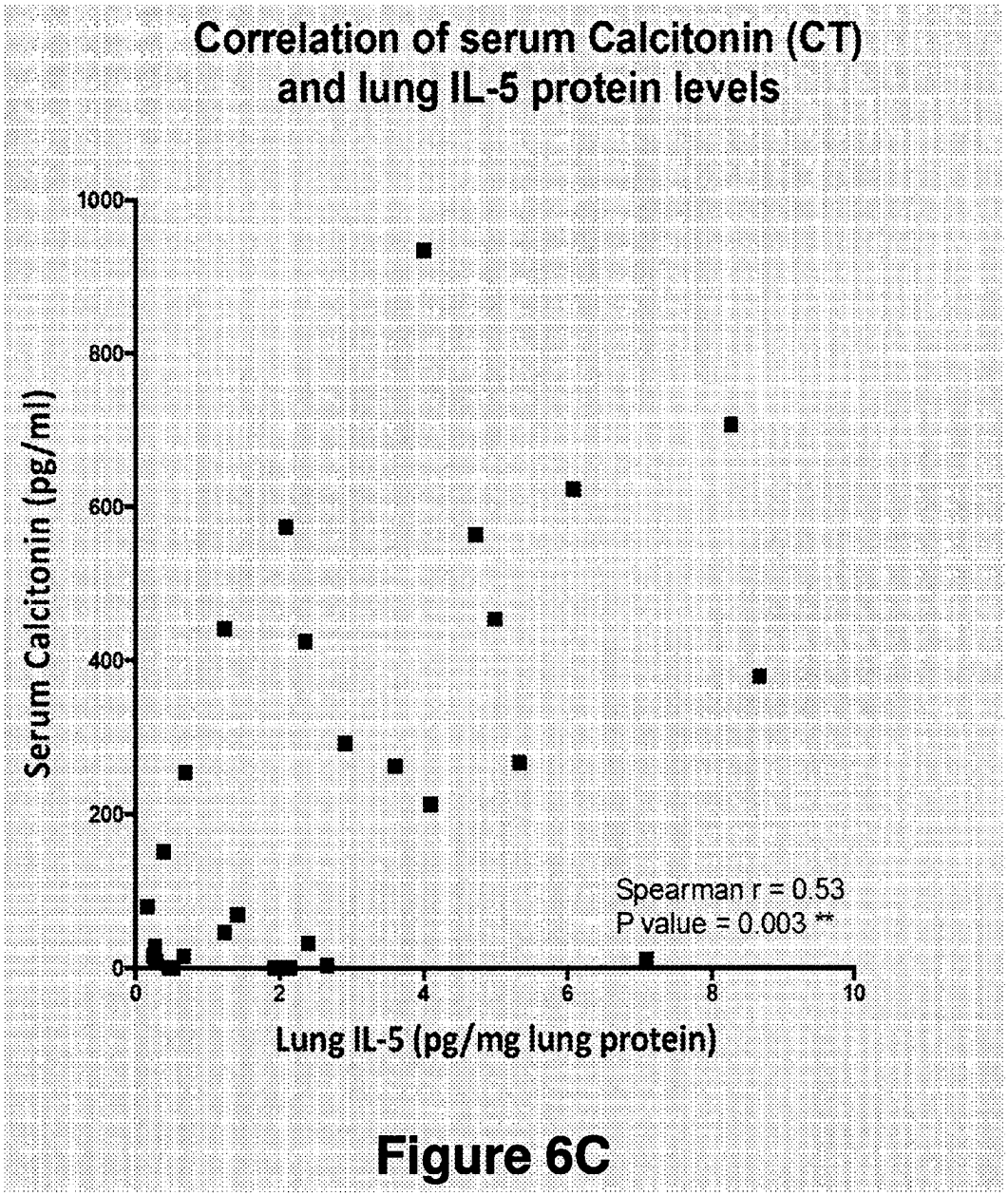

Correlation of serum Calcitonin (CT) and lung IL-5 protein levels
Serum Calcitonin (pg/ml)
1000
800
600
400
200
0
0
2
4
6
8
10
Lung IL-5 (pg/mg lung protein)
Spearman r = 0.53
P value = 0.003 **

Figure 6C

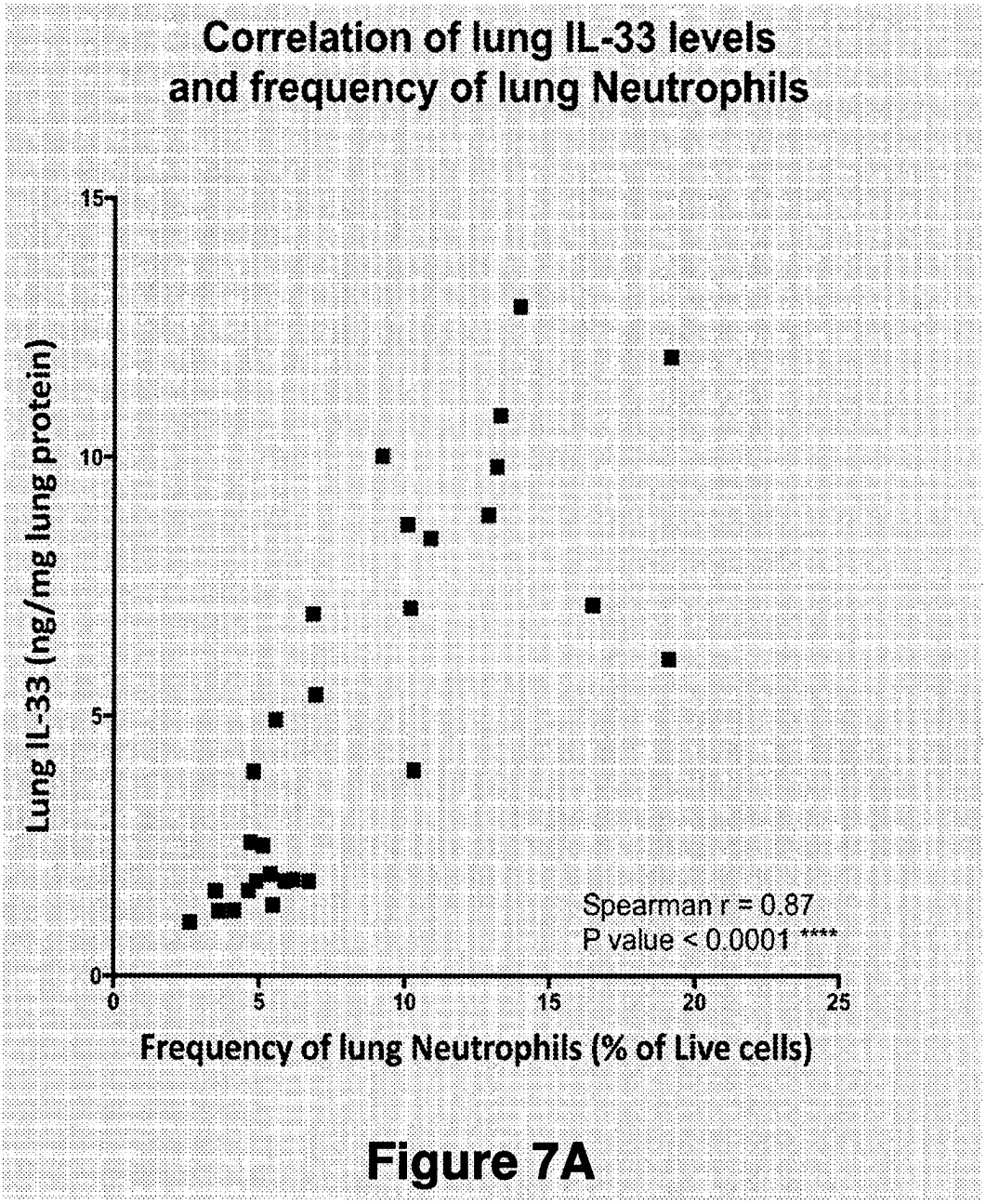

Correlation of lung IL-33 levels
and frequency of lung Neutrophils
Lung IL-33 (ng/mg lung protein)
15
10
5
0
0
5
10
15
20
25
Frequency of lung Neutrophils (% of Live cells)
Spearman r = 0.87
P value < 0.0001 ****

Figure 7A

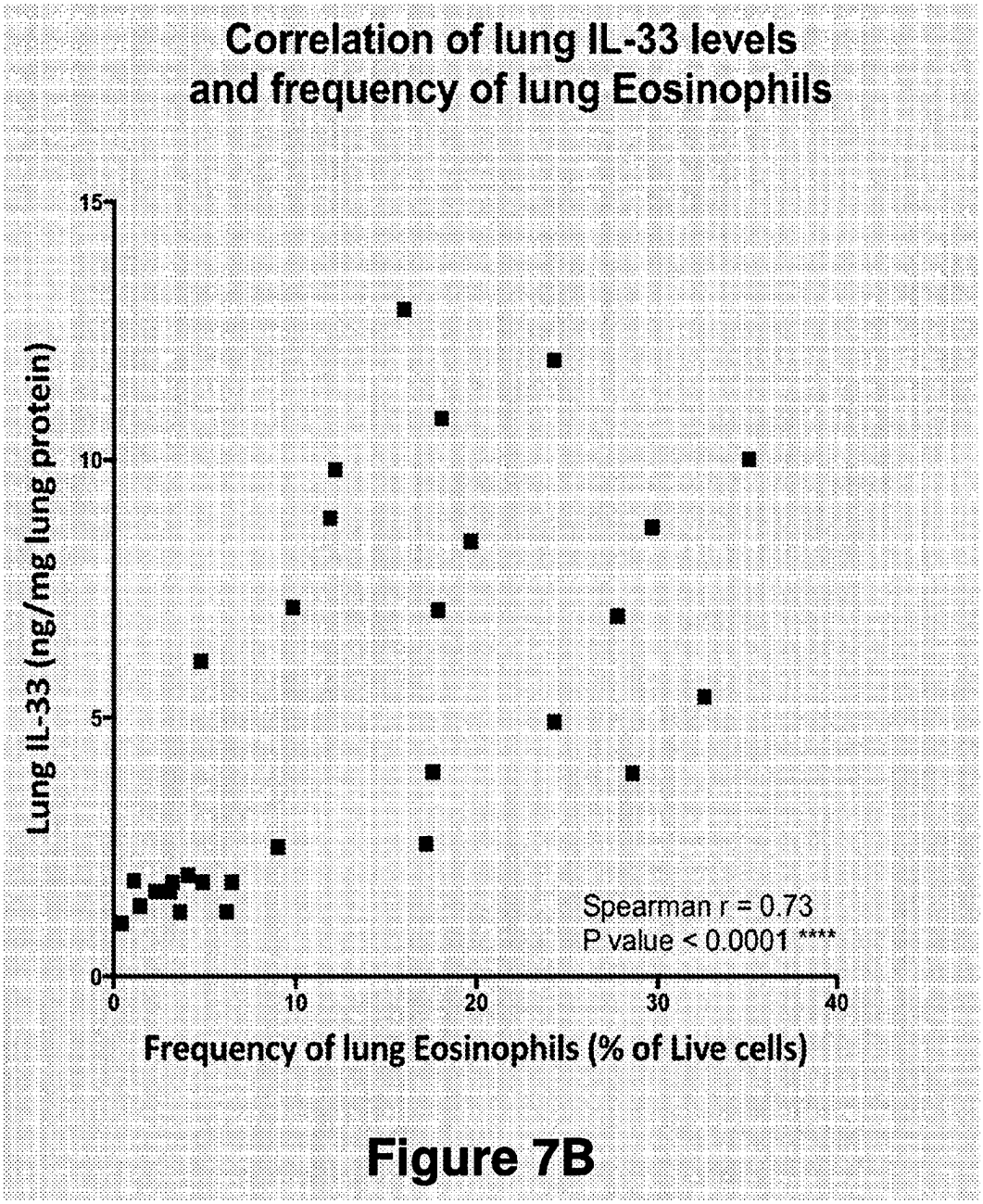

Correlation of lung IL-33 levels
and frequency of lung Eosinophils
Lung IL-33 (ng/mg lung protein)
15
10
5
0
0
10
20
30
40
Spearman r = 0.73
P value < 0.0001 ****
Frequency of lung Eosinophils (% of Live cells)

Figure 7B

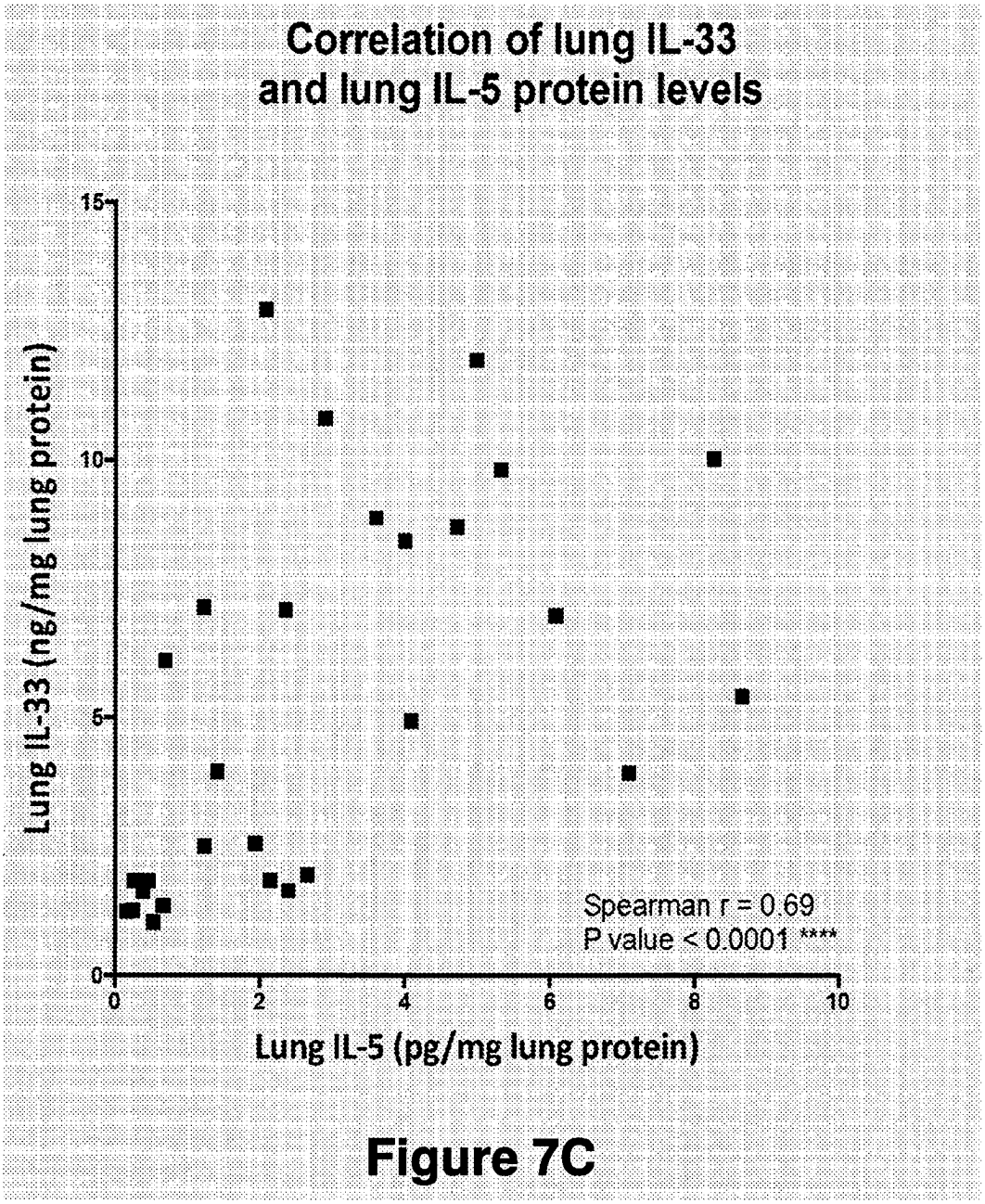

Correlation of lung IL-33
and lung IL-5 protein levels
Lung IL-33 (ng/mg lung protein)
15
10
5
0
0
2
4
6
8
10
Lung IL-5 (pg/mg lung protein)
Spearman r = 0.69
P value < 0.0001 ****

Figure 7C

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 April 2017 (13.04.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/062456 A3

(51) International Patent Classification:
C07K 16/24 (2006.01) *G01N 33/50* (2006.01)
C07K 14/715 (2006.01)

(21) International Application Number:
PCT/US2016/055502

(22) International Filing Date:
5 October 2016 (05.10.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/237,624 6 October 2015 (06.10.2015) US

(71) Applicant: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707 (US).

(72) Inventors: **ORENGO, Jamie M.**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **ALLINNE, Jeanne**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **FURY, Wen**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **BAI, Yu**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(74) Agents: **TERMES, Lance** et al.; Alston & Bird LLP, 101 South Tryon Street, Bank of America Plaza, Suite 4000, Charlotte, North Carolina 28280-4000 (US).

(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, 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,

[Continued on next page]

(54) Title: BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF

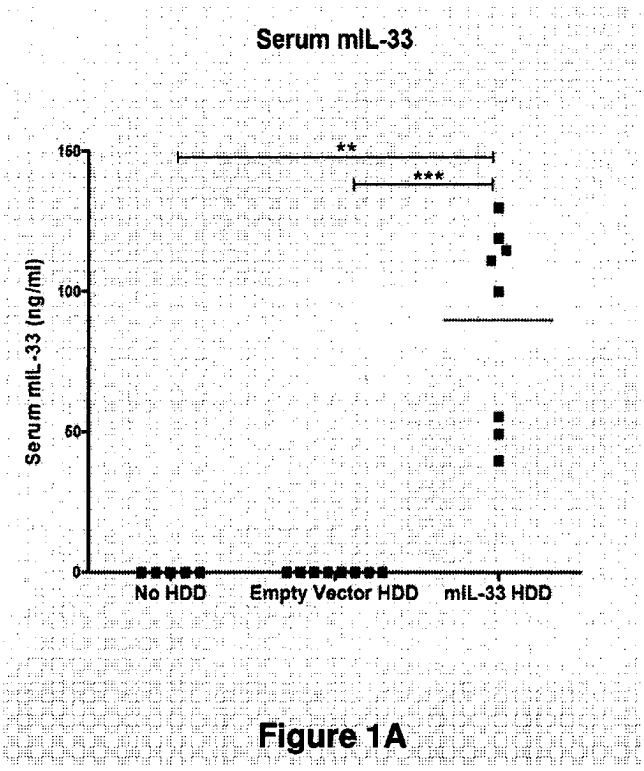


Figure 1A

(57) Abstract: The present invention relates to the identification of certain biomarkers for use in identifying patients who have, or are likely to develop an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein. Also provided are methods for decreasing the level of at least one biomarker in a subject suffering from an IL-33-mediated disease or disorder, and methods for treating such diseases or disorders according to the expression levels of one or more biomarkers. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising an interleukin-33 antagonist.

WO 2017/062456 A3

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).

Declarations under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
18 May 2017

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055502

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 C07K14/715 G01N33/50
ADD.

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 G01N A61K

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, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/061441 A1 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]) 30 April 2015 (2015-04-30)	1-3, 15-21, 31-39, 51,52
Y	page 2, paragraph [0006] - page 3, paragraph [0007] page 15, paragraph [0045]; claims	4-14, 22-30, 40-50
Y	US 2014/271658 A1 (MURPHY ANDREW J [US] ET AL) 18 September 2014 (2014-09-18) claims; examples	4-8,14, 22-26, 40-44, 49,50

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
22 December 2016	31/03/2017

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

3

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055502

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/271642 A1 (MURPHY ANDREW J [US] ET AL) 18 September 2014 (2014-09-18) claims; examples	4,9-14, 22, 27-30, 40,45-50
A	LOUTEN ET AL: "Biomarkers of Disease and Treatment in Murine and Cynomolgus Models of Chronic Asthma", BIOMARKER INSIGHTS, 1 July 2012 (2012-07-01), page 87, XP055186030, DOI: 10.4137/BMI.S9776 abstract; figure 4; tables 1-3 page 95, right-hand column, last paragraph - page 96, left-hand column, paragraph 1	1-52
A	JIA GUIQUAN ET AL: "Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients", JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 130, no. 3, 1 September 2012 (2012-09-01), pages 647-654, XP009164752, ISSN: 0091-6749, DOI: 10.1016/J.JACI.2012.06.025 abstract	1-52
A	KATHERINE J. BAINES ET AL: "Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes", JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 133, no. 4, 1 April 2014 (2014-04-01) , pages 997-1007, XP055286629, AMSTERDAM, NL ISSN: 0091-6749, DOI: 10.1016/j.jaci.2013.12.1091 figures 2,3; tables I,III,V	1-52
A	WEI LONG ET AL: "Procalcitonin guidance for reduction of antibiotic use in patients hospitalized with severe acute exacerbations of asthma: a randomized controlled study with 12-month follow-up", CRITICAL CARE, BIOMED CENTRAL LTD., LONDON, GB, vol. 18, no. 5, 5 September 2014 (2014-09-05), page 471, XP021198750, ISSN: 1364-8535, DOI: 10.1186/S13054-014-0471-7 abstract	1-52

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055502

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHANG DI ET AL: "[Changes in plasma interleukin-33 concentration in sepsis and its correlation with seriousness of sepsis]", CHINESE CRITICAL CARE MEDICINE - ZHONGHUA WEIZHONGBING JIJIU Y, ZHONGHUA YIXUEHUI, CN, vol. 27, no. 2, 1 February 2015 (2015-02-01), pages 138-142, XP009192820, ISSN: 2095-4352 abstract ----	1-52
A	JOSE C ALVES-FILHO ET AL: "Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection", NATURE MEDICINE., vol. 16, no. 6, 16 May 2010 (2010-05-16), pages 708-712, XP055331303, US ISSN: 1078-8956, DOI: 10.1038/nm.2156 abstract ----	1-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/055502

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.:
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:

see additional sheet

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 an 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.:

2, 3, 20-36, 51, 52(completely); 1, 4-19, 37-50(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2, 3, 20-36, 51, 52(completely); 1, 4-19, 37-50(partially)

Subject-matter of claims 1 to 52 wherein the biomarker is calcitonin, procalcitonin or calcitonin gene-related peptide.

2-10. claims: 1, 4-19, 37-50(all partially)

Subject-matter of claims 1, 4-19, 37-50 wherein the biomarker is resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), or serine (or cysteine) peptides (Serpina3f). Each marker defines a separate invention.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/055502

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015061441 A1	30-04-2015	AR 098155 A1	04-05-2016
		AU 2014340129 A1	26-05-2016
		CA 2924873 A1	30-04-2015
		CN 105849280 A	10-08-2016
		EP 3060685 A1	31-08-2016
		JP 2017501680 A	19-01-2017
		KR 20160068802 A	15-06-2016
		SG 11201603127W A	30-05-2016
		US 2017067108 A1	09-03-2017
		WO 2015061441 A1	30-04-2015
US 2014271658 A1	18-09-2014	AU 2014248839 A1	20-08-2015
		CA 2902172 A1	09-10-2014
		CL 2015002469 A1	10-06-2016
		CN 105051063 A	11-11-2015
		EA 201591716 A1	29-07-2016
		EP 2970460 A2	20-01-2016
		JP 2016513644 A	16-05-2016
		KR 20150126598 A	12-11-2015
		PH 12015501656 A1	19-10-2015
		SG 11201505745W A	28-08-2015
		TW 201522370 A	16-06-2015
		US 2014271658 A1	18-09-2014
		US 2016362487 A1	15-12-2016
		UY 35417 A	31-10-2014
		WO 2014164959 A2	09-10-2014
US 2014271642 A1	18-09-2014	AU 2014240101 A1	15-10-2015
		CA 2904377 A1	25-09-2014
		CN 105007929 A	28-10-2015
		EA 201591331 A1	29-01-2016
		EP 2968454 A1	20-01-2016
		JP 2016515130 A	26-05-2016
		KR 20150127591 A	17-11-2015
		US 2014271642 A1	18-09-2014
		WO 2014152195 A1	25-09-2014

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 108137682 A
(43)申请公布日 2018.06.08

(21)申请号 201680058610.4
(22)申请日 2016.10.05
(30)优先权数据
62/237,624 2015.10.06 US
(85)PCT国际申请进入国家阶段日
2018.04.08
(86)PCT国际申请的申请数据
PCT/US2016/055502 2016.10.05
(87)PCT国际申请的公布数据
WO2017/062456 EN 2017.04.13
(71)申请人 瑞泽恩制药公司
地址 美国纽约州
(72)发明人 J·M·奥兰格 J·阿林内 W·菲里 Y·白
(74)专利代理机构 北京市中咨律师事务所 11247
代理人 凌立 黄革生
(51)Int.Cl.
C07K 16/24(2006.01)
C07K 14/715(2006.01)
G01N 33/50(2006.01)

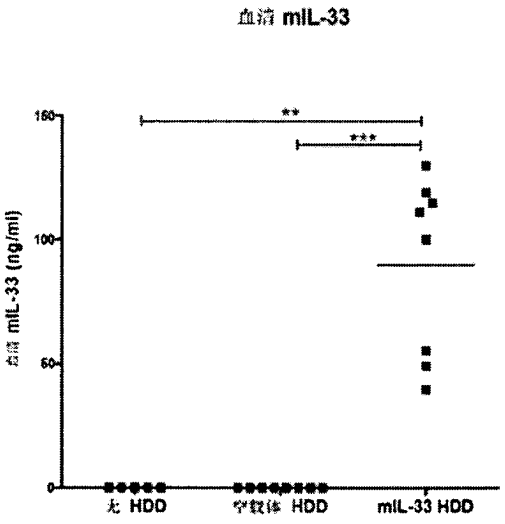
权利要求书5页 说明书35页
序列表119页 附图14页

(54)发明名称

与白介素-33(IL-33)介导的疾病有关的生物标志物及其用途

(57)摘要

本发明涉及某些生物标志物的鉴别，所述生物标志物用于鉴别具有或可能发生IL-33介导的疾病或障碍且更可能对使用IL-33拮抗剂的疗法做出应答的患者。本发明也涉及通过给有此需要的患者施用IL-33拮抗剂并监测使用本文描述的生物标志物的疗法的有效性来治疗患者中的IL-33介导的疾病或障碍的方法。还提供了用于降低遭受IL-33介导的疾病或障碍的受试者中的至少一种生物标志物的水平的方法，和根据一种或多种生物标志物的表达水平治疗这样的疾病或障碍的方法。本发明的方法包括给有此需要的受试者施用包含白介素-33拮抗剂的药物组合物。



CN 108137682 A

1.包含治疗有效量的白介素-33拮抗剂的药物组合物用于治疗受试者中白介素-33(IL-33)介导的疾病或障碍的用途,所述受试者表现出升高的与IL-33介导的疾病或障碍有关的至少一种生物标志物的水平。

2.根据权利要求1所述的用途,其中所述生物标志物是由CALCA基因编码的多肽且选自降钙素、原降钙素和降钙素基因相关肽(CGRP)。

3.根据权利要求2所述的用途,其中所述生物标志物是降钙素。

4.根据权利要求1所述的用途,其中所述IL-33拮抗剂包含抗-IL-33抗体或基于抗-IL-33受体的拮抗剂(IL-33阱)。

5.根据权利要求4所述的用途,其中所述抗-IL-33抗体是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:(a)重链可变区(HCVR)的互补性决定区(CDR),其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列;和(b)轻链可变区(LCVR)的CDR,其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

6.根据权利要求4或5所述的用途,其中所述抗-IL-33抗体或其抗原结合片段包含选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的HCVR/LCVR氨基酸序列对的重链和轻链CDR。

7.根据权利要求4-6中的任一项所述的用途,其中所述抗体或抗原结合片段包含HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3结构域,其分别选自SEQ ID NO:4-6-8-12-14-16;20-22-24-28-30-32;36-38-40-44-46-48;52-54-56-60-62-64;68-70-72-76-78-80;84-86-88-92-94-96;100-102-104-108-110-112;116-118-120-124-126-128;132-134-136-140-142-144:148-150-152-156-158-160:164-166-168-172-174-176;180-182-184-188-190-192;196-198-200-204-206-208;212-214-216-220-222-224;228-230-232-236-238-240;244-246-248-252-254-256;260-262-264-268-270-272;276-278-280-284-286-288;292-294-296-300-302-304;和310-312-314-318-320-322。

8.根据权利要求4-7中的任一项所述的用途,其中所述抗体或抗原结合片段包含:(a)重链可变区(HCVR),其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列;和(b)轻链可变区(LCVR),其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

9.根据权利要求4所述的用途,其中所述基于IL-33受体的拮抗剂包含与多聚化结构域(M)连接的第一个IL-33结合结构域(D1),其中D1包含ST2蛋白的IL-33-结合部分。

10.根据权利要求9所述的用途,其中所述基于IL-33受体的拮抗剂还包含一个或多个另外的选自D2、D3和D4的IL-33结合结构域。

11.根据权利要求4所述的用途,其中所述基于IL-33受体的拮抗剂包含ST2蛋白的IL-33-结合部分、IL-1RAcP蛋白的细胞外结构域或其它IL-33结合结构域。

12.根据权利要求4所述的用途,其中所述基于IL-33受体的拮抗剂选自SEQ ID NO:323、324、325、326和335。

13.根据权利要求1-12中的任一项所述的用途，其中所述药物组合物用于与有效量的第二种治疗剂联合使用，所述第二种治疗剂用于减轻IL-33介导的疾病或障碍的至少一种症状。

14.根据权利要求13所述的用途，其中所述第二种治疗剂选自非甾体类抗炎剂(NSAID)、皮质类固醇、支气管扩张剂、抗组胺剂、肾上腺素、解充血药、胸腺基质淋巴细胞生成素(TSLP)拮抗剂、IL-13拮抗剂、IL-4拮抗剂、IL-4/IL-13双拮抗剂、IL-5拮抗剂、IL-6拮抗剂、IL-12/23拮抗剂、IL-22拮抗剂、IL-25拮抗剂、IL-17拮抗剂、IL-31拮抗剂、口服PDE4抑制剂和另一种IL-33拮抗剂或不同的基于抗体或受体的IL-33拮抗剂。

15.根据权利要求1所述的用途，其中受试者中与IL-33介导的疾病或障碍有关的生物标志物选自降钙素、原降钙素、降钙素基因相关肽(CGRP)、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A 3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirg1)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸(或半胱氨酸)肽(Serpina3f)。

16.根据权利要求1所述的用途，其中所述生物标志物是降钙素，且其中所述降钙素水平在具有IL-33介导的疾病或障碍的所述受试者的血清中增加，且其中血清降钙素的增加与所述受试者的肺中增加的降钙素和IL-33水平相关。

17.根据权利要求16所述的用途，其中治疗所述IL-33介导的疾病或障碍包括将所述受试者的血清中的降钙素水平降低至正常水平。

18.根据权利要求1所述的用途，其中所述升高的至少一种生物标志物的水平对应于对于女性而言大于约5pg/mL和对于男性而言大于约10pg/mL的平均血清降钙素水平。

19.根据权利要求1所述的用途，其中所述IL-33介导的疾病或障碍是选自哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)的炎性疾病或障碍。

20.一种用于鉴别可能对使用IL-33拮抗剂的疗法做出有利应答的、遭受IL-33介导的疾病或障碍的受试者的方法，所述方法包括从所述受试者获得样品和测量所述样品中的降钙素、原降钙素或降钙素基因相关肽(CGRP)的水平；其中与没有遭受IL-33介导的疾病或障碍的受试者中的较低降钙素、原降钙素或CGRP水平相比升高的降钙素、原降钙素或CGRP水平将所述患者鉴别为可能对使用IL-33拮抗剂的疗法做出有利应答的患者。

21.根据权利要求20所述的方法，其中所述IL-33介导的疾病或障碍是选自哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)的炎性疾病或障碍。

22.根据权利要求20所述的方法，其中所述IL-33拮抗剂是抗-IL-33抗体或基于抗-IL-33受体的拮抗剂(IL-33阱)。

23.根据权利要求22所述的方法，其中所述抗-IL-33抗体是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段，其中所述抗体或抗原结合片段包含：(a)重链可变区(HCVR)的互补性决定区(CDR)，其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列；和(b)轻链可变区(LCVR)的CDR，其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

24.根据权利要求22或23的方法，其中所述抗-IL-33抗体或其抗原结合片段包含选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的HCVR/LCVR氨基酸序列对的重链和轻链CDR。

25.根据权利要求22-24中的任一项所述的方法，其中所述抗体或抗原结合片段包含HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3结构域，其分别选自SEQ ID NO:4-6-8-12-14-16；20-22-24-28-30-32；36-38-40-44-46-48；52-54-56-60-62-64；68-70-72-76-78-80；84-86-88-92-94-96；100-102-104-108-110-112；116-118-120-124-126-128；132-134-136-140-142-144；148-150-152-156-158-160；164-166-168-172-174-176；180-182-184-188-190-192；196-198-200-204-206-208；212-214-216-220-222-224；228-230-232-236-238-240；244-246-248-252-254-256；260-262-264-268-270-272；276-278-280-284-286-288；292-294-296-300-302-304；和310-312-314-318-320-322。

26.根据权利要求22-25中的任一项所述的方法，其中所述抗体或抗原结合片段包含：(a)重链可变区(HCVR)，其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列；和(b)轻链可变区(LCVR)，其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

27.根据权利要求22所述的方法，其中所述基于IL-33受体的拮抗剂包含与多聚化结构域(M)连接的第一个IL-33结合结构域(D1)，其中D1包含ST2蛋白的IL-33-结合部分。

28.根据权利要求27所述的方法，其中所述基于IL-33受体的拮抗剂还包含一个或多个另外的选自D2、D3和D4的IL-33结合结构域。

29.根据权利要求22所述的方法，其中所述基于IL-33受体的拮抗剂包含ST2蛋白的IL-33-结合部分、IL-1RAcP蛋白的细胞外结构域或其它IL-33结合结构域。

30.根据权利要求22所述的方法，其中所述基于IL-33受体的拮抗剂选自SEQ ID NO:323、324、325、326和335。

31.一种确定受试者是否可能具有或可能发生IL-33介导的疾病或障碍的方法，所述方法包括从所述受试者获得样品和测量所述样品中的降钙素、原降钙素或CGRP水平；其中与不可能具有或不可能发生IL-33介导的疾病或障碍的受试者中的较低降钙素、原降钙素或CGRP水平相比升高的降钙素、原降钙素或CGRP水平将所述患者鉴别为可能具有或发生IL-33介导的疾病或障碍的患者。

32.根据权利要求31所述的方法，其中所述IL-33介导的疾病或障碍是选自哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性

粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)的炎性疾病或障碍。

33.根据权利要求31所述的方法,其中来自所述受试者的样品是固体组织样品、细胞样品或血液样品。

34.根据权利要求33所述的方法,其中所述固体组织样品选自心脏、肾、肝、肺和脾。

35.根据权利要求33所述的方法,其中所述细胞样品是背根神经节细胞样品、痰细胞样品、支气管肺泡灌洗细胞样品、鼻息肉细胞样品、粪便细胞样品、肺、结肠、心脏、肾、皮肤活组织检查、或脾活组织检查细胞样品。

36.根据权利要求33所述的方法,其中所述血液样品是全血、血浆或血清。

37.包含治疗有效量的IL-33拮抗剂的药物组合物用于治疗受试者中的IL-33介导的疾病或障碍的用途,所述受试者已经被诊断出具有IL-33介导的疾病或障碍且已经基于表现出增强的至少一种生物标志物的治疗前表达被选择用于用IL-33拮抗剂治疗,其中基于与相应生物标志物的参比表达水平的对比来确定所述增强的生物标志物表达。

38.根据权利要求37所述的用途,其中所述IL-33介导的疾病或障碍是选自哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)的炎性疾病或障碍。

39.根据权利要求37所述的用途,其中受试者中与IL-33介导的疾病或障碍有关的生物标志物选自降钙素、原降钙素、CGRP、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A 3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirgl)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸(或半胱氨酸)肽(Serpina3f)。

40.根据权利要求37所述的用途,其中所述IL-33拮抗剂包含抗-IL-33抗体或基于抗-IL-33受体的拮抗剂(IL-33阱)。

41.根据权利要求40所述的用途,其中所述抗-IL-33抗体是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段,其中所述抗体或抗原结合片段包含:(a)重链可变区(HCVR)的互补性决定区(CDR),其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列;和(b)轻链可变区(LCVR)的CDR,其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

42.根据权利要求40或41的用途,其中所述抗-IL-33抗体或其抗原结合片段包含选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的HCVR/LCVR氨基酸序列对的重链和轻链CDR。

43.根据权利要求40-42中的任一项所述的用途,其中所述抗体或抗原结合片段包含HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3结构域,其分别选自SEQ ID NO:4-6-8-12-14-16;

20-22-24-28-30-32;36-38-40-44-46-48;52-54-56-60-62-64;68-70-72-76-78-80;84-86-88-92-94-96;100-102-104-108-110-112;116-118-120-124-126-128;132-134-136-140-142-144;148-150-152-156-158-160;164-166-168-172-174-176;180-182-184-188-190-192;196-198-200-204-206-208;212-214-216-220-222-224;228-230-232-236-238-240;244-246-248-252-254-256;260-262-264-268-270-272;276-278-280-284-286-288;292-294-296-300-302-304;和310-312-314-318-320-322。

44.根据权利要求40-43中的任一项所述的用途，其中所述抗体或抗原结合片段包含：(a)重链可变区(HCVR)，其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列；和(b)轻链可变区(LCVR)，其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

45.根据权利要求40所述的用途，其中所述基于IL-33受体的拮抗剂包含与多聚化结构域(M)连接的第一个IL-33结合结构域(D1)，其中D1包含ST2蛋白的IL-33-结合部分。

46.根据权利要求45所述的用途，其中所述基于IL-33受体的拮抗剂还包含一个或多个另外的选自D2、D3和D4的IL-33结合结构域。

47.根据权利要求40所述的用途，其中所述基于IL-33受体的拮抗剂包含ST2蛋白的IL-33-结合部分、IL-1RAcP蛋白的细胞外结构域或其它IL-33结合结构域。

48.根据权利要求40所述的用途，其中所述基于IL-33受体的拮抗剂选自SEQ ID NO:323、324、325、326和335。

49.根据权利要求40所述的用途，其中所述药物组合物用于与第二种治疗剂联合使用，所述第二种治疗剂用于减轻IL-33介导的疾病或障碍的至少一种症状。

50.根据权利要求49所述的用途，其中所述第二种治疗剂选自非甾体类抗炎剂(NSAID)、类固醇、皮质类固醇(吸入的或局部的)、免疫抑制剂(例如环磷酰胺)、抗胆碱能剂(例如噻托溴铵)、毒蕈碱样药剂(例如格隆铵(glycopyrronium))、磷酸二酯酶抑制剂(例如茶碱、罗氟司特、西洛司特)、β阻滞剂、环孢菌素、他克莫司、吡美莫司、硫唑嘌呤、甲氨蝶呤、色甘酸钠、蛋白酶抑制剂、支气管扩张剂、β-2-激动剂、抗组胺剂、肾上腺素、解充血药、白三烯抑制剂、肥大细胞抑制剂、胸腺基质淋巴细胞生成素(TSLP)拮抗剂、TNF拮抗剂、IgE拮抗剂、IL-1拮抗剂、IL-4或IL-4R拮抗剂、IL-13或IL-13R拮抗剂、IL-4/IL-13双拮抗剂、IL-5拮抗剂、IL-6或IL-6R拮抗剂、IL-8拮抗剂、IL-9拮抗剂、IL-12/23拮抗剂、IL-22拮抗剂、IL-17拮抗剂、IL-31拮抗剂、IL-33拮抗剂、口服PDE4抑制剂和不同的针对IL-25的抗体。

51.一种用于测量IL-33介导的疾病或障碍在受试者中的存在的血清生物标志物，其中所述血清生物标志物的水平与增强的体内IL-33水平相关，且其中所述血清生物标志物是由CALCA基因编码的多肽且选自降钙素、原降钙素和CGRP。

52.用于测量IL-33介导的疾病或障碍在受试者中的存在的根据权利要求51所述的血清生物标志物，其中所述血清生物标志物的水平与增强的体内IL-33水平相关，且其中所述生物标志物是降钙素。

与白介素-33(IL-33)介导的疾病有关的生物标志物及其用途

[0001] 对序列表的引用

[0002] 本申请通过引用并入作为文件10187W001-Sequence.txt(其创建于2016年10月4日且含有159,920字节)以计算机可读形式呈交的序列表。

技术领域

[0003] 本发明涉及与IL-33的表达有关的哺乳动物中的特定生物标志物的鉴别和这些生物标志物用于鉴别患者的用途,所述患者具有或可能发生IL-33介导的疾病或障碍且其更可能对使用IL-33拮抗剂的疗法做出应答。本发明也涉及通过将IL-33拮抗剂施用给有此需要的患者和使用本文描述的生物标志物监测疗法的有效性来治疗患者中的IL-33介导的疾病或障碍的方法。

背景技术

[0004] 白介素-33(IL-33)是细胞因子的IL-1超家族的一个成员,主要由间质细胞(诸如上皮和内皮细胞)在促炎症性刺激后表达。IL-33是ST2的配体,所述ST2是一种与辅助蛋白IL-1RAcP相关的toll-样/白介素-1受体超家族成员(关于综述,参见,例如,Kakkar和Lee,Nature Reviews-Drug Discovery 7(10):827-840(2008),Schmitz等人,Immunity 23:479-490(2005);Liew等人,Nature Reviews-Immunology 10:103-110(2010);US 2010/0260770;US 2009/0041718)。在ST2/IL-1RAcP被IL-33活化后,通过下游分子诸如MyD88(骨髓细胞样分化因子88)和TRAF6(TNF受体相关因子6)触发信号传递级联,从而导致NFκB(核因子-κB)的活化,以及其它。

[0005] IL-33信号传递已经作为一种因子涉入多种疾病和障碍(Liew等人,Nature Reviews-Immunology 10:103-110(2010))。例如,当IL-33在宿主中保护免于蠕虫感染并且也通过促进T_H2-型免疫应答来减少动脉粥样硬化时,它还可以通过扩增T_H2细胞而促进哮喘的发病机制和通过肥大细胞活化而介导关节炎症、特应性皮炎和过敏反应。这样,IL-33可以是多种疾病的治疗干预的新靶标;例如,IL-33信号传递的阻断可以提供改善某些炎性疾病的多种致病特征的潜力。

[0006] 在以下文献中描述了IL-33拮抗剂:美国专利号5,576,191;7,666,622;8,119,771;8,187,596;9,090,694;9,212,227;9,382,318;美国专利公开号2007/0042978;2009/0041718;2010/0260770;2012/0207752;2012/0263709;2014/0140954;2014/0271642;2014/0212412;欧洲专利或专利申请号1725261B1;2069784A1;2152740A1;2283860A2;2475388A1;和PCT公开号W005/079844;W008/132709;W008/144610;W009/053098;W011/031600;W014/152195和W014/164959。

[0007] 降钙素(CT)是一种多肽激素,据信主要由甲状腺的滤泡旁细胞产生(Foster,G.V.,等人,(1964),Nature 202:1303-1305)。CT是位于染色体11上的CALCA基因的产物。CALCA基因编码多肽原降钙素(PCT)和PCT基因相关肽α(proCGRPα),它们通过选择性剪接而差别表达(Christ-Crain,M.等人(2008),Crit Care Med 36:1684-1687;Hoff,AO,等人

(2002),J.Clin.Invest.110:1849-1857)。降钙素(CT)源自较大前体原降钙素(PCT,116个氨基酸),后者被切割成未成熟的降钙素(33个氨基酸)并然后被切割成成熟的降钙素,即由32个氨基酸组成的3.5-kd肽的单体。CT主要由神经内分泌细胞诸如甲状腺的C细胞和肺的神经内分泌细胞(PNEC)通过蛋白水解性裂解在生产细胞的分泌颗粒中产生,然后作为成熟的CT多肽释放。表达calca基因的其它细胞包括肥大细胞、背路神经节细胞(dorsal route ganglion cell,DRG)和脊髓细胞。循环降钙素前体的多种形式存在于健康和患病个体的血清中(Becker K.等人(2004),J Clin Endocrinol Metab 89:1512-1525)。

[0008] 降钙素基因相关肽(CGRP)是降钙素家族肽的一个成员。α-CGRP是从位于染色体11上的降钙素/CGRP基因的选择性剪接形成的37-氨基酸肽(Amara,SG,等人,(1982),Nature,298(5871):240-244)。

[0009] CT起减少血液钙的作用,抑制破骨细胞和骨质吸收,与甲状旁腺激素(PTH)的活性相反(Naot,D.和J.Cornish(2008),Bone 43(5):813-818)。体内和体外实验提示,CT的主要生理功能是在钙应激状态(诸如在妊娠、生长或哺乳过程中)与高钙血症做斗争(Hoff,A.O.,等人,(2002),J Clin Invest 110(12):1849-1857;Zaidi,M.,等人,(2002),J Clin Invest 110(12):1769-1771)。在诊断上,CT被用作甲状腺髓样癌(MTC)的生物标志物。CT肽的正常循环水平较低,但是在生理条件下,这些水平全身性地或局部地增加。高CT水平指示MTC的存在,并且被用于评价外科手术摘除的效力和复发。CT也在C-细胞增生、肺和胰腺肿瘤、肾功能衰竭和甲状腺自身免疫病中升高(Becker,K.L.,等人,(2004).J Clin Endocrinol Metab 89(4):1512-1525)。

[0010] 迄今为止,尚未鉴别出这样的生物标志物,其用于鉴别具有或易于发生IL-33介导的疾病或障碍的患者,或确定患者对使用IL-33拮抗剂的疗法做出应答的可能性。尽管已经鉴别出在炎性病症或变态反应的治疗中表现出希望的IL-33拮抗剂,但是需要可以预测抗-IL-33疗法的效力的生物标志物来有效地鉴别和选择对抗-IL-33疗法做出有利应答的患者亚群。因此,在本领域中存在一个未得到满足的关于鉴别和验证施用抗-IL-33疗法的具有炎性病症或变态反应的患者中的预测和预后生物标志物的需要。

发明内容

[0011] 根据本发明的某些方面,提供了用于治疗、预防和/或减轻受试者中白介素-33(IL-33)介导的疾病或障碍的症状的严重程度或持续时间的方法。本发明的方法包括给有此需要的受试者施用包含治疗有效量的白介素-33(IL-33)拮抗剂的药物组合物。

[0012] 在本发明的一个实施方案中,所述IL-33拮抗剂是特异性地结合IL-33的抗体或其抗原结合片段。

[0013] 在本发明的一个实施方案中,所述IL-33拮抗剂是如本文中所述的基于受体的IL-33的拮抗剂,诸如IL-33阱。

[0014] 本发明还提供了特定生物标志物的鉴别,所述特定生物标志物与遭受IL-33介导的疾病或障碍的受试者中的IL-33的表达有关。在本发明的上下文中可以评价和/或测量的与IL-33介导的疾病或障碍有关的示例性生物标志物包括、但不限于由CALCA基因编码的多肽,包括降钙素、原降钙素和降钙素基因相关肽(CGRP)。在一个实施方案中,在本发明的上下文中可以评价和/或测量的与IL-33介导的疾病或障碍有关的生物标志物是降钙素。在本

发明的上下文中可以评价和/或测量的与IL-33介导的疾病或障碍有关的其它生物标志物包括、但不限于：抵抗素-样α(RETNA)；趋化因子(C-C基序)配体8(Ccl8)；血清淀粉样蛋白A3(Saa3)；Gm1975(BC117090)；杀伤细胞凝集素-样受体(Kirgl)；stefin A1(Csta)；跨膜4-结构域(Ms4a8a)；趋化因子(C-C基序)配体11(Ccl11)；和丝氨酸(或半胱氨酸)肽(Serpina3f)。

[0015] 在一个有关的方面，本发明提供了遭受IL-33介导的疾病或障碍的受试者的血清中的至少一种生物标志物的表达，所述生物标志物与来自所述受试者的至少一个组织样品中的IL-33表达水平相关。

[0016] 在一个实施方案中，所述与至少一个组织样品中的IL-33表达水平有关的血清生物标志物是降钙素。

[0017] 在一个特定实施方案中，所述与至少一个组织样品中的IL-33表达水平有关的血清生物标志物是原降钙素。

[0018] 在一个有关的实施方案中，所述与至少一个组织样品中的IL-33表达水平有关的血清生物标志物是降钙素基因相关肽(CGRP)。

[0019] 在另一个有关的方面，本发明提供了一种用于治疗受试者中白介素-33(IL-33)介导的疾病或障碍的方法，所述方法包括：(a)选择受试者，其在治疗之前或在治疗时表现出升高的与IL-33介导的疾病或障碍有关的至少一种生物标志物的水平，和(b)给所述受试者施用包含治疗有效量的白介素-33拮抗剂的药物组合物。

[0020] 根据本发明的一个有关方面，提供了用于治疗IL-33介导的疾病或障碍的方法，其包括给受试者施用包含治疗有效量的IL-33拮抗剂的药物组合物，其中所述药物组合物向所述受试者的施用导致所述受试者中与IL-33介导的疾病或障碍有关的至少一种生物标志物的减少。

[0021] 在一个实施方案中，所述在遭受IL-33介导的疾病或障碍的受试者中升高的生物标志物是降钙素，且所述IL-33拮抗剂是特异性地结合IL-33的单克隆抗体。

[0022] 本发明还提供了用于降低受试者中一种或多种IL-33介导的疾病或障碍相关的生物标志物的水平的方法，其导致改善受试者中一种或多种IL-33介导的疾病或障碍相关的参数，其中所述方法包括给有此需要的受试者施用单个初始剂量的包含IL-33拮抗剂的药物组合物。在某些实施方案中，本发明提供了施用一个或多个第二剂量的包含IL-33拮抗剂的药物组合物，其中所述施用导致受试者中一种或多种本文描述的生物标志物的水平的进一步下降和一种或多种IL-33介导的疾病或障碍相关的参数的进一步改善。在一个实施方案中，在存在于具有IL-33介导的疾病或障碍的受试者的血清中的生物标志物的水平和疾病或障碍相关的参数(例如嗜酸性粒细胞或嗜中性粒细胞向受试者的肺中的浸润或受试者的肺中的细胞因子水平(例如IL-5)的增加)的严重程度之间存在强关联。在某些实施方案中，所述组织(例如肺)中的IL-33的水平与疾病或障碍相关的参数的严重程度相关。在一个实施方案中，所述IL-33拮抗剂是特异性地结合IL-33的人单克隆抗体。在一个实施方案中，所述IL-33拮抗剂是如本文中所述的IL-33阱。

[0023] 在一个有关的方面，本发明提供了用于治疗受试者中的IL-33介导的疾病或障碍的方法，所述方法包括给所述受试者施用药物组合物，其包含治疗有效量的特异性地结合IL-33的抗体或其抗原结合片段，其中所述受试者已经被诊断出具有IL-33介导的疾病或障

碍且还已经被选择进行治疗，所述选择基于与生物标志物的参比水平(例如，诊断出具有IL-33介导的疾病或障碍的受试者子集中的生物标志物的表达和/或健康受试者中的生物标志物的表达)相比，所述受试者在治疗之前表现出升高的至少一种IL-33介导的疾病或障碍相关的生物标志物的水平。

[0024] 在另一个有关的方面，本发明还提供了通过给所述受试者施用药物组合物来治疗IL-33介导的疾病或障碍的方法，所述药物组合物包含治疗有效量的特异性地结合IL-33的抗体或其抗原结合片段，其中所述受试者已经被诊断出具有IL-33介导的疾病或障碍，已经用抗-IL33拮抗剂治疗了确定的时间段，且已经被选择用IL-33拮抗剂进一步治疗，所述选择基于在治疗确定的时间段以后表现出至少一种生物标志物(例如，降钙素、原降钙素或降钙素基因相关肽(CGRP))的表达，其中基于与在用抗-IL-33拮抗剂治疗之前受试者中的相应生物标志物的表达水平的对比来确定所述生物标志物的表达。

[0025] 本发明还提供了一种用于鉴别遭受IL-33介导的疾病或障碍的受试者的方法，所述受试者可能对使用IL-33拮抗剂的疗法做出有利应答，所述方法包括从所述受试者获得样品和测量所述样品中的降钙素水平；其中与没有遭受IL-33介导的疾病或障碍的受试者中的较低降钙素水平相比升高的降钙素水平将所述患者鉴别为可能对使用IL-33拮抗剂的疗法做出有利应答的患者。

[0026] 在某些实施方案中，通过测量组织样品中原降钙素或降钙素基因相关肽(CGRP)的水平，可以鉴别可能对使用IL-33拮抗剂的疗法做出有利应答的受试者，其中与没有遭受IL-33介导的疾病或障碍的受试者中的较低原降钙素或CGRP水平相比升高的原降钙素或降钙素基因相关肽(CGRP)的水平将该患者鉴别为可能对使用IL-33拮抗剂的疗法做出有利应答的患者。

[0027] 在另一个有关的方面，本发明提供了一种确定受试者是否可能具有或可能发生IL-33介导的疾病或障碍的方法，所述方法包括从所述受试者获得样品和测量所述样品中的降钙素水平；其中与参比标准相比升高的降钙素水平将所述患者鉴别为可能具有或发生IL-33介导的疾病或障碍的患者。

[0028] 在某些实施方案中，本发明提供了一种确定受试者是否可能具有或可能发生IL-33介导的疾病或障碍的方法，所述方法包括从所述受试者获得样品和测量所述样品中的原降钙素或CGRP的水平；其中与两种生物标志物中的任一种的参比标准相比升高的原降钙素或CGRP水平将所述患者鉴别为可能具有或发生IL-33介导的疾病或障碍的患者。用于测量以上生物标志物中的任一种的水平的方法是本领域技术人员已知的。

[0029] 来自所述受试者的样品可以是固体组织样品、细胞样品或血液样品。所述固体组织样品可以选自心脏、肾、肝、肺、结肠和脾。所述细胞样品可以是背根神经节细胞样品、痰细胞样品、支气管肺泡灌洗细胞样品、鼻息肉细胞样品、粪便细胞样品、肺、结肠、心脏、肾、皮肤活组织检查或脾活组织检查细胞样品。所述血液样品可以是全血、血浆或血清。

[0030] 在一个实施方案中，要用在本发明的方法中的IL-33拮抗剂是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段，其中所述抗体或抗原结合片段包含：(a)重链可变区(HCVR)的互补性决定区(CDR)，其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列；和(b)轻链可变区(LCVR)的CDR，其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、

154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

[0031] 在一个实施方案中，要用在本发明的方法中的IL-33拮抗剂是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段，其中所述抗-IL-33抗体或其抗原结合片段包含选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的HCVR/LCVR氨基酸序列对的重链和轻链CDR。

[0032] 在一个实施方案中，要用在本发明的方法中的IL-33拮抗剂是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段，其中所述抗体或抗原结合片段包含分别选自SEQ ID NO:4-6-8-12-14-16、20-22-24-28-30-32、36-38-40-44-46-48、52-54-56-60-62-64、68-70-72-76-78-80、84-86-88-92-94-96、100-102-104-108-110-112、116-118-120-124-126-128、132-134-136-140-142-144、148-150-152-156-158-160、164-166-168-172-174-176、180-182-184-188-190-192、196-198-200-204-206-208、212-214-216-220-222-224、228-230-232-236-238-240、244-246-248-252-254-256、260-262-264-268-270-272、276-278-280-284-286-288、292-294-296-300-302-304、和310-312-314-318-320-322的HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3结构域。

[0033] 在一个实施方案中，要用在本发明的方法中的IL-33拮抗剂是分离的特异性地结合人白介素-33(IL-33)的人单克隆抗体或其抗原结合片段，其中所述抗体或抗原结合片段包含：(a)重链可变区(HCVR)，其具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列；和(b)轻链可变区(LCVR)，其具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。

[0034] 在一个实施方案中，要用在本发明的方法中的IL-33拮抗剂是分离的基于IL-33受体的拮抗剂(例如IL-33阱)，其包含与多聚化结构域(M)连接的第一个IL-33结合结构域(D1)，其中D1包含ST2蛋白的IL-33-结合部分。在一个实施方案中，所述IL-33阱还包含一个或多个另外的选自D2、D3和D4的IL-33结合结构域。

[0035] 在一个实施方案中，所述基于IL-33受体的拮抗剂包含ST2蛋白的IL-33-结合部分、IL-1RAcP蛋白的细胞外结构域或其它IL-33结合结构域。

[0036] 在一个实施方案中，所述基于IL-33受体的拮抗剂是包含选自SEQ ID NO:323、324、325、326和335的氨基酸序列的IL-33阱。

[0037] 在一个实施方案中，本发明的方法提供了有效量的第二种治疗剂的施用，所述第二种治疗剂可用于减少IL-33介导的疾病或障碍的至少一种症状。

[0038] 在一个实施方案中，所述第二种治疗剂选自非甾体类抗炎剂(NSAID)、皮质类固醇、支气管扩张剂、抗组胺剂、肾上腺素、解充血药、胸腺基质淋巴细胞生成素(TSLP)拮抗剂、IL-13拮抗剂、IL-4拮抗剂、IL-4/IL-13双拮抗剂、IL-5拮抗剂、IL-6拮抗剂、IL-12/23拮抗剂、IL-22拮抗剂、IL-25拮抗剂、IL-17拮抗剂、IL-31拮抗剂、口服PDE4抑制剂和另一种IL-33拮抗剂或不同的基于抗体或受体的IL-33拮抗剂。

[0039] 在一个实施方案中，本发明提供了用选自抗-IL-33抗体或IL-33阱的IL-33拮抗剂对遭受IL-33介导的疾病或障碍的受试者的治疗，其可以与上面指出的第二种治疗剂中的任意一种或多种联合使用，其中通过评估选自降钙素(Calca)、原降钙素、降钙素基因相关

肽(CGRP)、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A 3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirg1)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸(或半胱氨酸)肽(Serpina3f)的至少一种生物标志物的水平可以监测这些药剂的治疗的有效性。

[0040] 在一个实施方案中,所述生物标志物是具有IL-33介导的疾病或障碍的受试者的血清中的降钙素和降钙素水平增加,且血清降钙素的增加与受试者的肺中增加的降钙素和IL-33水平相关。

[0041] 在一个实施方案中,在用抗-IL-33拮抗剂治疗以后,具有IL-33介导的疾病或障碍的受试者的血清中增加的降钙素水平降低至参比范围水平。

[0042] 在一个实施方案中,基于在治疗之前或在治疗时表现出大于约5pg/ml、大于约10pg/ml、大于约50pg/ml或大于约100pg/ml的平均血清降钙素水平,选择遭受IL-33介导的疾病或障碍的受试者用IL-33拮抗剂治疗。

[0043] 在一个实施方案中,基于在治疗之前或在治疗时表现出大于约0.15ng/ml、大于约1.0ng/ml、大于约5ng/ml、大于约10ng/ml、大于约50ng/ml或大于约100ng/ml的平均血清原降钙素水平,选择遭受IL-33介导的疾病或障碍的受试者用IL-33拮抗剂治疗。

[0044] 在一个实施方案中,基于在治疗之前或在治疗时表现出大于约45pg/ml、大于约100pg/ml、大于约250pg/ml或大于约500pg/ml的平均血清降钙素基因相关肽(CGRP)水平,选择遭受IL-33介导的疾病或障碍的受试者用IL-33拮抗剂治疗。

[0045] 在某些实施方案中,本发明的方法提供了IL-33介导的疾病或障碍的治疗,所述IL-33介导的疾病或障碍是选自哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)的炎性疾病或障碍。

[0046] 在一个实施方案中,要治疗的IL-33介导的疾病或障碍是变态反应,且基于超过正常的降钙素水平的增加(例如基于生物标志物的参比水平,如通过健康受试者的确定值与诊断出IL-33介导的疾病或障碍的受试者的对比所确定的)选择为IL-33拮抗剂治疗而选择的受试者,其中所述IL-33拮抗剂治疗包括施用药物组合物,其包含治疗有效量的特异性地结合IL-33的单克隆抗体且包含SEQ ID NO:274/282的HCVR/LCVR氨基酸序列对,且其中所述施用导致至少一种与IL-33疾病或障碍有关的症状的改善或至少一种疾病相关参数的改善;且其中所述降钙素水平恢复至通过与参比标准的对比所确定的正常范围。

[0047] 在一个实施方案中,要治疗的IL-33介导的疾病或障碍是变态反应,且基于血清降钙素水平的增加(如通过与参比标准的对比所确定的)选择为IL-33拮抗剂治疗而选择的受试者,其中所述降钙素水平与所述受试者中IL-33水平的增加相关,或与所述受试者中至少一种疾病参数的增加相关,且其中使用包含抗体的结合测定确定IL-33水平的增加,所述抗体特异性地结合人IL-33且包含SEQ ID NO:274/282的HCVR/LCVR氨基酸序列对。

[0048] 本发明还包括在上面或在本文中讨论的IL-33拮抗剂(例如,IL-33阱)、抗-IL-33抗体和药物组合物用于治疗IL-33介导的疾病或障碍、或减轻IL-33介导的疾病或障碍的症状的用途。本发明还包括在上面或在本文中讨论的IL-33拮抗剂(例如,IL-33阱)、抗-IL-33

抗体和药物组合物在药物制备中的用途，所述药物用于治疗IL-33介导的疾病或障碍、或减轻IL-33介导的疾病或障碍的症状。

[0049] 本发明还包括在上面或在本文中讨论的实施方案的任意组合。本发明的特定实施方案将从接下来的详细描述的综述显而易见。

附图说明

[0050] 图1A和1B：显示了在小鼠IL-33HDD实验中的小鼠血清IL-33(图1A)和小鼠降钙素(CT)水平(图1B)。

[0051] 图2：显示了慢性HDM模型中的小鼠降钙素(Calca)基因表达。

[0052] 图3：显示了在慢性HDM模型中血清和肺降钙素(CT)水平之间的关联。

[0053] 图4：显示了在慢性HDM模型中血清降钙素(CT)和肺IL-33水平的关联。

[0054] 图5A、5B和5C：显示了在慢性HDM模型中受抗-IL-33治疗影响的参数。图5A：肺嗜中性粒细胞的频率；图5B：肺嗜酸性粒细胞的频率；图5C：肺IL-5水平。

[0055] 图6A、6B和6C：显示了在慢性HDM模型中血清降钙素(CT)和受抗-IL-33治疗影响的参数的关联。图6A：血清CT水平和肺嗜中性粒细胞的频率之间的关联；图6B：血清CT水平和肺嗜酸性粒细胞的频率之间的关联；图6C：血清CT和肺IL-5蛋白水平之间的关联。

[0056] 图7A、7B和7C：显示了在慢性HDM模型中肺IL-33水平和受抗-IL-33治疗影响的参数的关联。图7A：肺IL-33水平和肺嗜中性粒细胞的频率之间的关联；图7B：肺IL-33水平和肺嗜酸性粒细胞的频率之间的关联；图7C：肺IL-33和肺IL-5蛋白水平之间的关联。

具体实施方式

[0057] 在描述本发明之前，应当理解，本发明不限于描述的特定方法和实验条件，因为这样的方法和条件可以变化。还应当理解，在本文中使用的术语仅仅用于描述具体实施方案的目的，无意成为限制性的，因为本发明的范围仅由所附权利要求书限定。

[0058] 除非另外定义，在本文中使用的所有技术和科学术语具有与本发明所属领域的普通技术人员通常理解相同的含义。如本文中使用的，当参考特定列举的数值使用时，术语“约”是指，所述值可以从列举的值变化不超过1％。例如，如本文中使用的，表述“约100”包括99和101和在之间的所有值(例如，99.1、99.2、99.3、99.4等)。本文中使用的术语“治疗”、“处理”等是指减轻症状、消除症状的原因(暂时地或永久地)、或预防或减慢提及的障碍或病症的症状的出现。

[0059] 尽管与本文描述的那些类似或等同的任何方法和材料可以用于实践本发明，但是现在描述优选的方法和材料。

[0060] 与IL-33介导的疾病或障碍有关的生物标志物

[0061] 本发明包括涉及与IL-33介导的疾病或障碍有关的生物标志物的使用、定量和分析的方法。

[0062] “IL-33介导的疾病或障碍”可以选自任何炎性疾病或障碍，例如，但不限于，哮喘、变态反应、变应性鼻炎、变应性气道炎症、特应性皮炎(AD)、慢性阻塞性肺疾病(COPD)、炎性肠病(IBD)、多发性硬化、关节炎、银屑病、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性肺炎、嗜酸性粒细胞性银屑病、嗜酸细胞增多综合征、移植物抗宿主病、葡萄膜炎、心血管疾病、疼

痛、多发性硬化、狼疮、血管炎、慢性特发性荨麻疹和伴有多血管炎的嗜酸性粒细胞性肉芽肿病(丘斯综合征)。

[0063] 所述哮喘可以选自变应性哮喘、非变应性哮喘、严重的顽固性哮喘、哮喘恶化、病毒诱导的哮喘或病毒诱导的哮喘恶化、类固醇抵抗型哮喘、类固醇敏感型哮喘、嗜酸性粒细胞性哮喘或非嗜酸性粒细胞性哮喘和以气道炎症或气道高反应性(AHR)为特征的其它有关障碍。

[0064] 所述COPD可以是部分地与香烟烟气、空气污染、职业性化学物质、变态反应或气道高反应性有关或由其造成的疾病或障碍。

[0065] 所述变态反应可以与食品、花粉、霉菌、尘螨、动物或动物毛皮垢屑有关。

[0066] 所述IBD可以选自溃疡性结肠炎、克罗恩氏病、胶原性结肠炎、淋巴细胞性结肠炎、缺血性结肠炎、改道性结肠炎、贝赫切特综合征、感染性结肠炎、不确定性结肠炎和以大肠或结肠的粘膜层炎症为特征的其它障碍。

[0067] 所述关节炎可以选自骨关节炎、类风湿性关节炎和银屑病关节炎。

[0068] 本文中使用的术语“IL-33介导的疾病或障碍相关的生物标志物”、或“与IL-33介导的疾病或障碍有关的生物标志物”是指在遭受IL-33介导的疾病或障碍的患者中存在的或可检测的任何生物应答、细胞类型、参数、蛋白、多肽、酶、酶活性、代谢物、核酸、碳水化合物或其它生物分子,其水平或量不同于(例如,大于或小于)在非-IL-33介导的疾病或障碍患者中存在的或可检测的标志物的水平或量。示例性的IL-33介导的疾病或障碍相关的生物标志物包括、但不限于,例如,由CALCA基因编码的多肽,包括降钙素、原降钙素或降钙素基因相关肽(CGRP)。其它的与IL-33介导的疾病或障碍有关的生物标志物包括、但不限于:抵抗素-样α(RETNA);趋化因子(C-C基序)配体8(Ccl8);血清淀粉样蛋白A 3(Saa3);Gm1975(BC117090);杀伤细胞凝集素-样受体(Kirgl);stefin A1(Csta);跨膜4-结构域(Ms4a8a);趋化因子(C-C基序)配体11(Ccl11);和丝氨酸(或半胱氨酸)肽(Serpina3f)。

[0069] 用于检测和/或定量这样的生物标志物的方法是本领域已知的;用于测量这样的生物标志物的试剂盒可得自多种商业来源;并且多种商业诊断实验室提供服务,它们也提供这样的生物标志物的测量。

[0070] 在一个实施方案中,所述生物标志物是降钙素,且降钙素的水平在遭受或易于发生IL-33介导的疾病或障碍的受试者中增加。在一个实施方案中,所述生物标志物是原降钙素,且原降钙素的水平在遭受或易于发生IL-33介导的疾病或障碍的受试者中增加。在一个有关的实施方案中,所述生物标志物是降钙素基因相关肽(CGRP),且CGRP的水平在遭受或易于发生IL-33介导的疾病或障碍的受试者中增加。使用本领域技术人员已知的任意方法可以评估降钙素、原降钙素或CGRP的水平。检测方法包括使用任何检测标记(诸如放射性同位素标记、酶标记或化学发光标记)的免疫测定。结果的解释必须考虑使用的方法、受试者的年龄、性别和重量(参见d'Herbomez,M.等人,(2007),Eur.J.Endocrinology 157:749-755)。通常,男性中的降钙素的“正常”参考值小于约16pg/mL,且对于女性而言小于约8pg/mL。近年来,Camacho等人开发了一种新的针对血清降钙素的免疫荧光测定,并且已经在来自794位患者的样品中验证了它。使用该测定得到的结果表明,男性的正常截止范围小于11.1pg/mL,且对于女性而言小于5.5pg/mL(参见Camacho,等人,(2014),Eur.Thyroid J.,Jun;3(2):117-24。成年人和大于72小时的儿童中的原降钙素的“参比”范围是约0.15ng/mL

或更低。在健康的成年人中，原降钙素的参比范围低于检测水平（参见Dandona，P.等人(1994)，J.Clin.Endocrinol.Metab.Dec.79(6)：1605-8.）。健康个体中的CGRP的正常范围通常低于约45pg/ml。这样，在此以上的值可以视作指示疾病或障碍的存在。

[0071] "IL-33有关的疾病参数"可以包括、但不限于嗜中性粒细胞、嗜酸性粒细胞或ST2+CD4T细胞向组织（例如肺）的浸润的增加，杯形细胞组织转化，组织中ST2水平的增加，或组织中细胞因子（诸如IL-1β、IL-4、IL-5、IL-6、IL-9、IL-13、IL-33、MCP-1、TNFα）的水平的增加。

[0072] 根据本发明的某些方面，提供了用于治疗IL-33介导的疾病或障碍的方法，其包括：(a)选择受试者，其在治疗之前或在治疗时表现出预示疾病状态的至少一种生物标志物的水平，和(b)给所述受试者施用包含治疗有效量的IL-33拮抗剂的药物组合物。在本发明的该方面的一个实施方案中，基于升高的降钙素水平选择受试者。在一个实施方案中，所述IL-33拮抗剂是分离的特异性地结合IL-33的单克隆抗体或基于受体的IL-33拮抗剂（如本文中所述的IL-33阱）。

[0073] 根据本发明的其它方面，提供了用于治疗IL-33介导的疾病或障碍的方法，其包括给受试者施用包含治疗有效量的IL-33拮抗剂的药物组合物，其中所述药物组合物向所述受试者的施用导致与所述施用之前所述受试者中的生物标志物的水平相比，在所述药物组合物施用以后的时间至少一种生物标志物（例如，降钙素(Calca)、原降钙素、降钙素基因相关肽(CGRP)、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A 3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirg1)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸（或半胱氨酸）肽(Serpina3f)等）的减少。

[0074] 如本领域普通技术人员会明白的，通过对比以下(i)和(ii)，可以确定与IL-33介导的疾病或障碍有关的生物标志物的增加或减少：(i)在施用包含IL-33拮抗剂的药物组合物以后的确定时间点，在受试者中测得的生物标志物的水平，(ii)在施用包含IL-33拮抗剂的药物组合物之前，在患者中测得的生物标志物的水平（即，"基线测量"）。测量生物标志物的确定时间点可以是，例如，在施用包含IL-33拮抗剂的药物组合物以后约4小时、8小时、12小时、1天、2天、3天、4天、5天、6天、7天、8天、9天、10天、14天、15天、20天、35天、40天、50天、55天、60天、65天、70天、75天、80天、84天、85天或更久。

[0075] 根据本发明的某些特定实施方案，在施用包含IL-33拮抗剂（例如，抗-IL-33抗体或基于IL-33受体的拮抗剂（IL-33阱）的药物组合物以后，受试者可以表现出降钙素、原降钙素、CGRP、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirg1)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸（或半胱氨酸）肽(Serpina3f)中的一种或多种的水平的下降。例如，在施用第一、第二、第三或第四剂包含约抗-hIL-33拮抗剂的药物组合物以后约第4天、第8天、第14天、第15天、第22天、第25天、第29天、第36天、第43天、第50天、第57天、第64天、第71天、第84天或第85天。施用给患者的抗-IL-33拮抗剂的剂量可以随患者的年龄和大小、靶疾病、状况、施用途径等而变化。通常根据体重或体表面积计算优选剂量。当将IL-33拮抗剂用于治疗成年患者中与IL-33活性有关的病症或疾病时，可能有利的是静脉内地施用抗-IL-33拮抗剂，通常在约0.01至约20mg/kg体重的剂量，

更优选约0.02至约7、约0.03至约5、或约0.05至约3mg/kg体重。

[0076] 根据本发明的受试者可以表现出下述生物标志物中的至少一种的减少：由CALCA基因编码的多肽，其选自降钙素、原降钙素和CGRP，或可以表现出下述生物标志物中的至少一种的减少：抵抗素-样α(RETNA)；趋化因子(C-C基序)配体8(Ccl8)；血清淀粉样蛋白A 3(Saa3)；Gm1975(BC117090)；杀伤细胞凝集素-样受体(Kirg1)；stefin A1(Csta)；跨膜4-结构域(Ms4a8a)；趋化因子(C-C基序)配体11(Ccl11)；和丝氨酸(或半胱氨酸)肽(Serpina3f)，所述减少是相对于基线的约1%、2%、5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或更多(其中将“基线”定义为在即将第一次施用之前受试者中的生物标志物的水平)。类似地，在施用第一、第二、第三或第四剂抗-hIL-33拮抗剂的药物组合物以后约第4天、第8天、第14天、第15天、第22天、第25天、第29天、第36天、第43天、第50天、第57天、第64天、第71天、第84天或第85天，根据本发明的受试者可以表现出至少一种上述生物标志物相对于基线约1%、2%、5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或更多的减少(其中将“基线”定义为在即将第一次施用之前受试者中的生物标志物的水平)。

[0077] 本发明还包括用于确定受试者是否是包含IL-33拮抗剂的药物组合物的施用将对其有益的合适受试者(例如，可能对使用IL-33拮抗剂的疗法做出有利应答的受试者)的方法。例如，如果个体在接受包含IL-33拮抗剂的药物组合物之前表现出预示疾病状态的与IL-33介导的疾病或障碍有关的生物标志物的水平，因此将所述个体鉴别为本发明的药物组合物(包含抗-IL-33拮抗剂的组合物)的施用将对其有益的合适患者。根据某些示例性实施方案，如果个体表现出以下一种或多种，可以将个体鉴别为抗-IL-33疗法的好候选人：(i)大于约5pg/mL、大于约10pg/mL、大于约20pg/mL、大于约30pg/mL、大于约40pg/mL、大于约50pg/mL、大于约60pg/mL、大于约70pg/mL、大于约80pg/mL、大于约90pg/mL、大于约100pg/mL或大于约200pg/mL的降钙素水平。另外的标准(诸如IL-33介导的疾病或障碍的其它临床指标)可以与本文描述的生物标志物中的任一种联合使用，以将个体鉴别为如本文别处所述的抗-IL-33疗法的合适候选人(例如，嗜中性粒细胞、嗜酸性粒细胞或ST2+CD4T细胞向组织(例如肺)的浸润的增加，杯形细胞组织转化，组织中ST2水平的增加，或组织中细胞因子(诸如IL-1β、IL-4、IL-5、IL-6、IL-9、IL-13、IL-33、MCP-1、TNFα)以及干扰素γ的水平的增加)。

[0078] 在本发明的其它方面，使用治疗的生物标志物的水平和/或生物标志物的水平的变化可以具有使用特定抗-IL33药剂的抗-IL33疗法的效力的预测价值。

[0079] 本发明的另一个方面提供了确定受试者是否可能具有或可能发生IL-33介导的疾病或障碍的方法，其中所述方法包括从所述受试者得到生物样品和测量本文描述的生物标志物中的至少一种的水平；其中与来自不具有IL-33介导的疾病或障碍或不可能发生IL-33介导的疾病或障碍的受试者的生物标志物的水平相比升高的至少一种生物标志物的水平将所述受试者鉴别为可能具有或发生IL-33介导的疾病或障碍的受试者。

[0080] 本发明的另一个方面表征了包括施用抗-IL33拮抗剂的治疗受试者中的IL-33介导的疾病或障碍的方法，其中所述受试者已经被诊断出具有IL-33介导的疾病或障碍，已经用抗-IL33拮抗剂治疗，且已经被选择用IL-33拮抗剂进一步治疗，所述选择基于表现出减

少的生物标志物(例如降钙素)的表达,其中基于与在用抗-IL-33拮抗剂治疗之前所述受试者中的相应生物标志物的表达水平的对比来确定所述减少的生物标志物表达。本发明的一个特定方面表征了包括施用抗-IL33拮抗剂的治疗受试者中的IL-33介导的疾病或障碍的方法,其中所述受试者已经被诊断出具有IL-33介导的疾病或障碍,已经用抗-IL33拮抗剂治疗,且已经被选择用IL-33拮抗剂进一步治疗,所述选择基于表现出减少的生物标志物表达,其中与治疗前表达水平相比减少的生物标志物表达等于或大于生物标志物表达的约10%、20%、30%、40%、50%、60%、70%、80%或90%减少。

[0081] 白介素-33拮抗剂

[0082] 如上面详细公开的,本发明包括方法,其包括给有此需要的受试者施用包含IL-33拮抗剂的治疗组合物。本文中使用的"IL-33拮抗剂"是,当IL-33在体外或体内细胞上表达时,结合IL-33或者与IL-33相互作用、或者结合它的受体或与它的受体相互作用并抑制IL-33的正常生物信号传递功能的任何药剂。IL-33拮抗剂的类型的非限制性例子包括小分子IL-33拮抗剂、抗-IL-33适体、基于肽的IL-33拮抗剂(例如,"肽体(peptibody)"分子)、特异性地结合人IL-33的抗体或抗体的抗原结合片段、或基于受体的IL-33拮抗剂(例如IL-3trap),诸如本文描述的那些。

[0083] 在一个实施方案中,本发明包括方法,其包括给患者施用特异性地结合hIL-33的人抗体或其抗原结合片段。本文中使用的术语"hIL-33"是指特异性地结合它的受体ST2的人细胞因子。人ST2的氨基酸序列显示在SEQ ID NO:334中。人IL-33的氨基酸序列显示在SEQ ID NO:306中。编码人IL-33的核酸显示在SEQ ID NO:305中。抗-IL-33抗体可以选自具有在表1中所述的氨基酸序列的那些抗体中的任意一种或多种。

[0084] 在一个实施方案中,本发明包括方法,其包括给患者施用基于受体的IL-33拮抗剂,例如,IL-33阱,诸如本文描述的那些。使用标准的分子生物学技术,构建了5种不同的示例性的本发明的IL-33阱。表2a阐述了不同的IL-33阱和它们的组成部分的总结性描述。表2b阐述了IL-33阱和它们的组成部分的氨基酸序列。

[0085] 本文中使用的术语"抗体"意图表示包含通过二硫键相互连接的四个多肽链(2个重(H)链和2个轻(L)链)的免疫球蛋白分子及其多聚体(例如,IgM)。每个重链包含重链可变区(在本文中缩写为HCVR或V_H)和重链恒定区。重链恒定区包含三个结构域,C_H1、C_H2和C_H3。每个轻链包含轻链可变区(文中缩写为LCVR或V_L)和轻链恒定区。轻链恒定区包含一个结构域(C_L1)。V_H和V_L区可以进一步细分为被称为互补性决定区(CDR)的高变异性区域,其散布有更保守的被称为框架区(FR)的区域。每个V_H和V_L由3个CDR和4个FR组成,从氨基端至羧基端以下列顺序排列:FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4。在本发明的不同实施方案中,抗-IL-33抗体(或其抗原结合部分)的FR可以与人种系序列相同,或可以是天然地或人工地修饰的。可以基于两个或更多个CDR的并排(side-by-side)分析来定义氨基酸共有序列。

[0086] 本文中使用的术语"抗体"也包括完整抗体分子的抗原结合片段。本文中使用的术语"抗体的抗原结合部分"、"抗体的抗原结合片段"等包括特异性地结合抗原以形成复合物的任何天然存在的、酶促可获得的、合成的或遗传工程改造的多肽或糖蛋白。使用任意合适的标准技术诸如蛋白水解消化或重组基因工程技术(其涉及操作和表达编码抗体可变结构域和任选的恒定结构域的DNA),可以从例如完整抗体分子衍生出抗体的抗原结合片段。这样的DNA是已知的,和/或可以容易地从例如商业来源、DNA文库(包括,例如噬菌体-抗体文

库)获得,或者可以被合成。可以对DNA测序,并化学地或通过使用分子生物学技术进行操作,例如,以将一个或多个可变结构域和/或恒定结构域排列为合适的构型,或导入密码子,产生半胱氨酸残基,修饰、添加或删除氨基酸等。

[0087] 抗原结合片段的非限制性例子包括:(i)Fab片段;(ii)F(ab′)2片段;(iii)Fd片段;(iv)Fv片段;(v)单链Fv(scFv)分子;(vi)dAb片段;和(vii)由模拟抗体高变区的氨基酸残基组成的最小识别单位(例如,分离的互补性决定区(CDR)诸如CDR3肽)或受限制的FR3-CDR3-FR4肽。在本文中使用的表述"抗原结合片段"内还涵盖其它经工程改造的分子,诸如结构域-特异性抗体、单结构域抗体、结构域缺失的抗体、嵌合抗体、CDR-移植抗体、双体、三体、四体、微体、纳米抗体(例如,单价纳米抗体、二价纳米抗体等)、小模块免疫药物(SMIP)和鲨鱼可变IgNAR结构域。

[0088] 抗体的抗原结合片段通常包括至少一个可变结构域。所述可变结构域可以具有任何大小或氨基酸组成,并且通常包含至少一个CDR,所述CDR与一个或多个框架序列相邻或同框。在具有与V_L结构域结合的V_H结构域的抗原结合片段中,V_H和V_L结构域可以相对于彼此以任何适合的排列定位。例如,所述可变区可以是二聚体并含有V_H-V_H、V_H-V_L或V_L-V_L二聚体。可替换地,抗体的抗原结合片段可以含有单体V_H或V_L结构域。

[0089] 在某些实施方案中,抗体的抗原结合片段可以含有与至少一个恒定结构域共价连接的至少一个可变结构域。可在本发明的抗体的抗原结合片段中找到的可变结构域和恒定结构域的非限制性示例性构型包括:(i)V_H-C_H1;(ii)V_H-C_H2;(iii)V_H-C_H3;(iv)V_H-C_H1-C_H2;(v)V_H-C_H1-C_H2-C_H3;(vi)V_H-C_H2-C_H3;(vii)V_H-C_L;(viii)V_L-C_H1;(ix)V_L-C_H2;(x)V_L-C_H3;(xi)V_L-C_H1-C_H2;(xii)V_L-C_H1-C_H2-C_H3;(xiii)V_L-C_H2-C_H3;和(xiv)V_L-C_L。在可变结构域和恒定结构域的任何构型(包括上面列出的任何示例性构型)中,可变结构域和恒定结构域可以彼此直接连接或者可以通过全长或部分铰链或接头区相连。铰链区可以由至少2个(例如5、10、15、20、40、60或更多个)氨基酸组成,其导致在单个多肽分子中的相邻可变结构域和/或恒定结构域之间的柔性的或半柔性的连接。此外,本发明的抗体的抗原结合片段可以包含彼此和/或与一个或多个单体V_H或V_L结构域非共价地结合的上面列出的可变结构域和恒定结构域构型中的任一种的同二聚体或异二聚体(或其它多聚体)(例如,通过二硫键)。

[0090] 与完整抗体分子一样,抗原结合片段可以是单特异性的或多特异性的(例如,双特异性的)。抗体的多特异性抗原结合片段通常包含至少两个不同的可变结构域,其中每个可变结构域能够特异性地结合单独的抗原或相同抗原上的不同表位。任何多特异性抗体形式可以使用本领域可利用的常规技术改造,使其适用于本发明的抗体的抗原结合片段的背景。

[0091] 抗体的恒定区在抗体的固定补体并介导细胞依赖性细胞毒性的能力中是重要的。因此,可以基于是否需要抗体介导细胞毒性来选择抗体的同种型。

[0092] 本文中使用的术语"人抗体"意图包括非天然存在的人抗体。该术语包括在非人哺乳动物中、或在非人哺乳动物的细胞中重组地生产的抗体。该术语无意包括从人受试者分离或在人受试者中产生的抗体。

[0093] 本文中使用的术语"重组人抗体"意图包括通过重组方式制备、表达、建立或分离的所有人抗体,诸如使用转染进宿主细胞中的重组表达载体(在下面进一步描述)表达的抗体,从重组的组合人抗体文库(在下面进一步描述)分离的抗体,从人免疫球蛋白基因转基

因的动物(例如小鼠)分离的抗体(参见例如,Taylor等人(1992)Nucl.Acids Res.20:6287-6295),或通过涉及将人免疫球蛋白基因序列剪接至其它DNA序列的任何其它方式制备、表达、建立或分离的抗体。这样的重组人抗体具有从人种系免疫球蛋白序列衍生出的可变区和恒定区。但是,在某些实施方案中,对这样的重组人抗体进行体外诱变(或,当使用人Ig序列转基因的动物时,体内体细胞诱变),且因而重组抗体的V_H和V_L区域的氨基酸序列是这样的序列:其尽管源自人种系V_H和V_L序列和与人种系V_H和V_L序列有关,但是不可能天然存在于体内人抗体种系谱系内。

[0094] 人抗体可以以与铰链异质性相关的两种形式存在。在一种形式中,免疫球蛋白分子包含大约150-160kDa的稳定四链构建体,其中二聚体通过链间重链二硫键保持一起。在第二种形式中,二聚体不通过链间二硫键连接,并且约75-80kDa的分子由共价地偶联的轻链和重链组成(半抗体)。这些形式极难以分离,即使在亲和纯化以后。

[0095] 所述第二种形式在各种完整IgG同种型中的出现频率是由于、但不限于与抗体的铰链区同种型相关的结构差异。在人IgG4铰链的铰链区中的单个氨基酸置换可以显著地减少第二种形式的出现(Angal等人(1993)Molecular Immunology 30:105)至使用人IgG1铰链通常观察到的水平。本发明涵盖在铰链、C_H2或C_H3区域中具有一个或多个突变的抗体,所述突变可以例如在生产中是合乎需要的,以提高所需抗体形式的收率。

[0096] 本文中使用的"分离的抗体"是指已经被鉴定并从它的天然环境的至少一种组分分离和/或回收的抗体。例如,已经从生物体的至少一种组分、或从抗体天然地存在于其中或在其中天然地产生抗体的组织或细胞分离或移出的抗体,是就本发明的目的而言"分离的抗体"。分离的抗体还包括在重组细胞内的原位抗体。分离的抗体是已经进行至少一个纯化或分离步骤的抗体。根据某些实施方案,分离的抗体可以基本上不含有其它细胞物质和/或化学物质。

[0097] 术语"特异性地结合"等是指,抗体或其抗原结合片段与抗原形成在生理条件下相对稳定的复合物。用于确定抗体是否特异性地结合抗原的方法是本领域众所周知的,且包括,例如,平衡透析、表面等离子体共振等。例如,在本发明的上下文中使用的"特异性地结合"IL-33的抗体包括以小于约1000nM、小于约500nM、小于约300nM、小于约200nM、小于约100nM、小于约90nM、小于约80nM、小于约70nM、小于约60nM、小于约50nM、小于约40nM、小于约30nM、小于约20nM、小于约10nM、小于约5nM、小于约4nM、小于约3nM、小于约2nM、小于约1nM或小于约0.5nM的K_D结合IL-33或其部分的抗体,如在表面等离子体共振测定中所测得的。但是,分离的特异性地结合人IL-33的抗体可以具有与其它抗原(诸如来自其它(非-人)物种的IL-33分子)的交叉反应性。

[0098] 在本发明的方法中有用的抗-IL-33抗体可以包含与衍生出所述抗体的对应种系序列相比在重链和轻链可变结构域的框架区和/或CDR区中的一个或多个氨基酸置换、插入和/或缺失。通过将本文公开的氨基酸序列与可得自例如公共抗体序列数据库的种系序列进行对比,可以容易地确定这样的突变。本发明包括方法,其涉及使用从本文中公开的任何氨基酸序列衍生出的抗体及其抗原结合片段,其中在一个或多个框架区和/或CDR区内的一个或多个氨基酸被突变成衍生出所述抗体的种系序列的对应残基,或被突变成另一个人种系序列的对应残基,或被突变成对应种系残基的保守氨基酸置换(这样的序列变化在本文中统称为"种系突变")。本领域普通技术人员从本文公开的重链和轻链可变区序列出发可

容易地产生众多包含一个或多个单独种系突变或其组合的抗体和抗原结合片段。在某些实施方案中，在V_H和/或V_L结构域内的所有框架和/或CDR残基被回复突变为在衍生出所述抗体的原始种系序列中发现的残基。在其它实施方案中，仅某些残基被回复突变为原始种系序列，例如，仅在FR1的前8个氨基酸内或FR4的后8个氨基酸内存在的突变残基，或仅在CDR1、CDR2或CDR3内存在的突变残基。在其它实施方案中，框架和/或CDR残基中的一个或多个被突变成不同种系序列的对应残基(即，与最初衍生出所述抗体的种系序列不同的种系序列)。此外，本发明的抗体可以在框架区和/或CDR区内含有两个或更多个种系突变的任何组合，例如，其中某些单独残基被突变成特定种系序列的对应残基，而与原始种系序列不同的某些其它残基被维持或被突变成不同种系序列的对应残基。一旦获得，可以针对一种或多种期望的性质容易地试验含有一个或多个种系突变的抗体和抗原结合片段，例如提高的结合特异性、增加的结合亲和力、提高或增强的拮抗或激动生物学特性(视情况而定)、降低的免疫原性等。本发明的应用包括以该一般方式得到的抗体和抗原结合片段。

[0099] 本发明还包括涉及使用抗-IL-33抗体的方法，所述抗-IL-33抗体包含本文中公开的HCVR、LCVR和/或CDR氨基酸序列中的任一种的变体，其具有一个或多个保守置换。例如，本发明包括具有HCVR、LCVR和/或CDR氨基酸序列的抗-IL-33抗体的应用，所述HCVR、LCVR和/或CDR氨基酸序列相对于本文中公开的HCVR、LCVR和/或CDR氨基酸序列中的任一个具有例如10个或更少、8个或更少、6个或更少、4个或更少等的保守氨基酸置换。

[0100] 本文中使用的术语“表面等离子体共振”表示光学现象，其允许通过检测生物传感器基质内的蛋白浓度的改变来分析实时相互作用，例如使用BIAcore™系统(Biacore Life Sciences division of GE Healthcare,Piscataway,NJ)。

[0101] 本文中使用的术语“K_D”意图表示特定抗体-抗原相互作用的平衡解离常数。

[0102] 术语“表位”表示与抗体分子的可变区中被称为互补位的特定抗原结合位点相互作用的抗原决定簇。单个抗原可具有不止一个表位。因而，不同的抗体可以结合抗原上的不同区域并可以具有不同的生物学效应。表位可以是构象的或线性的。构象表位由线性多肽链的不同区段的在空间上邻接的氨基酸产生。线性表位是由多肽链中的相邻氨基酸残基产生的表位。在某些情况下，表位可以包括在抗原上的糖类部分、磷酰基或磺酰基。

[0103] 人抗体的制备

[0104] 用于在转基因小鼠中产生人抗体的方法是本领域已知的。任何这样的已知方法可以用在本发明的上下文中以制备特异性地结合人IL-33的人抗体。

[0105] 使用VELOCIMMUNE™技术(参见，例如，US 6,596,541,Regeneron Pharmaceuticals)或用于产生单克隆抗体的任意其它已知方法，最初分离出具有人可变区和小鼠恒定区的针对IL-33的高亲和力嵌合抗体。VELOCIMMUNE®技术涉及制备转基因小鼠，其具有与内源性小鼠恒定区基因座可操作地连接的包含人重链和轻链可变区的基因组，使得所述小鼠响应于抗原刺激产生包含人可变区和小鼠恒定区的抗体。将编码抗体的重链和轻链的可变区的DNA分离，并可操作地连接至编码人重链和轻链恒定区的DNA。然后将所述DNA在能够表达全人抗体的细胞中表达。

[0106] 通常，用目标抗原攻击VELOCIMMUNE®小鼠，并从表达抗体的小鼠回收淋巴细胞(诸如B-细胞)。可以将所述淋巴细胞与骨髓瘤细胞系融合以制备永生的杂交瘤细胞系，并筛选和选择这样的杂交瘤细胞系以鉴别产生对目标抗原特异性的抗体的杂交瘤细胞

系。可以将编码重链和轻链的可变区的DNA分离并连接至重链和轻链的合乎需要的同种型恒定区。可以在细胞、诸如CHO细胞中生产这样的抗体蛋白。可替换地，可以从抗原特异性的淋巴细胞直接分离编码抗原特异性嵌合抗体或轻链和重链的可变结构域的DNA。

[0107] 最初，分离具有人可变区和小鼠恒定区的高亲和力嵌合抗体。使用本领域技术人员已知的标准操作，针对合乎需要的特征(包括亲和力、选择性、表位等)表征和选择所述抗体。将小鼠恒定区用期望的人恒定区替换以产生本发明的全人抗体，例如野生型或经修饰的IgG1或IgG4。尽管选择的恒定区可以随特定用途变化，高亲和力抗原结合和靶标特异性特征存在于可变区中。

[0108] 一般而言，当通过与固定化在固相上或在溶液相中的抗原的结合测量时，可以用在本发明的方法中的抗体具有高亲和力，如上所述。将小鼠恒定区用期望的人恒定区替换以产生本发明的全人抗体。尽管选择的恒定区可以随特定用途变化，高亲和力抗原结合和靶标特异性特征存在于可变区中。

[0109] 可以用在本发明的方法的上下文中的特异性地结合IL-33的人抗体或抗体的抗原结合片段的具体例子包括包含三个重链CDR(HCDR1、HCDR2和HCDR3)的任何抗体或抗原结合片段，所述重链CDR被包含在重链可变区(HCVR)内且具有选自SEQ ID NO:2、18、34、50、66、82、98、114、130、146、162、178、194、210、226、242、258、274、290和308的氨基酸序列。所述抗体或抗原结合片段可以包含三个轻链CDR(LCVR1、LCVR2、LCVR3)，所述轻链CDR被包含在轻链可变区(LCVR)内且具有选自SEQ ID NO:10、26、42、58、74、90、106、122、138、154、170、186、202、218、234、250、266、282、298和316的氨基酸序列。用于鉴别在HCVR和LCVR氨基酸序列内的CDR的方法和技术是本领域众所周知的，且可以用于鉴别在本文公开的指定HCVR和/或LCVR氨基酸序列内的CDR。可以用于鉴别CDR的边界的示例性惯例包括，例如，Kabat定义、Chothia定义和AbM定义。一般而言，Kabat定义是基于序列变异性，Chothia定义是基于结构环区域的位置，且AbM定义是Kabat和Chothia方案之间的折中。参见，例如，Kabat，“Sequences of Proteins of Immunological Interest,”National Institutes of Health,Bethesda,Md.(1991)；Al-Lazikani等人，J.Mol.Biol.273:927-948(1997)；和Martin等人，Proc.Natl.Acad.Sci.USA 86:9268-9272(1989)。公开的数据库也可用于鉴别抗体内的CDR序列。

[0110] 在本发明的某些实施方案中，所述抗体或其抗原结合片段包含六个CDR(HCDR1、HCDR2、HCDR3、LCDR1、LCDR2和LCDR3)，它们来自选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的重链和轻链可变区氨基酸序列对(HCVR/LCVR)。

[0111] 在本发明的某些实施方案中，所述抗体或其抗原结合片段包含选自SEQ ID NO:2/10、18/26、34/42、50/58、66/74、82/90、98/106、114/122、130/138、146/154、162/170、178/186、194/202、210/218、226/234、242/250、258/266、274/282、290/298和308/316的HCVR/LCVR氨基酸序列对。

[0112] 在附带的实施例中总结的研究利用了被称作H4H9675P的抗-hIL-33抗体。该抗体包含具有SEQ ID NO:274/282的HCVR/LCVR氨基酸序列对和由SEQ ID NO:276-278-280/SEQ ID NO:284-286-288表不的HCDR1-HCDR2-HCDR3/LCDR1-LCDR2-LCDR3结构域。但是，使用本

文中公开的任何抗-IL-33抗体、以及这样的抗体的变体和抗原结合片段，可以实践本发明的方法。

[0113] 药物组合物

[0114] 本发明包括方法，其包括将IL-33拮抗剂施用给患者，其中所述IL-33拮抗剂被包含在药物组合物内。用合适的载体、赋形剂和提供合适转移、递送、耐受性等的其它试剂来配制本发明的药物组合物。可以在所有药物化学家已知的处方中发现大量的许多适当的制剂:Remington's Pharmaceutical Sciences,Mack Publishing Company,Easton,PA。这些制剂包括，例如，粉剂、糊剂、软膏剂、凝胶剂、蜡状物、油、脂质、含脂质(阳离子的或阴离子的)的囊泡(诸如LIPOFECTIN™)、DNA缀合物、无水吸收糊剂、水包油和油包水乳剂、乳剂碳蜡(不同分子量的聚乙二醇)、半固体凝胶和含有碳蜡的半固体混合物。也参见Powell等人."Compendium of excipients for parenteral formulations"PDA(1998)J Pharm Sci Technol 52:238-311。

[0115] 根据本发明的方法施用给患者的抗体的剂量可以随患者的年龄和大小、症状、状况、施用途径等而变化。通常根据体重或体表面积计算所述剂量。取决于病症的严重程度，可以调节治疗的频率和持续时间。可以经验地确定关于施用包含抗-IL-33抗体的药物组合物的有效剂量和计划;例如，通过定期评估可以监测患者进展，并相应地调节剂量。此外，使用本领域众所周知的方法(例如，Mordenti等人，1991，Pharmaceut.Res.8:1351)，可以执行剂量的种间缩放。在本文别处公开了可以用在本发明的上下文中的抗-IL33抗体的具体示例性剂量和包含它们的施用方案。

[0116] 多种递送系统是已知的，并且可以用于施用本发明的药物组合物，例如，包囊在脂质体、微粒、微胶囊中、能够表达突变体病毒的重组细胞、受体介导的胞吞作用(参见，例如，Wu等人，1987，J.Biol.Chem.262:4429-4432)。施用方法包括、但不限于真皮内、肌肉内、腹膜内、静脉内、皮下、鼻内、硬膜外和口服途径。可以通过任何方便的途径施用所述组合物，例如通过输注或快速推注，通过经上皮或皮肤粘膜内衬(例如，口腔粘膜、直肠粘膜和肠粘膜等)的吸收，且可以与其它生物活性剂一起施用。

[0117] 可以用标准针头和注射器皮下地或静脉内地递送本发明的药物组合物。另外，就皮下递送而言，笔式递送装置容易地在递送本发明的药物组合物中具有用途。这样的笔式递送装置可以是可重复使用的或一次用弃的。可重复使用的笔式递送装置通常利用含有药物组合物的可替换筒。一旦所述筒内的所有药物组合物已经被施用且所述筒是空的，可以将空筒容易地抛弃和用含有药物组合物的新筒替换。然后可以重复使用笔式递送装置。在一次用弃的笔式递送装置中，不存在可替换的筒。相反，一次用弃的笔式递送装置是预填充的，其中将药物组合物保持在所述装置内的蓄池中。一旦蓄池被排空药物组合物，就抛弃整个装置。

[0118] 众多可重复使用的笔式和自动注射器递送装置在本发明的药物组合物的皮下递送中具有用途。例子包括、但不限于AUTOPEN™(Owen Mumford,Inc.,Woodstock,UK)、DISETRONIC™笔(Disetronic Medical Systems,Bergdorf,瑞士)、HUMALOG MIX 75/25™笔、HUMALOG™笔、HUMALIN 70/30™笔(Eli Lilly and Co.,Indianapolis,IN)、NOVOPEN™I、II和III(Novo Nordisk,哥本哈根，丹麦)、NOVOPEN JUNIOR™(Novo Nordisk,哥本哈根，丹麦)、BD™笔(Becton Dickinson,Franklin Lakes,NJ)、OPTIPEN™、OPTIPEN PRO™、OPTIPEN

STARLET™和OPTICLIK™(sanofi-aventis,法兰克福,德国),仅举几个例子。在本发明的药物组合物的皮下递送中具有用途的一次用弃的笔式递送装置的例子包括、但不限于SOLOSTAR™笔(sanofi-aventis)、FLEXPEN™(Novo Nordisk)和KWIKPEN™(Eli Lilly)、SURECLICK™自动注射器(Amgen,Thousand Oaks,CA)、PENLET™(Haselmeier,斯图加特,德国)、EPI笔(Dey,L.P.)和HUMIRA™笔(Abbott Labs,Abbott Park IL),仅举几个例子。

[0119] 在某些情形下,可以在控释系统中递送药物组合物。在一个实施方案中,可以使用泵(参见Langer,出处同上;Sefion,1987,CRC Crit.Ref.Biomed.Eng.14:201)。在另一个实施方案中,可以使用聚合材料;参见,MedicalApplications of Controlled Release,Langer和Wise(编),1974,CRC Pres.,Boca Raton,Florida。在另一个实施方案中,可以将控释系统放在所述组合物的靶标附近,因而仅需要全身剂量的一部分(参见,例如,Goodson,1984,Medical Applications of Controlled Release,出处同上,第2卷,第115-138页)。在Langer,1990,Science 249:1527-1533的综述中讨论了其它控释系统。

[0120] 所述可注射制剂可以包括用于静脉内、皮下、皮内和肌肉内注射、点滴输注等的剂型。这些可注射制剂可以通过已知方法制备。例如,可以如下制备可注射制剂:例如,将上述抗体或其盐溶解、悬浮或乳化在常规地用于注射剂的无菌水性介质或油性介质中。作为用于注射剂的水性介质,存在例如生理盐水、包含葡萄糖和其它助剂的等渗溶液等,其可与适当的增溶剂组合使用,所述增溶剂是诸如醇(例如,乙醇)、多元醇(例如,丙二醇、聚乙二醇)、非离子表面活性剂[例如,聚山梨醇80、HCO-50(氢化蓖麻油的聚氧乙烯(50mol)加成化合物)]等。作为油性介质,使用例如芝麻油、大豆油等,其可与增溶剂(诸如苯甲酸苄酯、苯甲醇等)组合使用。如此制备的注射剂可以装填在适当的安瓿中。

[0121] 有利地,将用于上述口服或胃肠外使用的药物组合物制备成适合配合活性成分的剂量的单位剂量的剂型。这样的单位剂量的剂型包括,例如,片剂、丸剂、胶囊剂、注射剂(安瓿)、栓剂等。

[0122] 在例如美国专利申请公开号2014/0271658中公开了可以用在本发明的上下文中的包含抗-IL-33抗体的示例性药物组合物。

[0123] 剂量

[0124] 根据本发明的方法施用给受试者的IL-33拮抗剂(例如,抗-IL-33抗体)的量通常是治疗有效量。本文中使用的短语"治疗有效量"是指导致IL-33介导的疾病或障碍的一种或多种症状或指标的可检测改善的IL-33拮抗剂的量。"治疗有效量"也包括抑制、预防、减轻受试者中这样的疾病或障碍、或者延迟其进展的IL-33拮抗剂的量。

[0125] 在抗-IL-33抗体的情况下,治疗有效量可以是约0.05mg至约600mg,例如,约0.05mg、约0.1mg、约1.0mg、约1.5mg、约2.0mg、约10mg、约20mg、约30mg、约40mg、约50mg、约60mg、约70mg、约80mg、约90mg、约100mg、约110mg、约120mg、约130mg、约140mg、约150mg、约160mg、约170mg、约180mg、约190mg、约200mg、约210mg、约220mg、约230mg、约240mg、约250mg、约260mg、约270mg、约280mg、约290mg、约300mg、约310mg、约320mg、约330mg、约340mg、约350mg、约360mg、约370mg、约380mg、约390mg、约400mg、约410mg、约420mg、约430mg、约440mg、约450mg、约460mg、约470mg、约480mg、约490mg、约500mg、约510mg、约520mg、约530mg、约540mg、约550mg、约560mg、约570mg、约580mg、约590mg或约600mg、ofthe抗-IL-33抗体。在某些实施方案中,将75mg、150mg、200mg或300mg抗-IL-33抗体施用给受试

者。

[0126] 可以以毫克抗体/千克患者体重(即,mg/kg)的方式表达在各个剂量中所含的IL-33拮抗剂的量。例如,可以以约0.0001至约10mg/kg患者体重的剂量将IL-33拮抗剂施用给患者。

[0127] 联合治疗

[0128] 根据某些实施方案,本发明的方法包括给所述受试者施用与IL-33拮抗剂组合的一种或多种另外的治疗剂。本文中使用的表述"与.....组合"是指,在包含IL-33拮抗剂的药物组合物之前、之后或同时施用另外的治疗剂。

[0129] 例如,当在包含IL-33拮抗剂的药物组合物"之前"施用时,可以在施用包含IL-33拮抗剂的药物组合物之前约72小时、约60小时、约48小时、约36小时、约24小时、约12小时、约10小时、约8小时、约6小时、约4小时、约2小时、约1小时、约30分钟、约15分钟或约10分钟施用另外的治疗剂。当在包含IL-33拮抗剂的药物组合物"之后"施用时,可以在施用包含IL-33拮抗剂的药物组合物之后约10分钟、约15分钟、约30分钟、约1小时、约2小时、约4小时、约6小时、约8小时、约10小时、约12小时、约24小时、约36小时、约48小时、约60小时或约72小时施用另外的治疗剂。

[0130] 与包含IL-33拮抗剂的药物组合物"同时"施用是指,在施用包含IL-33拮抗剂的药物组合物的小于5分钟(之前、之后或同时)内在单独剂型中将另外的治疗剂施用给受试者,或作为包含另外的治疗剂和IL-33拮抗剂的单一组合剂量制剂施用给受试者。

[0131] 本发明包括治疗IL-33介导的疾病或障碍的方法,所述方法包括给需要这种治疗的患者施用与至少一种另外的治疗剂组合的抗-hIL-33抗体或IL-33阱。可以在本发明的方法的实践中与抗-hIL-33抗体组合施用的另外的治疗剂的例子包括、但不限于非甾体类抗炎剂(NSAID)、类固醇、皮质类固醇(吸入的或局部的)、免疫抑制剂(例如环磷酰胺)、抗胆碱能剂(例如噻托溴铵)、毒蕈碱样药剂(例如格隆铵(glycopyrronium))、磷酸二酯酶抑制剂(例如茶碱、罗氟司特、西洛司特)、β阻滞剂、环孢菌素、他克莫司、吡美莫司、硫唑嘌呤、甲氨蝶呤、色甘酸钠、蛋白酶抑制剂、支气管扩张剂、β-2-激动剂、抗组胺剂、肾上腺素、解充血药、白三烯抑制剂、肥大细胞抑制剂、胸腺基质淋巴细胞生成素(TSLP)拮抗剂、TNF拮抗剂、IgE拮抗剂、IL-1拮抗剂、IL-4或IL-4R拮抗剂、IL-13或IL-13R拮抗剂、IL-4/IL-13双拮抗剂、IL-5拮抗剂、IL-6或IL-6R拮抗剂、IL-8拮抗剂、IL-9拮抗剂、IL-12/23拮抗剂、IL-22拮抗剂、IL-17拮抗剂、IL-31拮抗剂、口服PDE4抑制剂、IL-25拮抗剂和另一种IL-33拮抗剂或不同的基于抗体或受体的IL-33拮抗剂,和已知治疗、预防或改善人受试者中的IL-33介导的疾病或障碍的任意其它化合物。例如,对于同时施用,可以制备含有抗-hIL-33抗体和至少一种另外的治疗剂的药物制剂。使用本领域中已知的和容易得到的常规方法,可以容易地确定在本发明的方法的实践中与抗-hIL-33抗体组合施用的另外的治疗剂的量。

[0132] 施用方案

[0133] 本发明包括方法,其包括以每周约4次、每周2次、每周1次、每2周1次、每3周1次、每4周1次、每5周1次、每6周1次、每8周1次、每12周1次的给药频率或更低频率将包含IL-33拮抗剂的药物组合物施用给受试者,只要实现治疗应答。在某些涉及施用包含抗-IL-33抗体的药物组合物的实施方案中,以约0.01至约20mg/kg体重、更优选约0.02至约7、约0.03至约5、或约0.05至约3mg/kg体重的量每周1次给药。

[0134] 根据本发明的某些实施方案，可以在确定的时程中将多剂量的IL-33拮抗剂施用给受试者。根据本发明的该方面的方法包括依次给受试者施用多剂量的IL-33拮抗剂。本文中使用的“依次施用”是指，在不同的时间点，例如，在被预定间隔(例如，数小时、数天、数周或数月)隔开的不同天，将每剂IL-33拮抗剂施用给受试者。本发明包括方法，其包括依次给患者施用单个初始剂量的IL-33拮抗剂，继之以一个或多个第二剂量的IL-33拮抗剂，并任选地继之以一个或多个第三剂量的IL-33拮抗剂。

[0135] 术语“初始剂量”、“第二剂量”和“第三剂量”表示IL-33拮抗剂的施用的时间顺序。因而，“初始剂量”是在治疗方案开始时施用的剂量(也被称作“基线剂量”)；“第二剂量”是在初始剂量之后施用的剂量；且“第三剂量”是在第二剂量之后施用的剂量。初始剂量、第二剂量和第三剂量可以都含有相同量的IL-33拮抗剂，但是通常可以在施用频率方面彼此不同。但是，在某些实施方案中，在初始剂量、第二剂量和/或第三剂量中所含的IL-33拮抗剂的量在治疗过程中彼此不同(例如，在适当时向上或向下调节)。在某些实施方案中，两个或更多个(例如，2、3、4或5个)剂量是在治疗方案开始时作为“负荷剂量”，随后是以更低频率施用的后续剂量(例如，“维持剂量”)。

[0136] 在本发明的一个示例性实施方案中，在紧挨着的前一个剂量之后1-14(例如，1、$1^1/_2$、2、$2^1/_2$、3、$3^1/_2$、4、$4^1/_2$、5、$5^1/_2$、6、$6^1/_2$、7、$7^1/_2$、8、$8^1/_2$、9、$9^1/_2$、10、$10^1/_2$、11、$11^1/_2$、12、$12^1/_2$、13、$13^1/_2$、14、$14^1/_2$或更多)周施用每个第二和/或第三剂量。本文中使用的短语“紧挨着的前一个剂量”是指，在多次施用的顺序中，在所述顺序中在接着的下一个剂量施用之前施用给患者的IL-33拮抗剂的剂量，没有插入剂量。

[0137] 根据本发明的该方面的方法可以包括，给患者施用任意数目的第二和/或第三剂量的IL-33拮抗剂。例如，在某些实施方案中，将仅单个第二剂量施用给患者。在其它实施方案中，将两个或更多个(例如，2、3、4、5、6、7、8或更多个)第二剂量施用给患者。同样地，在某些实施方案中，将仅单个第三剂量施用给患者。在其它实施方案中，将两个或更多个(例如，2、3、4、5、6、7、8或更多个)第三剂量施用给患者。

[0138] 在涉及多个第二剂量的实施方案中，每个第二剂量可以以与其它第二剂量相同的频率施用。例如，每个第二剂量可以在紧挨着的前一个剂量之后1-2周施用给患者。类似地，在涉及多个第三剂量的实施方案中，每个第三剂量可以以与其它第三剂量相同的频率施用。例如，每个第三剂量可以在紧挨着的前一个剂量之后2-4周施用给患者。可替换地，将第二和/或第三剂量施用给患者的频率可以在治疗方案过程中变化。医师也可以在治疗过程中调节施用频率，取决于在临床检查以后各个患者的需要。

[0139] 本发明包括包含向上滴定选项(在本文中也被称作“剂量改变”)的施用方案。本文中使用的“向上滴定选项”是指，在接受特定数目的IL-33抑制剂剂量以后，如果患者尚未实现一个或多个确定的治疗参数的特定改善(例如，至少20％改善)，或以其它方式表现出在医师或保健提供者的判断下效力的明确缺乏，那么此后增加IL-33抑制剂的剂量。例如，就包含以每2周1次的频率给患者施用150mg剂量的抗-IL-33抗体的治疗方案而言，如果在8、10、12、14、16或更多周以后，所述患者尚未实现一个参数的至少20％改善，或如果所述患者表现出在医师或保健提供者的判断下效力的明确缺乏，那么将抗-IL-33抗体的剂量增加至例如此后(例如，在第10、12、14、16、18周或以后开始)每2周1次施用的200mg、300mg或更多。

[0140] 治疗群体

[0141] 本发明包括方法，其包括给有此需要的受试者施用包含IL-33拮抗剂的治疗组合物。本文中使用的表述“有此需要的受试者”是指这样的人或非人动物，其表现出IL-33介导的疾病或障碍的一种或多种症状或指标，诸如本文描述的那些(例如，炎症、肺中的嗜酸性粒细胞增多症、向肺中的嗜中性粒细胞浸润、肺中升高的某些细胞因子水平，例如，但不限于IL-5等)，和/或其已经被诊断出具有本文描述的IL-33介导的疾病或障碍中的任一种。

[0142] 实施例

[0143] 提出下述实施例从而给本领域普通技术人员提供如何制备和使用本发明的方法和组合物的完整公开和描述，且无意将发明人考虑的内容的范围限制为他们的发明。已经做出努力来确保关于使用的数字(例如，量、温度等)的准确度，但是应当考虑一些实验误差和偏差。除非另外指出，否则份数是重量份，分子量是平均分子量，温度是以摄氏度为单位，且压强是在或接近大气压。

[0144] 实施例1.针对人IL-33的人抗体的制备

[0145] 将包含人IL-33的免疫原与佐剂(以刺激免疫应答)一起直接施用给包含编码人免疫球蛋白重链和κ轻链可变区的DNA的VELOCIMMUNE®小鼠。通过IL-33-特异性的免疫测定监测抗体免疫应答。当实现期望的免疫应答时，将脾细胞收获并与小鼠骨髓瘤细胞融合以维持它们的生存力和形成杂交瘤细胞系。将杂交瘤细胞系筛选和选择以鉴别产生IL-33-特异性抗体的细胞系。使用该技术，得到几种抗-IL-33嵌合抗体(即，具有人可变结构域和小鼠恒定结构域的抗体)；如下命名以此方式产生的示例性抗体：H1M9559N、H1M9566N、H1M9568N和H1M9565N。随后将来自嵌合抗体的人可变结构域克隆到人恒定结构域上以制备如本文中所述的全人抗-IL-33抗体。

[0146] 如在US 2007/0280945A1中所述，还从没有与骨髓瘤细胞融合的抗原阳性的B细胞直接分离抗-IL-33抗体。使用该方法，得到几种全人抗-IL-33抗体(即，具有人可变结构域和人恒定结构域的抗体)；如下命名以此方式产生的示例性抗体：H4H9629P、H4H9633P、H4H6940P、H4H9659P、H4H9660P、H4H9662P、H4H9663P、H4H9664P、H4H9665P、H4H9666P、H4H9667P、H4H9670P、H4H9671P、H4H9672P、H4H9675P和H4H9676P。

[0147] 在下面阐述的实施例中详细描述了根据本实施例的方法制备的示例性抗-IL-33抗体的某些生物学特性。

[0148] 实施例2.重链和轻链可变区氨基酸序列

[0149] 表1阐述了选择的抗-IL-33抗体的重链和轻链可变区氨基酸序列对和CDR序列、以及它们的对应抗体标识符。

[0150] 表1

[0151]

	SEQ ID NO:							
抗体命名	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1M9559N	2	4	6	8	10	12	14	16
H1M9566N	18	20	22	24	26	28	30	32
H1M9568N	34	36	38	40	42	44	46	48
H4H9629P	50	52	54	56	58	60	62	64
H4H9633P	66	68	70	72	74	76	78	80

[0152]

H4H9640P	82	84	86	88	90	92	94	96
H4H9659P	98	100	102	104	106	108	110	112
H4H9660P	114	116	118	120	122	124	126	128
H4H9662P	130	132	134	136	138	140	142	144
H4H9663P	146	148	150	152	154	156	158	160
H4H9664P	162	164	166	168	170	172	174	176
H4H9665P	178	180	182	184	186	188	190	192
H4H9666P	194	196	198	200	202	204	206	208
H4H9667P	210	212	214	216	218	220	222	224
H4H9670P	226	228	230	232	234	236	238	240
H4H9671P	242	244	246	248	250	252	254	256
H4H9672P	258	260	262	264	266	268	270	272
H4H9675P	274	276	278	280	282	284	286	288
H4H9676P	290	292	294	296	298	300	302	304
H1M9565N	308	310	312	314	316	318	320	322

[0153] 在本文中通常根据下述命名法提及抗体:Fc前缀(例如"H1M"或"H4H"),继之以数

字标识符(例如“9559”、“9566”或“9629”,如在表1中所示),继之以“P”或“N”后缀。因而,根据该命名法,抗体在本文中可以被称作,例如,“H1M9559N”、“H1M9566N”、“H4H9629P”等。在本文使用的抗体命名上的H1M和H4H前缀指示所述抗体的特定Fc区同种型。例如,“H1M”抗体具有小鼠IgG1 Fc,而“H4H”抗体具有人IgG4Fc。如本领域普通技术人员会明白的,具有特定Fc同种型的抗体可以转化成具有不同Fc同种型的抗体(例如,具有小鼠IgG1 Fc的抗体可以转化成具有人IgG4的抗体,等),但是在任何情况下,可变结构域(包括CDR)-其用表1中所示的数字标识符指示-将保持相同,且预期结合性质是相同的或基本上类似的,不论Fc结构域的性质。

[0154] 实施例3.IL-33阱的构建

[0155] 使用标准的分子生物学技术构建本发明的5种不同的示例性IL-33拮抗剂(IL-33阱)。第一种IL-33拮抗剂(hST2-hFc,SEQ ID NO:323)由在它的C-端与人IgG1 Fc区(SEQ ID NO:331)的N-端融合的人ST2(SEQ ID NO:327)的可溶性细胞外区域组成。第二种IL-33拮抗剂(hST2-mFc,SEQ ID NO:324)由在它的C-端与小鼠IgG2a Fc区(SEQ ID NO:332)的N-端融合的人ST2(SEQ ID NO:327)的可溶性细胞外区域组成。第三种IL-33拮抗剂(hST2-hIL1RAcP-mFc,SEQ ID NO:325)由线内融合体组成,所述线内融合体具有在它的N-端处的人ST2(SEQ ID NO:327),继之以人IL-1RAcP(SEQ ID NO:329)的细胞外区域,继之以在它的C-端处的小鼠IgG2a Fc(SEQ ID NO:332)。第四种IL-33拮抗剂(mST2-mIL1RAcP-mFc,SEQ ID NO:326)由线内融合体组成,所述线内融合体具有在它的N-端处的小鼠ST2(SEQ ID NO:328),继之以小鼠IL-1RAcP(SEQ ID NO:330)的细胞外区域,继之以在它的C-端处的小鼠IgG2a Fc(SEQ ID NO:332)。第五种IL-33拮抗剂(hST2-hIL1RAcP-hFc,SEQ ID NO:335)由线内融合体组成,所述线内融合体具有在它的N-端处的SEQ ID NO:327的人ST2,继之以人IL-1RAcP(SEQ ID NO:329)的细胞外区域,继之以在它的C端处的人IgG1 Fc(SEQ ID NO:331)。表2a阐述了不同IL-33拮抗剂和它们的组成部分的总结性描述。表2b阐述了IL-33拮抗剂和它们的组成部分的氨基酸序列。

[0156] 表2a:IL-33拮抗剂(IL-33阱)的总结

[0157]

IL-33 拮抗剂	完整拮抗剂分子的氨基酸序列	D1 组分	D2 组分	M 组分
hST2-hFc	SEQ ID NO:323	人 ST2 细胞外(SEQ ID NO:327)	不存在	人 IgG1 Fc (SEQ ID NO:331)
hST2-mFc	SEQ ID NO:324	人 ST2 细胞外(SEQ ID NO:327)	不存在	小鼠 IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-mFc	SEQ ID NO:325	人 ST2 细胞外(SEQ ID NO:327)	人 IL-1RAcP 细胞外(SEQ ID NO:329)	小鼠 IgG2a Fc (SEQ ID NO:332)
mST2-mIL1RAcP-mFc	SEQ ID NO:326	小鼠 ST2 细胞外(SEQ ID NO:328)	小鼠 IL-1RAcP 细胞外(SEQ ID NO:330)	小鼠 IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-hFc	SEQ ID NO: 335	人 ST2 细胞外(SEQ ID NO:327)	人 IL-1RAcP 细胞外(SEQ ID NO:329)	人 IgG1 Fc (SEQ ID NO:331)

[0158] 表2b:氨基酸序列

[0159]

标识符	序列
SEQ ID NO:323 (hST2-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT QERNRVFASGQLLKFLPAAVADSGIYTCIVRSPTFNRTGYAN VTIYKKQSDCNVPDYLMYSTVSGSEKNSKIYCPTIDLYNWT

[0160]

标识符	序列
	APLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYTCK FIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKE VEIGKNANLTCSACFGKGTQFLAAVLWQLNGTKITDFGEPR IQQEEGQNQSFSNGLACLDMVLRIADVKEEDLLLQYDCLAL NLHGLRRHTVRLSRKNPIDHHSDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
SEQ ID NO:324 (hST2-mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT QERNRVFASGQLLKFLPAAVADSGIYTCIVRSPTFNRTGYAN VTIYKKQSDCNVPDYLMYSTVSGSEKNSKIYCPTIDLYNWT APLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYTCK FIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKE VEIGKNANLTCSACFGKGTQFLAAVLWQLNGTKITDFGEPR IQQEEGQNQSFSNGLACLDMVLRIADVKEEDLLLQYDCLAL NLHGLRRHTVRLSRKNPIDHHSEPRGPTIKPCPPCKCPAPNL LGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGK EFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMT KKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLD SDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTK SFSRTPGK

[0161]

标识符	序列
SEQ ID NO:325 (hST2-hIL1RAcP-mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT QERNRVFASGQLLKFLPAAVADSGIYTCIVRSPTFNRTGYAN VTIYKKQSDCNVPDYLMYSTVSGSEKNSKIYCPTIDLYNWT APLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYTCK FIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKE VEIGKNANLTCSACFGKGTQFLAAVLWQLNGTKITDFGEPR IQQEEGQNQSFSNGLACLDMVLRIADVKEEDLLLQYDCLAL NLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQIQVFE DEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLE EPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYC SKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVD GYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFLIALISN NGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHS PNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVC HARSAKGEVAKAAKVKQKVPAPRYTVESGEPRGPTIKPCPP CKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSED DPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVL PPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNY KNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEG LHNHHTTKSFSRTPGK
SEQ ID NO:326 (mST2-mIL1RAcP-mFc)	SKSSWGLENEALIVRCPQRGRSTYPVEWYYSDTNESIPTQKR NRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIH KKPPSCNIPDYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQ WFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQFTHA ENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGK PASIACSACFGKGSHFLADVLWQINKTVVGNFGEARIQEEE GRNESSSNDMDCLTSVLRITGVTEKDLSLEYDCLALNLHGM IRHTIRLRRKQPIDHRSERCDDWGLDTMRQIQVFEDEPARIK

[0162]

标识符	序列
	CPLFEHFLKYNYSTAHSSGLTLIWYWTRQDRDLEEPINFRLP ENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVVQKDSCFNSAMRFPVHKMYIEHGIHKITCPNVDGYFPSS VKPSVTWYKGCTEIVDFHNVLPEGMNLSFFIPLVSNNGNYT CVVTYPENGRLFHLTRTVTVKVVGSPKDALPPQIYSPNDRV VYEKEPGEELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDVT VDITINESVSYSSTEDETRTQILSIKKVTPEDLRRNYVCHARN TKGEAEQAAKVKQKVIPPRYTVESGEPRGPTIKPCPPCKCPA PNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQ ISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWM SGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEE EMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEP VLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGK
SEQ ID NO:327 (人 ST2 细胞外结构域)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT QERNRVFASGQLLKFLPAAVADSGIYTCIVRSPTFNRTGYAN VTIYKKQSDCNVPDYLMYSTVSGSEKNSKIYCPTIDLYNWT APLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYTCK FIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKE VEIGKNANLTCSACFGKGTQFLAAVLWQLNGTKITDFGEPR IQQEEGQNQSFSNGLACLDMVLRIADVKEEDLLLQYDCLAL NLHGLRRHTVRLSRKNPIDHHS
SEQ ID NO:328 (小鼠 ST2 细胞外结构域)	SKSSWGLENEALIVRCPQRGRSTYPVEWYYSDTNESIPTQKR NRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIH KKPPSCNIPDYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQ WFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQFTHA ENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGK PASIACSACFGKGSHFLADVLWQINKTVVGNFGEARIQEEE GRNESSSNDMDCLTSVLRITGVTEKDLSLEYDCLALNLHGM IRHTIRLRRKQPIDHR

[0163]

标识符	序列
SEQ ID NO:329 (人 IL1RAcP 细胞外结构域)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTA HSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRP TLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMK LPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKIQ NFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTR TLTVKVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVY FSFLMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDETR TQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPA PRYTVE
SEQ ID NO:330 (小鼠 IL1RAcP 细胞外结构域)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKYNYSTA HSSGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRP TLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSAMR FPVHKMYIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTEIV DFHNVLPEGMNLSFFIPLVSNNGNYTCVVTYPENGRLFHLT RTVTVKVVGSPKDALPPQIYSPNDRVVYEKEPGEELVIPCKV YFSFIMDSHNEVWWTIDGKKPDDVTVDITINESVSYSSTEDE TRTQILSIKKVTPEDLRRNYVCHARNTKGEAEQAAKVKQKV IPPRYTVE
SEQ ID NO:331 (人 IgG1 Fc)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:332 (小鼠 IgG2a Fc)	EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIV TCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNST LRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPK GSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVE WTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE

[0164]

标识符	序列
	RNSYSCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO:333 (成束猴（*M. fascicularis*）IL-33-6His)	SITGISPITESLASLSTYNDQSITFALEDESYEIYVEDLKKDKK KDKVLLSYYESQHPSSESGDGVDGKMLMVTLSPTKDFWLQ ANNKEHSVELHKCEKPLPDQAFFVLHNRSFNCVSFECKTDP GVFIGVKDNHLALIKVDYSENLGSENILFKLSEILEHHHHHH
SEQ ID NO:335 (hST2-hIL1RAcP-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT QERNRVFASGQLLKFLPAAVADSGIYTCIVRSPTFNRTGYAN VTIYKKQSDCNVPDYLMYSTVSGSEKNSKIYCPTIDLYNWT APLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYTCK FIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKE VEIGKNANLTCSACFGKGTQFLAAVLWQLNGTKITDFGEPR IQQEEGQNQSFSNGLACLDMVLRIADVKEEDLLLQYDCLAL NLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQIQVFE DEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLE EPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYC SKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVD GYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFLIALISN NGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHS PNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVC HARSAKGEVAKAAKVKQKVPAPRYTVEDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK

[0165] 实施例4：IL-33活性的生物标志物

[0166] 进行研究来确定哪些基因在过表达IL-33的小鼠中升高。下述的研究总结了用于确定哪些基因与体内IL-33表达相关和哪些基因在用IL-33拮抗剂治疗动物以后受到调节

的方法和动物模型。

[0167] 流体动力学DNA递送

[0168] 通过流体动力学DNA递送(HDD)在野生型(WT)小鼠中过表达小鼠IL-33。对于HDD实验，给WT小鼠注射50μg或25ug表达全长小鼠IL-33的质粒(参见GenBank登录号NM_001164724;mIL-33)或50μg或25ug不含编码序列的相同质粒(空载体)。在HDD注射以后7天处死小鼠，并收集血液、背根神经节(DRG)、心脏、肾、肝、肺和脾。在这些组织中表达的前10个基因显示在下面表4中。

[0169] 收集的组织的微阵列分析

[0170] 使用QuickAmp RNA Amplification Kit(Agilent)将Cy3-CTP掺入来自500ng总RNA的扩增的cRNA中。然后将来自每个样品的Cy3标记的cRNA与包含43538个覆盖小鼠转录组的60聚体寡物的定制Agilent阵列杂交。根据Agilent方案执行阵列的杂交和洗涤，并在Agilent Microarray扫描仪上扫描阵列。使用Agilent Feature Extraction Software 9.5从扫描的阵列图像提取数据。

[0171] 血清收集

[0172] 在研究结束时通过心脏穿刺将全血收集进Microtainer®试管中。通过将血液在室温静置至少30分钟，允许血液凝结。通过在冷却离心机中在18,000x g在4℃离心10分钟，使凝结的血液和细胞沉淀。将得到的上清液(命名为血清)转移进干净的聚丙烯平板中并适当地处理或储存。

[0173] 血清或细胞裂解物提取物中的IL-33蛋白浓度的确定

[0174] 根据生产商的说明书，使用来自R&D系统的ELISA试剂盒确定人(DY3625)和小鼠(DY3626)IL-33的IL-33浓度。

[0175] 血清或细胞裂解物提取物中的小鼠降钙素(CT)浓度的确定

[0176] 使用来自CusaBio(Through ARP)(目录号CSB-E05133m)的小鼠降钙素(CT)ELISA试剂盒，在小鼠血清中测量小鼠降钙素。根据与试剂盒一起提供的生产商的说明书进行测定操作。

[0177] 细胞裂解物提取物中的小鼠IL-5浓度的确定

[0178] 根据生产商的说明书，使用V-Plex定制小鼠多路免疫测定试剂盒(MesoScale Discovery,#K152A41)测量肺蛋白提取物中的细胞因子浓度。

[0179] 屋尘螨诱导的慢性肺部炎症模型

[0180] 给IL-33HumIn小鼠鼻内地施用在20μL 1X磷酸盐缓冲盐水(PBS)中稀释的50μg屋尘螨提取物(HDM;Greer,#XPB70D3A2.5)或20μL 1X PBS，每周3天共15周。给IL33HumIn小鼠的第二个对照组施用在20μL 1X PBS中稀释的50μgHDM提取物，每周3天共4或11周，以评估在抗体治疗开始时疾病的严重程度。在HDM攻击的4或11周以后开始给两组HDM攻击的小鼠皮下地注射25mg/kg的抗-IL-33抗体、H4H9675P或同种型对照抗体，然后每周2次直到HDM攻击结束(8或4周的抗体治疗)。在研究的第85天，将所有小鼠处死，并收获它们的肺。小鼠组的实验性给药和治疗方案显示在表3中。

[0181] 给IL-33HumIn小鼠鼻内地施用在20μL 1X磷酸盐缓冲盐水(PBS)中稀释的50μg屋尘螨提取物(HDM;Greer,#XPB70D3A2.5)或20μL 1X PBS，每周3天共15周。给IL33HumIn小鼠的第二个对照组施用在20μL 1X PBS中稀释的50μgHDM提取物，每周3天共11周，以评估在抗

体治疗开始时疾病的严重程度。在HDM攻击的11周以后开始给两组HDM攻击的小鼠皮下地注射25mg/kg的抗-IL-33抗体、H4H9675P或同种型对照抗体，并然后每周2次直到HDM攻击结束(8周的抗体治疗)。在研究的第85天，将所有小鼠处死，并收获它们的肺。小鼠组的实验性给药和治疗方案显示在表3中。

[0182] 表3:小鼠组的实验性给药和治疗方案

[0183]

组	小鼠	鼻内攻击	鼻内攻击的持续时间	抗体
1	IL-33 HumIn 小鼠	1X PBS	15 周	无
2	IL-33 HumIn 小鼠	50 μg HDM 在 20 μL 1X PBS 中	4 或 11 周	无
3	IL-33 HumIn 小鼠	50 μg HDM 在 20 μL 1X PBS 中	15 周	无
4	IL-33 HumIn 小鼠	50 μg HDM 在 20 μL 1X PBS 中	15 周	同种型对照抗体
5	IL-33 HumIn 小鼠	50 μg HDM 在 20 μL 1X PBS 中	15 周	抗-IL-33 抗体 (H4H9675P)

[0184]

[0185] 组织收获用于基因表达分析

[0186] 在放血以后，将肺取出，放入含有400μL RNA Later(Ambion，目录号AM720)的试管中，并在处理之前在-20℃储存。将组织在TRIzol中匀浆化，并将氯仿用于相分离。根据生产商的说明书，使用用于Microarrays Total RNA Isolation Kit的MagMAX™-96纯化含有总RNA的水相。使用来自上面列出的MagMAX试剂盒的MagMAX™Turbo™DNase Buffer和TURBO DNase，除去基因组DNA。使用SuperScript®VILO™Master Mix将mRNA(多达2.5μg)反转录成cDNA。将cDNA稀释至2ng/μL，并使用ABI 7900HT Sequence Detection System(Applied Biosystems)用TaqMan®Gene Expression Master Mix和有关的探针(小鼠B2m，小鼠Calca，人IL33)扩增10ng cDNA。使用B2m探针扩增β-2microblobulin(B2m)基因作为内部对照，以便将cDNA输入差异归一化。使用Microsoft Excel和GraphPad Prism™软件执行数据分析。将每个基因的表达归一化至相同样品内的B2m表达。

[0187] 肺收获用于细胞因子分析

[0188] 在放血以后，从每只小鼠取出右肺的前叶和中叶(cranial and middle lobes)，并放入含有补充了1X Halt蛋白酶抑制剂混合液(Pierce，#78430)的组织蛋白提取试剂(1X T-PER试剂；Pierce，#78510)的溶液的试管中。在冰上执行所有其它步骤。为每个样品调节T-PER试剂(含有蛋白酶抑制剂混合液)的体积以匹配1:8(w/v)的组织:T-PER比率。使用Tissue Lyser II(Qiagen，目录号85300)，将肺样品在试管中匀浆化。将得到的裂解物离心以沉淀碎片。将含有可溶性蛋白提取物的上清液转移至新鲜试管并在进一步分析之前在4℃储存。

[0189] 使用Bradford测定来测量肺蛋白提取物中的总蛋白质含量。对于测定，将10μL稀释的提取物样品一式两份地放入96孔板中，并与200μL 1X Dye Reagent(Biorad，#500-0006)混合。将牛血清白蛋白(BSA；Sigma，#A7979)在1X T-Per试剂中的系列稀释物(在700μg/mL开始)用作标准以确定提取物的蛋白浓度。在室温温育5-分钟以后，在Molecular Devices SpectraMax M5平板读数器上测量在595nm的吸光度。使用GraphPad Prism™软件执行数据分析以基于BSA标准确定总肺提取物蛋白质含量。

[0190] 将来自每组的所有小鼠的肺总蛋白提取物中的每种细胞因子浓度归一化至通过Bradford测定测得的提取物的总蛋白质含量，并为每组表达为平均pg细胞因子/mg总肺蛋白(pg/mg肺蛋白，±SD)。

[0191] 表4:前列IL-33紊乱基因

[0192]

DrgIL33	心脏 IL33	肾 IL33	肝 IL33	肺 IL33	脾 IL33	基因符号	描述
10.2	415.9	33.5	72.7	5.0	86.3	Calca	降钙素/降钙素基因有关肽(CGRP)
36.2	114.8	51.7	572.8	24.9	13.8	Retnla	抵抗素样α
6.5	194.0	19.5	152.2	4.5	19.4	Ccl8	趋化因子(C-C基序)配体8
6.5	17.8	251.0	14.9	8.0	45.9	Saa3	血清淀粉样蛋白A3

[0193]

38.3	28.2	8.3	66.6	53.2	29.3	BC117090	Gm1975
4.9	32.8	61.2	40.4	15.3	3.6	Klrg1	杀伤细胞凝集素-样受体
15.4	11.7	6.8	86.7	27.1	13.8	Csta	Stefin A1
5.7	46.1	18.2	38.4	1.8	4.5	Ms4a8a	跨膜4-结构域
1.7	19.0	23.3	81.6	9.4	6.6	Ccl11	趋化因子(C-C基序)配体11
3.7	17.9	49.0	18.0	25.8	10.5	Serpina3f	丝氨酸(或半胱氨酸)肽

[0194] 肺收获用于肺细胞浸润分析

[0195] 在放血以后，从每只小鼠取出右肺的尾叶，放入含有在汉克平衡盐溶液(HBSS)中稀释的20μg/mL DNA酶和0.7U/mL Liberase TH的溶液的试管中，并切成大约2-3mm大小的碎块。然后将含有切碎的肺的试管在37℃水浴中温育20分钟。通过以10mM的终浓度加入乙二胺四乙酸(EDTA)，停止反应。然后将样品转移至gentleMACS C Tubes。用MACS缓冲液使C Tubes中的体积达到3mL，并随后使用gentleMACS dissociator®(Miltenyi Biotec)解离样品以形成单细胞悬浮液。然后将试管离心并将得到的沉淀再悬浮于4mL 1X红血细胞裂解缓冲液中以裂解红血细胞。在室温温育3分钟以后，将10mL 1X DPBS加入以灭活红血细胞裂解缓冲液。然后将细胞悬浮液离心，并将得到的细胞沉淀物再悬浮于1mL 1X DPBS中。将再悬浮的样品穿过100μm过滤板离心过滤(在400x g离心1分钟)，并将大约$1.5x10^6$个细胞/孔铺板在96-孔U-底板中。然后将细胞离心，并将细胞沉淀物再悬浮于100μL在1X DPBS中1:500稀释的Near-IR LIVE/DEAD®Fixable Dead Cell Stain(Invitrogen目录号:L34976，批号:1647137)中以确定细胞生存力。将细胞与生存力染料一起在室温温育20分钟，同时避光。在1X DPBS中洗涤1次以后，将细胞在50μμL含有10μμg/mL纯化的大鼠抗-小鼠CD16/CD32 Fc Block的MACS缓冲液中在4℃温育10分钟。然后将细胞于在亮蓝缓冲液(在表5中描述)中稀释的适当2x抗体混合物中在4℃温育30分钟，同时避光。抗体温育以后，将细胞在MACS缓冲液中洗涤2次，再悬浮于已经在1X DPBS中1:4稀释的BDCytoFix中，并然后在4℃温育15分钟，同时避光。随后将细胞洗涤并再悬浮于MACS缓冲液中。然后将细胞悬浮液穿过AcroPrep Advance 96Filter Plate 30-40μm过滤进新U-底板中。使用HTS连接(BD Biosciences)在LSR Fortessa X-20细胞分析仪上获取样品数据。使用FlowJo X Software(Tree Star,OR)执行数据分析，并使用GraphPad Prism™(GraphPad Software,CA)执行统计分析。将嗜酸性粒细胞定义为活的(细胞生存染料阴性的)，单峰，$CD45^+$，$F4/80^+$，$Ly6G^-$，$CD11c^{lo-Int}$，$SiglecF^{hi}$。将嗜中性粒细胞定义为活的，单峰，$CD45^+$，$F4/80^-$，$Ly6G^+$。将嗜酸性粒细胞和嗜中性粒细胞的数据表达为活细胞的频率。

[0196] 表5:用于流式细胞计量术分析的抗体

[0197]

抗体	荧光染料	生产商	目录号，批号	最终的稀释度
CD45	Alexa Fluor 700	BioLegend	103128, B191240	1/200
Siglec-F	BV 421	BD	562681, 4234913	1/200
F4/80	PE	BD	56410, 5054900	1/500
Ly6G	BUV395	BD	563978, 4178621	1/200
CD11c	APC	BD	550261, 5016523	1/200

[0198] 统计分析

[0199] 使用GraphPad Prism™6.0版(GraphPad Software,CA)执行统计分析。

[0200] 使用Kolmogorov-Smimov检验评价数据的正态性。如果数据通过正态性检验并且不同组的标准差没有统计上不同于彼此(如通过Brown-Forsythe检验评估的),则通过单向方差分析(ANOVA)、继之以Tukey事后检验来解释结果进行多重比较。如果数据没有通过正态性检验或标准差是显著不同的,则使用Kruskal-Wallis检验、继之以Dunn氏事后检验来解释结果进行多重比较。当P值<0.05时,认为差异是统计上显著的。

[0201] 使用双尾Spearman非参数相关计算相关系数和显著性。当P值<0.05时,认为相关是统计上显著的。

[0202] 结果的总结:

[0203] 结果证实,在过表达IL-33的动物中紊乱的前10个基因是降钙素(Calca)、抵抗素-样α(RETNA)、趋化因子(C-C基序)配体8(Ccl8)、血清淀粉样蛋白A 3(Saa3)、Gm1975(BC117090)、杀伤细胞凝集素-样受体(Kirgl)、stefin A1(Csta)、跨膜4-结构域(Ms4a8a)、趋化因子(C-C基序)配体11(Ccl11)和丝氨酸(或半胱氨酸)肽(Serpina3f)(参见表4)。此外,数据也表明,血清IL-33和血清降钙素在该小鼠模型中显著升高(关于血清IL-33水平,参见图1A;关于血清降钙素水平,参见图1B)。血清IL-33的增加与降钙素的血清水平的增加相关。

[0204] 另外,慢性屋尘螨(HDM)攻击模型(其用作暴露于变应原以后严重进行性肺部炎症的模型)表明,降钙素基因表达在慢性HDM攻击后在小鼠的肺中上调,但是降钙素表达在已经用IL-33拮抗剂(H4H9675P抗-IL-33抗体)治疗过的小鼠的肺中显著地减少。使用同种型匹配的阴性对照抗体的治疗对降钙素基因表达的水平没有影响(图2)。

[0205] 另外,血清降钙素也在该HDM小鼠模型中升高,并且血清降钙素的水平与在这些小鼠的肺中看到的降钙素水平相关(参见图3)。此外,血清降钙素的水平与在该HDM小鼠模型中观察到的升高的IL-33水平相关(参见图4),从而提示,降钙素可以用作IL-33介导的疾病过程(例如变应性应答)中的IL-33的潜在生物标志物。

[0206] 关于IL-33介导的疾病参数,在该HDM模型中研究了肺嗜中性粒细胞、肺嗜酸性粒细胞的频率和IL5水平。在用屋尘螨变应原攻击以后15周之前,发现所有三个参数都升高(参见图5A(嗜中性粒细胞)、5B(嗜酸性粒细胞)和5C(IL-5))。但是,与用变应原攻击并保持不治疗或施用阴性同种型对照抗体的小鼠相比,接受IL-33抗体H4H9675P的小鼠表现出在它们的肺中显著更少的嗜中性粒细胞、嗜酸性粒细胞和IL-5(参见图5A、5B和5C)。

[0207] 此外,在血清降钙素水平和研究的三种疾病参数之间存在显著的关联。图6A显示了血清降钙素和肺嗜中性粒细胞之间的关联。图6B显示了血清降钙素和肺嗜酸性粒细胞之间的关联,且图6C显示了血清降钙素和肺IL-5水平之间的关联。

[0208] 另外,在肺IL-33水平和肺嗜中性粒细胞的频率(图7A)、肺嗜酸性粒细胞(图7B)和肺IL-5水平(图7C)之间存在显著的关联。

[0209] 总之,从屋尘螨模型得到的数据指示,在IL-33的水平、与该慢性变态反应的模型有关的至少三种疾病参数的频率和降钙素的表达之间存在显著关联。这样,该信息提示,降钙素可以是哺乳动物中的疾病严重程度和/或进展的一种生物标志物,且此外,所述数据提示,降钙素可以用于评估如本文中所述的使用IL-33拮抗剂的疗法的有效性。

[0210] 本发明在范围上不受本文描述的具体实施方案限制。实际上，除了本文描述的那些内容以外，本领域技术人员从前述描述和附图会明白本发明的不同改变。这样的改变意图落入所附权利要求的范围内。

序列表

<110> Regeneron Pharmaceuticals, Inc.
Jamie Orengo
Jeanne Allinne
Wen Fury
Yu Bai

<120> 与白介素-33 (IL-33)介导的疾病有关的生物标志物及其用途

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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Arg Asn Lys Tyr Tyr Thr Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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gggaaagccc ctaaggtcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
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50 55 60
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35 40 45
Ser Val Ile Ser Gly Ser Gly Ser Ser Thr Asp Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Arg Asp Thr Leu His

65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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gggaaagttc ctaaggtcct aatctatgct gcatccactt tgcaatcagg ggtcccatct 180
cggttcagtg gcagtggatc tgggacagtt ttcactctca ccatcagcag cctgcagact 240

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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Val Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Val Phe Thr Leu Thr Ile Ser Ser Leu Gln Thr
65                  70                  75                  80
Glu Asp Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Ser Ser Ala Pro Phe
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cctggacaag ggcttgagtg gatgggatgg atcaacccta acaatggtgg cacaaactat 180
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35 40 45
Gly Trp Ile Asn Pro Asn Asn Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
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ctctcctgca gggccagtca gagtgttggc aggcctact tagcctggta ccaacagata 120
cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tgacatccca 180
gacaggttca gtggcaatgg gtctgggaca gacttcactc tcaccatcag tagactggag 240
cctgaagatt ttgcagtgta ttactgtcag cagtatgata attccccta tacttttggc 300
caggggacca ggctggagat caaa 324

<210> 42
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 42
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Val Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Arg Pro
20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Asp Ile Pro Asp Arg Phe Ser
50 55 60
Gly Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asp Asn Ser Pro
85 90 95
Tyr Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

[0014]

<210> 43
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 43
cagagtgttg gcaggccctа c 21

<210> 44
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 44
Gln Ser Val Gly Arg Pro Tyr
1 5

<210> 45
<211> 9

<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 45
ggtgcatcc 9

<210> 46
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 46
Gly Ala Ser
1

<210> 47
[0015] <211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 47
cagcagtatg ataattcccc ttatact 27

<210> 48
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 48
Gln Gln Tyr Asp Asn Ser Pro Tyr Thr
1 5

<210> 49

<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 49
gaggtgcagc tggtggagtc tgggggaggc ttggtacaac ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctttaga agctttgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggaatt ggtctcagat ctcaggacta gtggtggtag tacatactac 180
gcagactccg tgaagggccg gctcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaagccac 300
tatagcacca gctggttcgg gggctttgac tactggggcc agggaaccct ggtcactgtc 360
tcctca 366

<210> 50
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

[0016]

<400> 50
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45
Ser Asp Leu Arg Thr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Ser His Tyr Ser Thr Ser Trp Phe Gly Gly Phe Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 51
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 51
ggattcacct ttagaagctt tgcc 24

<210> 52
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 52
Gly Phe Thr Phe Arg Ser Phe Ala
1 5

<210> 53
<211> 24
<212> DNA
<213> 人工序列

[0017]

<220>
<223> 合成的

<400> 53
ctcaggacta gtggtggtag taca 24

<210> 54
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 54
Leu Arg Thr Ser Gly Gly Ser Thr
1 5

<210> 55
<211> 45
<212> DNA

<213> 人工序列

<220>
<223> 合成的

<400> 55
gcgaaaagcc actatagcac cagctggttc gggggctttg actac 45

<210> 56
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 56
Ala Lys Ser His Tyr Ser Thr Ser Trp Phe Gly Gly Phe Asp Tyr
1 5 10 15

<210> 57
<211> 321
[0018] <212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 57
gacatccaga tgacccagtc tccatcttcc gtgtctgctt ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggttttagc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaagctcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcaccaa cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 58
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 58
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly

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 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Phe Ser Ser Trp
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 59
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0019]

<400> 59
cagggtttta gcagctgg 18

<210> 60
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 60
Gln Gly Phe Ser Ser Trp
 1   5

<210> 61
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 61
gctgcatcc 9

<210> 62
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 62
Ala Ala Ser
1

<210> 63
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0020]

<400> 63
caacaggcta acagtttccc tctcact 27

<210> 64
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 64
Gln Gln Ala Asn Ser Phe Pro Leu Thr
1 5

<210> 65
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 65
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacgtttagc agctatgtca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcaagt attagtggta atggtggtag cacaaactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgttt 240
ctggaaatga acagcctgag agccgaggac acggccgtat attactgtgc gaaatcactg 300
ggaactacca cgacttttt ggggtttgac tattggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 66
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 66
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
[0021] Val Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ser Ile Ser Gly Asn Gly Gly Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80
Leu Glu Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Ser Leu Gly Thr Thr Thr Thr Phe Leu Gly Phe Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 67
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 67
ggattcacgt ttagcagcta tgtc 24

<210> 68
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 68
Gly Phe Thr Phe Ser Ser Tyr Val
1 5

<210> 69
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 69
[0022] attagtggta atggtggtag caca 24

<210> 70
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 70
Ile Ser Gly Asn Gly Gly Ser Thr
1 5

<210> 71
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 71

gcgaaatcac tgggaactac cacgactttt ttggggtttg actat 45

<210> 72
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 72
Ala Lys Ser Leu Gly Thr Thr Thr Thr Phe Leu Gly Phe Asp Tyr
1 5 10 15

<210> 73
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0023] <400> 73
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattagc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaaactcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacatat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 74
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 74
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Thr Tyr Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 75
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 75
cagggtatta gcagctgg 18

<210> 76
<211> 6
<212> PRT
[0024] <213> 人工序列

<220>
<223> 合成的

<400> 76
Gln Gly Ile Ser Ser Trp
1 5

<210> 77
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 77
gctgcatcc 9

<210> 78
<211> 3
<212> PRT

<213> 人工序列

<220>
<223> 合成的

<400> 78
Ala Ala Ser
1

<210> 79
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 79
caacaggcta acagtttccc tctcact 27

<210> 80
<211> 9
[0025] <212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 80
Gln Gln Ala Asn Ser Phe Pro Leu Thr
1 5

<210> 81
<211> 363
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 81
caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagt agttattact ggagctggat ccggcagccc 120
ccagggaagg gactggagtt gattgggtat atttattaca gtgggagcac caattataac 180
ccctccctca agagtcgagt caccatatct gtagacacgt ccaagaacca cttctccctg 240

aagctgagct ctgtgaccgc tgcggacacg gccgtatatt actgtgcgag atcccagtat 300
accagtagtt ggtacggttc ttttgatatc tggggccaag ggacaatggt caccgtctct 360
tca 363

<210> 82
<211> 121
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 82
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Leu Ile
35 40 45
Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn His Phe Ser Leu
65 70 75 80
[0026]
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Ser Gln Tyr Thr Ser Ser Trp Tyr Gly Ser Phe Asp Ile Trp Gly
100 105 110
Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 83
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 83
ggtggctcca tcagtagtta ttac 24

<210> 84
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 84
Gly Gly Ser Ile Ser Ser Tyr Tyr
1 5

<210> 85
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 85
atttattaca gtgggagcac c 21

<210> 86
<211> 7
<212> PRT
<213> 人工序列

[0027]

<220>
<223> 合成的

<400> 86
Ile Tyr Tyr Ser Gly Ser Thr
1 5

<210> 87
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 87
gcgagatccc agtataccag tagttggtac ggttcttttg atatc 45

<210> 88
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 88
Ala Arg Ser Gln Tyr Thr Ser Ser Trp Tyr Gly Ser Phe Asp Ile
1 5 10 15

<210> 89
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 89
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattagc acctggttag cctggtttca gcagaaacca 120
gggaaagccc ctaagctcct gatctatgct gcatccactt tacaaggtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggccagaa ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt tcccgtggac gttcggccaa 300
gggaccaagg tggaaatcaa a 321

[0028]

<210> 90
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 90
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Thr Trp
20 25 30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Thr Leu Gln Gly Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Pro Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

100 105

<210> 91
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 91
cagggtatta gcacctgg 18

<210> 92
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 92
[0029] Gln Gly Ile Ser Thr Trp
1 5

<210> 93
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 93
gctgcatcc 9

<210> 94
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 94

Ala Ala Ser
1

<210> 95
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 95
caacaggcta acagtttccc gtggacg 27

<210> 96
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

[0030] <400> 96
Gln Gln Ala Asn Ser Phe Pro Trp Thr
1 5

<210> 97
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 97
caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cctctggtta cacctttaac agctatggta tcagctgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggatgg atcagctccc acaatggtaa cagtcactat 180
gtacagaagt tccagggcag agtctccatg accacagaca catccacgag tacagcctac 240
atggaactga ggagccttag atctgacgac acggccgtgt attactgtgc gagacacteg 300
tataccacca gctggtacgg gggttttgac tattggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 98
<211> 122

<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 98
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr
20 25 30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Ser Ser His Asn Gly Asn Ser His Tyr Val Gln Lys Phe
50 55 60
Gln Gly Arg Val Ser Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg His Ser Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

[0031]

<210> 99
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 99
ggttacacct ttaacagcta tggt 24

<210> 100
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 100
Gly Tyr Thr Phe Asn Ser Tyr Gly
1 5

<210> 101
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 101
atcagctccc acaatggtaa cagt 24

<210> 102
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 102
Ile Ser Ser His Asn Gly Asn Ser
1 5

[0032]

<210> 103
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 103
gcgagacact cgtataccac cagctggtac gggggttttg actat 45

<210> 104
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 104
Ala Arg His Ser Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Tyr

1 5 10 15

<210> 105
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 105
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggttttagc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctcagctcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggtcagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 106
<211> 107
<212> PRT
<213> 人工序列

[0033]

<220>
<223> 合成的

<400> 106
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Phe Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 107
<211> 18
<212> DNA

<213> 人工序列

<220>
<223> 合成的

<400> 107
cagggtttta gcagctgg 18

<210> 108
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 108
Gln Gly Phe Ser Ser Trp
1 5

<210> 109
<211> 9
[0034] <212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 109
gctgcatcc 9

<210> 110
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 110
Ala Ala Ser
1

<210> 111
<211> 27

<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 111
caacaggcta acagtttccc tctcact 27

<210> 112
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 112
Gln Gln Ala Asn Ser Phe Pro Leu Thr
1 5

[0035]

<210> 113
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 113
gaggtgcagc tggtggagtc cgggggaggc ttggttcagc cgggggggtc cctgagactc 60
tcctgtgcag cctctggaat caccttgagc agctatggca tgagctgggt ccgccaggct 120
ccagggaagg gactggagtg ggtcgcatcc atttttggta gtggtggtgg cccatactac 180
gcagactccg tgaagggccg gttcaccatg tccagagaca attccaagaa cacgctgtat 240
ttgcaaatga acagcctgag agccgaggac acggccgtat attattgtgc gaaagatcga 300
tacagtggga gctactacgg aggttttgac tactggggcc ggggaaccct ggtcaccgtc 360
tcctca 366

<210> 114
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 114
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ile Thr Leu Ser Ser Tyr
20 25 30
Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Ser Ile Phe Gly Ser Gly Gly Gly Pro Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Met Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Asp Arg Tyr Ser Gly Ser Tyr Tyr Gly Gly Phe Asp Tyr Trp
100 105 110
Gly Arg Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 115
<211> 24
<212> DNA
<213> 人工序列

[0036]

<220>
<223> 合成的

<400> 115
ggaatcacct tgagcagcta tggc 24

<210> 116
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 116
Gly Ile Thr Leu Ser Ser Tyr Gly
1 5

<210> 117
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 117
attttggta gtggtggtgg ccca 24

<210> 118
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 118
Ile Phe Gly Ser Gly Gly Gly Pro
1 5

<210> 119
<211> 45
<212> DNA
<213> 人工序列

[0037]

<220>
<223> 合成的

<400> 119
gcgaaagatc gatacagtgg gagctactac ggaggttttg actac 45

<210> 120
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 120
Ala Lys Asp Arg Tyr Ser Gly Ser Tyr Tyr Gly Gly Phe Asp Tyr
1 5 10 15

<210> 121
<211> 321
<212> DNA

<213> 人工序列

<220>
<223> 合成的

<400> 121
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattacc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctacactcct gatctatgct gcatccagtt tgcaaactgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaacattttg caacttacta ttgtcaacag gctaacagtt tccctcctac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 122
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 122
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
[0038] 1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Thr Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Thr Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu His Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Pro
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 123
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 123

cagggtatta ccagctgg 18

<210> 124
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 124
Gln Gly Ile Thr Ser Trp
1 5

<210> 125
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0039]

<400> 125
gctgcatcc 9

<210> 126
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 126
Ala Ala Ser
1

<210> 127
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 127
caacaggcta acagtttccc tcctact 27

<210> 128
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 128
Gln Gln Ala Asn Ser Phe Pro Pro Thr
1 5

<210> 129
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0040]

<400> 129
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctggggggtc cctaagactc 60
tcctgtgcag cctctggatt cacctttagc agttatgcct tgacctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctctttt attagtggta gtggtggtag gccattctac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa catgctgtat 240
ctgcaaatga acagcctgag agccgaggac acggccatat attactgtgc gaagtccctg 300
tataccacca gctggtacgg ggggttcgac tcctggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 130
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 130
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Leu Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

```
        35                  40                  45
Ser Phe Ile Ser Gly Ser Gly Gly Arg Pro Phe Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Met Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
                85                  90                  95
Ala Lys Ser Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Ser Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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<210> 131
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 131
ggattcacct ttagcagtta tgcc 24

[0041]

<210> 132
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 132
Gly Phe Thr Phe Ser Ser Tyr Ala
 1   5

<210> 133
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 133
attagtggta gtggtggtag gcca 24

<210> 134
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 134
Ile Ser Gly Ser Gly Gly Arg Pro
1 5

<210> 135
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 135
[0042] gcgaagtccc tgtataccac cagctggtac gggggttcg actcc 45

<210> 136
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 136
Ala Lys Ser Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Ser
1 5 10 15

<210> 137
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 137

gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtgtcgtc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaagctcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttatta ttgtcaacag tctaacagtt tccctttcac tctcggccct 300
gggaccaaag tggatatcaa a 321

<210> 138
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 138
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Val Val Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[0043] 50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Ser Phe Pro Phe
85 90 95
Thr Leu Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 139
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 139
cagggtgtcg tcagctgg 18

<210> 140
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 140
Gln Gly Val Val Ser Trp
1 5

<210> 141
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 141
gctgcatcc 9

<210> 142
<211> 3
<212> PRT
<213> 人工序列

[0044]

<220>
<223> 合成的

<400> 142
Ala Ala Ser
1

<210> 143
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 143
caacagtcta acagtttccc tttc 24

<210> 144
<211> 8
<212> PRT

<213> 人工序列

<220>
<223> 合成的

<400> 144
Gln Gln Ser Asn Ser Phe Pro Phe
1 5

<210> 145
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 145
caggtgcagc tggtgcagtc tggggctgaa gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggata caccttcacc ggccactata tgtactggat gcgacaggcc 120
cctggacaag ggcttgagtg gatgggatgg atcaacccta acagtggtgg cacaaactat 180
gcacagaagt ttcaggacag ggtcaccatg accagggaca cgtccatcag cacagcctac 240
[0045] atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagggaga 300
tatggcagta gctggtacgg ggggtttgag tactggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 146
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 146
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly His
20 25 30
Tyr Met Tyr Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Asp Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys

85 90 95
Ala Arg Gly Arg Tyr Gly Ser Ser Trp Tyr Gly Gly Phe Glu Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 147
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 147
ggatacacct tcaccggcca ctat 24

<210> 148
<211> 8
<212> PRT
<213> 人工序列

[0046]

<220>
<223> 合成的

<400> 148
Gly Tyr Thr Phe Thr Gly His Tyr
1 5

<210> 149
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 149
atcaacccta acagtggtgg caca 24

<210> 150
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 150
Ile Asn Pro Asn Ser Gly Gly Thr
1 5

<210> 151
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 151
gcgagaggga gatatggcag tagctggtac gggggtttg agtac 45

<210> 152
<211> 15
<212> PRT
<213> 人工序列

[0047]

<220>
<223> 合成的

<400> 152
Ala Arg Gly Arg Tyr Gly Ser Ser Trp Tyr Gly Gly Phe Glu Tyr
1 5 10 15

<210> 153
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 153
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgttggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattacc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaacctcct gatctatgct gcagccagtt tacaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacggat ttcactctca ccatcagcag cctgcagcct 240
gaagacttta caacttacta ttgtcaacag gcttacagtc tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 154
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 154
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Thr Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
35 40 45
Tyr Ala Ala Ala Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Thr Thr Tyr Tyr Cys Gln Gln Ala Tyr Ser Leu Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

[0048]

<210> 155
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 155
cagggtatta ccagctgg 18

<210> 156
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 156
Gln Gly Ile Thr Ser Trp

1 5

<210> 157
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 157
gctgcagcc 9

<210> 158
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 158
[0049] Ala Ala Ala
1

<210> 159
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 159
caacaggctt acagtctccc tctcact 27

<210> 160
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 160

Gln Gln Ala Tyr Ser Leu Pro Leu Thr
1 5

<210> 161
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 161
caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt agctatggct tgcactgggt ccgccagtct 120
ccaggcaagg ggctggaatg ggtggcactt atatcatatg acggaagtaa taaatactat 180
gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag acctgaggac acggctggat atttctgtgc gaaatcccta 300
tatacaacca gctggtacgg gggctttgac tattggggcc agggaaccct ggtcaccgtc 360
tcctca 366

[0050]
<210> 162
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 162
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Leu His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Leu Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Gly Tyr Phe Cys
85 90 95
Ala Lys Ser Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 163
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 163
ggattcacct tcagtagcta tggc 24

<210> 164
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 164
Gly Phe Thr Phe Ser Ser Tyr Gly
1 5

[0051]

<210> 165
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 165
atatcatatg acggaagtaa taaa 24

<210> 166
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 166
Ile Ser Tyr Asp Gly Ser Asn Lys
1 5

<210> 167
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 167
gcgaaatccc tatatacaac cagctggtac gggggctttg actat 45

<210> 168
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 168
Ala Lys Ser Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Asp Tyr
1 5 10 15

[0052]

<210> 169
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 169
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattaga agctggttag cctggtatca gcaaaaacca 120
gggaaagccc ctaacctcct gatctatgct gcgtccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt tccctcccac tttcggccct 300
gggaccaaag tggatatcaa a 321

<210> 170
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 170
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Pro
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 171
<211> 18
<212> DNA
[0053] <213> 人工序列

<220>
<223> 合成的

<400> 171
cagggtatta gaagctgg 18

<210> 172
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 172
Gln Gly Ile Arg Ser Trp
1 5

<210> 173
<211> 9
<212> DNA

<213> 人工序列

<220>
<223> 合成的

<400> 173
gctgcgtcc 9

<210> 174
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 174
Ala Ala Ser
1

<210> 175
<211> 27
[0054] <212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 175
caacaggcta acagtttccc tcccact 27

<210> 176
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 176
Gln Gln Ala Asn Ser Phe Pro Pro Thr
1 5

<210> 177
<211> 366

<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 177
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGTC cctgagactc 60
tcctgtgcag cctctgggtt caccttcagc aactatgcca tgacctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcaact atcagtggca gtggtgataa cacatactac 180
gcagactccg tgcagggccg gttcaccatc tccagaggcc attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac acggccgtat attactgtgc gaaacctacg 300
tatagcagaa gctggtacgg tgcttttgat ttctggggcc aagggacaat ggtcaccgtc 360
tcttca 366

<210> 178
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

[0055]
<400> 178

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25				30			
Ala	Met	Thr	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ser	Thr	Ile	Ser	Gly	Ser	Gly	Asp	Asn	Thr	Tyr	Tyr	Ala	Asp	Ser	Val
	50					55					60				
Gln	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Gly	His	Ser	Lys	Asn	Thr	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Lys	Pro	Thr	Tyr	Ser	Arg	Ser	Trp	Tyr	Gly	Ala	Phe	Asp	Phe	Trp
			100					105					110		
Gly	Gln	Gly	Thr	Met	Val	Thr	Val	Ser	Ser						
		115					120								

<210> 179
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 179
gggttcacct tcagcaacta tgcc 24

<210> 180
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 180
Gly Phe Thr Phe Ser Asn Tyr Ala
1 5

<210> 181
<211> 24
<212> DNA
<213> 人工序列

[0056]

<220>
<223> 合成的

<400> 181
atcagtggca gtggtgataa caca 24

<210> 182
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 182
Ile Ser Gly Ser Gly Asp Asn Thr
1 5

<210> 183
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 183
gcgaaacctа cgtatagcag aagctggtac ggtgcttttg atttc 45

<210> 184
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 184
Ala Lys Pro Thr Tyr Ser Arg Ser Trp Tyr Gly Ala Phe Asp Phe
1 5 10 15

<210> 185
<211> 321
<212> DNA
<213> 人工序列

[0057]

<220>
<223> 合成的

<400> 185
gacatccaga tgacccagtc tccatcctcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattagc agctggttag cctggtatca gcagaaaccg 120
gggaaagccc ctcaactcct gatctatgct gcatccagat tgcaaagtgg ggtcccatca 180
aggttctggg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacaatt tcccattcac tttcggccct 300
gggaccaaag tggatatcaa a 321

<210> 186
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 186
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Trp Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Asn Phe Pro Phe
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 187
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 187
[0058] cagggtatta gcagctgg 18

<210> 188
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 188
Gln Gly Ile Ser Ser Trp
1 5

<210> 189
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 189

gctgcatcc 9

<210> 190
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 190
Ala Ala Ser
1

<210> 191
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0059]

<400> 191
caacaggcta acaatttccc attcact 27

<210> 192
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 192
Gln Gln Ala Asn Asn Phe Pro Phe Thr
1 5

<210> 193
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 193
caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggtta cacctttacc agttatggta tcagctgggt gcgacaggcc 120
cctggacaag gccttgagtg gatgggatgg atccgcgctt acaatggtta cacaaactat 180
gcacagaagt ttcagggcag agtcaccatg accacagaca catccacgaa cacgcctac 240
atggagctga ggaccctgaa ttctgacgat acggccgttt attactgtgc gagagatcga 300
tatagtggga gcttccacgg taactttgac tactggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 194
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 194
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
[0060] Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Arg Ala Tyr Asn Gly Tyr Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Thr Leu Asn Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asp Arg Tyr Ser Gly Ser Phe His Gly Asn Phe Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 195
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 195
ggttacacct ttaccagtta tggt 24

<210> 196
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 196
Gly Tyr Thr Phe Thr Ser Tyr Gly
1 5

<210> 197
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 197
atccgcgctt acaatggtta caca 24

[0061]

<210> 198
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 198
Ile Arg Ala Tyr Asn Gly Tyr Thr
1 5

<210> 199
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 199
gcgagagatc gatatagtgg gagcttccac ggtaactttg actac 45

<210> 200
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 200
Ala Arg Asp Arg Tyr Ser Gly Ser Phe His Gly Asn Phe Asp Tyr
1 5 10 15

<210> 201
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 201
[0062] gacatccaga tgacccagtc tccatcttcc gtgtctgcgt ctgtaggaga cagagtgacc 60
atcacttgtc gggcgagtca gggtattttc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaaggtcct aatctatgct gcatccaatt tggaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag gctaacagtt taccgctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 202
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 202
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Phe Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45
Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Leu Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 203
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 203
cagggtattt tcagctgg 18

<210> 204
<211> 6
<212> PRT
<213> 人工序列

[0063]

<220>
<223> 合成的

<400> 204
Gln Gly Ile Phe Ser Trp
1 5

<210> 205
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 205
gctgcatcc 9

<210> 206
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 206
Ala Ala Ser
1

<210> 207
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 207
caacaggcta acagtttacc gctcact 27

<210> 208
<211> 9
<212> PRT
<213> 人工序列

[0064]

<220>
<223> 合成的

<400> 208
Gln Gln Ala Asn Ser Leu Pro Leu Thr
1 5

<210> 209
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 209
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt acctattcta tgcactgggt ccgccaggct 120
ccagggaagg gactggaata tgtttcaact attaataata atggggatac cacatattat 180
gcagactctg tgaagggcag attcaccatc tccagagaca attccaagaa cacgctgtat 240
cttcaactgg gcagcctgag acctgaggac atggctgtgt attactgtgc gagacagacg 300

tataccagca gctggtacgg ggggttcgac tcctggggcc agggaaccct ggtcaccgtc 360
tcctca 366

<210> 210
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 210
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
35 40 45
Ser Thr Ile Asn Asn Asn Gly Asp Thr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Leu Gly Ser Leu Arg Pro Glu Asp Met Ala Val Tyr Tyr Cys
[0065] 85 90 95
Ala Arg Gln Thr Tyr Thr Ser Ser Trp Tyr Gly Gly Phe Asp Ser Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 211
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 211
ggattcacct tcagtaccta ttct 24

<210> 212
<211> 8
<212> PRT
<213> 人工序列

<220>

<223> 合成的

<400> 212
Gly Phe Thr Phe Ser Thr Tyr Ser
1 5

<210> 213
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 213
attaataata atggggatac caca 24

<210> 214
<211> 8
<212> PRT
<213> 人工序列

[0066]

<220>
<223> 合成的

<400> 214
Ile Asn Asn Asn Gly Asp Thr Thr
1 5

<210> 215
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 215
gcgagacaga cgtataccag cagctggtac gggggttcg actcc 45

<210> 216
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 216
Ala Arg Gln Thr Tyr Thr Ser Ser Trp Tyr Gly Gly Phe Asp Ser
1 5 10 15

<210> 217
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 217
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggcga cagagtcacc 60
atcacttgtc gggcgagtca gggtattacc agctggttag cctggtatca gcagaaacca 120
gggaaagccc ctaaactcct gatctatgct gcatccaatt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcaccag cctgcagcct 240
gaggattttg caacttacta ttgtcaacag gctaacagtc tcccattcac tttcggccct 300
gggaccaaag tggatatcaa a 321

[0067]

<210> 218
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 218
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Thr Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Leu Pro Phe
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 219
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 219
cagggtatta ccagctgg 18

<210> 220
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 220
Gln Gly Ile Thr Ser Trp
1 5

[0068]

<210> 221
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 221
gctgcatcc 9

<210> 222
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 222
Ala Ala Ser

1

<210> 223
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 223
caacaggcta acagtctccc attcact 27

<210> 224
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 224
[0069] Gln Gln Ala Asn Ser Leu Pro Phe Thr
1 5

<210> 225
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 225
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGTC cctgagactc 60
tcctgtgcag cctctggatt cacccttagc agctatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggcag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa ctcgctgtat 240
ctgcaattga acagcctgag agccgaggac acggccgtat attactgtgc gaagacgctg 300
tatactacca gctggtacgg gggcttccag cactggggcc agggcaccct ggtcactgtc 360
tcctca 366

<210> 226
<211> 122
<212> PRT

<213> 人工序列

<220>
<223> 合成的

<400> 226

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Ser Tyr
            20                  25                  30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Lys Thr Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Gln His Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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[0070]

<210> 227
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 227

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ggattcaccc ttagcagcta tgcc                                          24
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<210> 228
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 228

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Gly Phe Thr Leu Ser Ser Tyr Ala
 1               5
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<210> 229
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 229
attagtggta gtggtggcag caca 24

<210> 230
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 230
Ile Ser Gly Ser Gly Gly Ser Thr
1 5

[0071]

<210> 231
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 231
gcgaagacgc tgtatactac cagctggtac gggggcttcc agcac 45

<210> 232
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 232
Ala Lys Thr Leu Tyr Thr Thr Ser Trp Tyr Gly Gly Phe Gln His
1 5 10 15

<210> 233
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 233
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctataggaga cagagtcacc 60
atcacttgtc gggcgagtca gggaatcagc agttggttag cctggtatca gcagaaacca 120
gggaaagtcc ctaagctcct gatctatgct gcgtcctctt tgcaaagtgg gttcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagtag cctgcagccc 240
gaagattttg caacttacta ttgtcaacag actcacagtt tcccgtggac ggtcggccaa 300
gggaccaagg tggaaatcaa a 321

<210> 234
<211> 107
<212> PRT
<213> 人工序列

<220>
[0072] <223> 合成的

<400> 234
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Ile Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Phe Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr His Ser Phe Pro Trp
85 90 95
Thr Val Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 235
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 235
cagggaatca gcagttgg 18

<210> 236
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 236
Gln Gly Ile Ser Ser Trp
1 5

<210> 237
<211> 9
<212> DNA
<213> 人工序列

[0073]

<220>
<223> 合成的

<400> 237
gctgcgtcc 9

<210> 238
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 238
Ala Ala Ser
1

<210> 239
<211> 24
<212> DNA

<213> 人工序列

<220>
<223> 合成的

<400> 239
caacagactc acagtttccc gtgg 24

<210> 240
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 240
Gln Gln Thr His Ser Phe Pro Trp
1 5

<210> 241
<211> 366
[0074] <212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 241
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGTC cctgagactc 60
tcctgtgcag cctctggatt cacccttagg agctatttca tgacctgggt ccgccaggtt 120
ccagggaagg ggctggaggg ggtctcagct attagtggca ttagtggtgg cacatactac 180
acagactccg ttaagggccg gttcaccatc tccagagaca attccaagaa cacgctgttt 240
ctgcaaatga acagcctgag agccgaggac acggccgtat atttctgtgc gagaacggtg 300
tatagtagta gttactacgg gggcttccag cactggggcc agggcaccct ggtcaccgtc 360
tcctca 366

<210> 242
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 242

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Arg Ser Tyr
20 25 30
Phe Met Thr Trp Val Arg Gln Val Pro Gly Lys Gly Leu Glu Gly Val
35 40 45
Ser Ala Ile Ser Gly Ile Ser Gly Gly Thr Tyr Tyr Thr Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95
Ala Arg Thr Val Tyr Ser Ser Ser Tyr Tyr Gly Gly Phe Gln His Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 243
<211> 24
<212> DNA
<213> 人工序列

[0075]

<220>
<223> 合成的

<400> 243
ggattcaccc ttaggagcta tttc 24

<210> 244
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 244
Gly Phe Thr Leu Arg Ser Tyr Phe
1 5

<210> 245
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 245
attagtggca ttagtggtgg caca 24

<210> 246
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 246
Ile Ser Gly Ile Ser Gly Gly Thr
1 5

<210> 247
<211> 45
<212> DNA
<213> 人工序列

[0076]

<220>
<223> 合成的

<400> 247
gcgagaacgg tgtatagtag tagttactac gggggcttcc agcac 45

<210> 248
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 248
Ala Arg Thr Val Tyr Ser Ser Ser Tyr Tyr Gly Gly Phe Gln His
1 5 10 15

<210> 249
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 249
gacatccaga tgacccagtc tccatcttcc gtgtctgtat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattagc agttggttag cctggtatca gcagaaacca 120
gggaaagccc ctaagctcct gatctatgtt gcatccagtt tacaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttacta ttgtcaacag actaacagtt tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 250
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 250
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Val Ser Val Gly
1 5 10 15
[0077] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Val Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Asn Ser Phe Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 251
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 251
cagggtatta gcagttgg 18

<210> 252
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 252
Gln Gly Ile Ser Ser Trp
1 5

<210> 253
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 253
[0078] gttgcatcc 9

<210> 254
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 254
Val Ala Ser
1

<210> 255
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 255

caacagacta acagtttccc tctcact 27

<210> 256
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 256
Gln Gln Thr Asn Ser Phe Pro Leu Thr
1 5

<210> 257
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

[0079] <400> 257
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt cacccttagg agttatgtca tgtactgggt ccgccagggt 120
ccagggaagg ggctggaggg ggtctcaggt attagtggca gtagtggtgg cacatactac 180
acagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgttt 240
ctgcaaatga acagcctgag agccgaggac acggccgtat atttctgtgc gagatcggtg 300
tatagtacca cctggtacgg gggcttccag cactggggcc agggcaccct ggtcaccgtc 360
tcctca 366

<210> 258
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 258
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Arg Ser Tyr
20 25 30
Val Met Tyr Trp Val Arg Gln Gly Pro Gly Lys Gly Leu Glu Gly Val
35 40 45

Ser Gly Ile Ser Gly Ser Ser Gly Gly Thr Tyr Tyr Thr Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95
Ala Arg Ser Val Tyr Ser Thr Thr Trp Tyr Gly Gly Phe Gln His Trp
100 105 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 259
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 259
ggattcaccc ttaggagtta tgtc 24

[0080]

<210> 260
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 260
Gly Phe Thr Leu Arg Ser Tyr Val
1 5

<210> 261
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 261
attagtggca gtagtggtgg caca 24

<210> 262
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 262
Ile Ser Gly Ser Ser Gly Gly Thr
1 5

<210> 263
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 263
gcgagatcgg tgtatagtac cacctggtac gggggcttcc agcac 45

[0081]

<210> 264
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 264
Ala Arg Ser Val Tyr Ser Thr Thr Trp Tyr Gly Gly Phe Gln His
1 5 10 15

<210> 265
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 265
gacatccaga tgacccagtc tccatcttcc gtgtctgtat ctgtgggaga cagagtcacc 60

atcacttgtc gggcgagtca ggttattagc agttggttag cctggtatca gctgaaacca 120
gggaaagccc ctaaactcct gatctatgct gcatccagtt tacaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcgg cctgcagcct 240
gaagattttg cagtttacta ttgtcaacag actaacagtt tccctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 266
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 266
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Val Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Leu Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
[0082] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Asn Ser Phe Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 267
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 267
caggttatta gcagttgg 18

<210> 268
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 268
Gln Val Ile Ser Ser Trp
1 5

<210> 269
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 269
gctgcatcc 9

<210> 270
<211> 3
<212> PRT
<213> 人工序列

[0083]

<220>
<223> 合成的

<400> 270
Ala Ala Ser
1

<210> 271
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 271
caacagacta acagtttccc tctcact 27

<210> 272
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 272
Gln Gln Thr Asn Ser Phe Pro Leu Thr
1 5

<210> 273
<211> 366
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 273
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tcctgtacag cctctggatt cacctttagc agatctgcca tgaactgggt ccgccgggct 120
ccagggaagg ggctggagtg ggtctcagga attagtggta gtggtggtcg aacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa tacgctatat 240
ctgcaaatga acagcctgag cgccgaggac acggccgcat attactgtgc gaaagattcg 300
[0084] tatactacca gttggtacgg aggtatggac gtctggggcc acgggaccac ggtcaccgtc 360
tcctca 366

<210> 274
<211> 122
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 274
Glu Val Gln Leu Val Glu Ser Gly Gly Asn Leu Glu Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Arg Ser
20 25 30
Ala Met Asn Trp Val Arg Arg Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Gly Ile Ser Gly Ser Gly Gly Arg Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Ser Ala Glu Asp Thr Ala Ala Tyr Tyr Cys
85 90 95

Ala Lys Asp Ser Tyr Thr Thr Ser Trp Tyr Gly Gly Met Asp Val Trp
100 105 110
Gly His Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 275
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 275
ggattcacct ttagcagatc tgcc 24

<210> 276
<211> 8
<212> PRT
<213> 人工序列

<220>
[0085] <223> 合成的

<400> 276
Gly Phe Thr Phe Ser Arg Ser Ala
1 5

<210> 277
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 277
attagtggta gtggtggtcg aaca 24

<210> 278
<211> 8
<212> PRT
<213> 人工序列

<220>

<223> 合成的

<400> 278
Ile Ser Gly Ser Gly Gly Arg Thr
1 5

<210> 279
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 279
gcgaaagatt cgtatactac cagttggtac ggaggtatgg acgtc 45

<210> 280
<211> 15
<212> PRT
<213> 人工序列

[0086]

<220>
<223> 合成的

<400> 280
Ala Lys Asp Ser Tyr Thr Thr Ser Trp Tyr Gly Gly Met Asp Val
1 5 10 15

<210> 281
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 281
gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtatttta agctggttag cctggtatca gcagaaacca 120
ggaaaagccc ctaagctcct gatctatgct gcttccagtt tacaaagtgg ggtcccatca 180
agattcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaggattttg caatttacta ttgtcaacag gctaacagtg tcccgatcac cttcggccaa 300
gggacacgac tggagattaa a 321

<210> 282
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 282
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Phe Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Ile Tyr Tyr Cys Gln Gln Ala Asn Ser Val Pro Ile
85 90 95
Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

[0087]

<210> 283
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 283
cagggtattt tcagctgg 18

<210> 284
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 284
Gln Gly Ile Phe Ser Trp
1 5

<210> 285
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 285
gctgcttcc 9

<210> 286
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 286
Ala Ala Ser
1

[0088]

<210> 287
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 287
caacaggcta acagtgtccc gatcacc 27

<210> 288
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 288
Gln Gln Ala Asn Ser Val Pro Ile Thr

1 5

<210> 289
<211> 366
<212> DNA
<213> 人工序列

<220>
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<400> 289
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ccagggaagg ggctggagtg ggtcaccgct attagtggca gtggtggtgg cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa ctcgctgttt 240
ctgcaattga acagcctgag agccgaggac acggccgtgt attactgtgc gaaacaaacg 300
tataccagca gctggtacgg tggctttgat atctggggcc aggggacaat ggtcaccgtc 360
tcttca 366

<210> 290
<211> 122
[0089] <212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 290
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Thr Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Ser Leu Phe
65 70 75 80
Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Gln Thr Tyr Thr Ser Ser Trp Tyr Gly Gly Phe Asp Ile Trp
100 105 110
Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 291
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 291
ggattcacct ttagcagcta tgcc 24

<210> 292
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 292
Gly Phe Thr Phe Ser Ser Tyr Ala
1 5

[0090]

<210> 293
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 293
attagtggca gtggtggtgg caca 24

<210> 294
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 294
Ile Ser Gly Ser Gly Gly Gly Thr
1 5

<210> 295
<211> 45
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 295
gcgaaacaaa cgtataccag cagctggtac ggtggctttg atatc 45

<210> 296
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 296
Ala Lys Gln Thr Tyr Thr Ser Ser Trp Tyr Gly Gly Phe Asp Ile
1 5 10 15

[0091]

<210> 297
<211> 321
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 297
gacatccaga tgacccagtc gccatcttcc gtgtccgcgt ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggttttagt tcctggttag cctggtatca gcagatacca 120
gggaaagccc ccaagctcct gatctatgct gcatcaaggt tgcaaagtgg ggtccccatcc 180
aggttccgcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaggattttg caacttacta ttgtcaacag gctaacagtt tccccgctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 298
<211> 107
<212> PRT
<213> 人工序列

<220>

<223> 合成的

<400> 298

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Phe Ser Ser Trp
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Ile Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Arg Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 299
<211> 18
<212> DNA
<213> 人工序列

[0092]

<220>
<223> 合成的

<400> 299

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cagggtttta gttcctgg                                                  18
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<210> 300
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 300

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Gln Gly Phe Ser Ser Trp
 1               5
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<210> 301
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 301
gctgcatca 9

<210> 302
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 302
Ala Ala Ser
1

<210> 303
<211> 27
<212> DNA
<213> 人工序列

[0093]

<220>
<223> 合成的

<400> 303
caacaggcta acagtttccc gctcact 27

<210> 304
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 304
Gln Gln Ala Asn Ser Phe Pro Leu Thr
1 5

<210> 305
<211> 2718
<212> DNA

<213> 人工序列

<220>
<223> GenBank NM_033439.3: 人 IL-33 核酸

<400> 305

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tcacaagaat actgaaaaat gaagcctaaa atgaagtatt caaccaacaa aatttccaca 120
gcaaagtgga agaacacagc aagcaaagcc ttgtgtttca agctgggaaa atcccaacag 180
aaggccaaag aagtttgccc catgtacttt atgaagctcc gctctggcct tatgataaaa 240
aaggaggcct gttactttag gagagaaacc accaaaaggc cttcactgaa aacaggtaga 300
aagcacaaaa gacatctggt actcgctgcc tgtcaacagc agtctactgt ggagtgcttt 360
gcctttggta tatcaggggt ccagaaatat actagagcac ttcatgattc aagtatcaca 420
ggaatttcac ctattacaga gtatcttgct tctctaagca catacaatga tcaatccatt 480
acttttgctt tggaggatga aagttatgag atatatgttg aagacttgaa aaaagatgaa 540
aagaaagata aggtgttact gagttactat gagtctcaac acccctcaaa tgaatcaggt 600
gacggtgttg atggtaagat gttaatggta accctgagtc ctacaaaaga cttctggttg 660
catgccaaca acaaggaaca ctctgtggag ctccataagt gtgaaaaacc actgccagac 720
caggccttct ttgtccttca taatatgcac tccaactgtg tttcatttga atgcaagact 780
gatcctggag tgtttatagg tgtaaaggat aatcatcttg ctctgattaa agtagactct 840
tctgagaatt tgtgtactga aaatatcttg tttaagctct ctgaaactta gttgatggaa 900
acctgtgagt cttgggttga gtacccaaat gctaccactg gagaaggaat gagagataaa 960
gaaagagaca ggtgacatct aagggaaatg aagagtgctt agcatgtgtg gaatgtttte 1020
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aacattctaa agagatacag tctgaccttt acttttctct agtttcagtc cagaaagaac 1200
ttcatattta gagctaaggc cactgaggaa agagccatag cttaagtctc tatgtagaca 1260
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caaacactag agctgctagt aaaaagaaga ccagatgctt cacagaatta tcatttttte 1380
aactggaata aaacaccagg tttgtttgta gatgtcttag gcaacactca gagcagatct 1440
cccttactgt caggggatat ggaacttcaa aggcccacat ggcaagccag gtaacataaa 1500
tgtgtgaaaa agtaaagata actaaaaaat ttagaaaaat aaatccagta tttgtaaagt 1560
gaataacttc atttctaatt gtttaatttt taaaattctg atttttatat attgagttta 1620
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tattaggaat atgtaacatg ctaaaacttt tttttttta aagagtactg agtcacaaca 1740
tgttttagag catccaagta ccatataatc caactatcat ggtaaggcca gaaatcttct 1800
aacctaccag agcctagatg agacaccgaa ttaacattaa aatttcagta actgactgtc 1860
cctcatgtcc atggcctacc atcccttctg accctggctt ccagggacct atgtctttta 1920
atactcactg tcacattggg caaagttgct tctaatcctt atttcccatg tgcacaagtc 1980
tttttgtatt ccagcttcct gataacactg cttactgtgg aatattcatt tgacatctgt 2040
ctcttttcat ttcttttaac taccatgccc ttgatatatc ttttgcacct gctgaacttc 2100
atttctgtat cacctgacct ctggatgcca aaacgtttat tctgctttgt ctgttgtaga 2160
attttagata aagctattaa tggcaatatt tttttgctaa acgtttttgt tttttactgt 2220
cactagggca ataaaattta tactcaacca tataataaca ttttttaact actaaaggag 2280
tagttttat tttaaagtct tagcaatttc tattacaact tttcttagac ttaacactta 2340
tgataaatga ctaacatagt aacagaatct ttatgaaata tgacctttte tgaaaatata 2400
tacttttaca tttctacttt attgagacct attagatgta agtgctagta gaatataaga 2460

taaaagaggc tgagaattac catacaaggg tattacaact gtaaaacaat ttatctttgt 2520
ttcattgttc tgtcaataat tgttaccaaa gagataaaaa taaaagcaga atgtatatca 2580
tcccatctga aaaacactaa ttattgacat gtgcatctgt acaataaact taaaatgatt 2640
attaaataat caaatatatc tactacattg tttatattat tgaataaagt atattttcca 2700
aatgtaaaaa aaaaaaaa 2718

<210> 306
<211> 270
<212> PRT
<213> 人工序列

<220>
<223> 人 IL-33 蛋白 GenBank 095760

<400> 306

Met Lys Pro Lys Met Lys Tyr Ser Thr Asn Lys Ile Ser Thr Ala Lys
1 5 10 15
Trp Lys Asn Thr Ala Ser Lys Ala Leu Cys Phe Lys Leu Gly Lys Ser
20 25 30
Gln Gln Lys Ala Lys Glu Val Cys Pro Met Tyr Phe Met Lys Leu Arg
35 40 45
Ser Gly Leu Met Ile Lys Lys Glu Ala Cys Tyr Phe Arg Arg Glu Thr
50 55 60

[0095]

Thr Lys Arg Pro Ser Leu Lys Thr Gly Arg Lys His Lys Arg His Leu
65 70 75 80
Val Leu Ala Ala Cys Gln Gln Gln Ser Thr Val Glu Cys Phe Ala Phe
85 90 95
Gly Ile Ser Gly Val Gln Lys Tyr Thr Arg Ala Leu His Asp Ser Ser
100 105 110
Ile Thr Gly Ile Ser Pro Ile Thr Glu Tyr Leu Ala Ser Leu Ser Thr
115 120 125
Tyr Asn Asp Gln Ser Ile Thr Phe Ala Leu Glu Asp Glu Ser Tyr Glu
130 135 140
Ile Tyr Val Glu Asp Leu Lys Lys Asp Glu Lys Lys Asp Lys Val Leu
145 150 155 160
Leu Ser Tyr Tyr Glu Ser Gln His Pro Ser Asn Glu Ser Gly Asp Gly
165 170 175
Val Asp Gly Lys Met Leu Met Val Thr Leu Ser Pro Thr Lys Asp Phe
180 185 190
Trp Leu His Ala Asn Asn Lys Glu His Ser Val Glu Leu His Lys Cys
195 200 205
Glu Lys Pro Leu Pro Asp Gln Ala Phe Phe Val Leu His Asn Met His
210 215 220
Ser Asn Cys Val Ser Phe Glu Cys Lys Thr Asp Pro Gly Val Phe Ile
225 230 235 240
Gly Val Lys Asp Asn His Leu Ala Leu Ile Lys Val Asp Ser Ser Glu
245 250 255

Asn Leu Cys Thr Glu Asn Ile Leu Phe Lys Leu Ser Glu Thr
260 265 270

<210> 307
<211> 354
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 307
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acctgctccg tctctggatt ctcactcagt aatgttagaa tgggtgtgag ctggatccgt 120
cagtccccag ggaaggccct ggagtggctt gcacacattt tttcgaatga cgaaaaatcc 180
tacaccacat ctctgaagac caggctcacc atctccaagg acacctccag aagccaggtg 240
gtccttacca tgaccgacat ggaccctggg gacacagcca catattactg tgcacggata 300
cggaatttgg cctttaatta ctggggccag ggaaccctgg tcaccgtctc ctca 354

<210> 308
<211> 118
[0096] <212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 308
Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15
Ser Leu Thr Leu Thr Cys Ser Val Ser Gly Phe Ser Leu Ser Asn Val
20 25 30
Arg Met Gly Val Ser Trp Ile Arg Gln Ser Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Phe Ser Asn Asp Glu Lys Ser Tyr Thr Thr Ser
50 55 60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Ser Gln Val
65 70 75 80
Val Leu Thr Met Thr Asp Met Asp Pro Gly Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Arg Asn Leu Ala Phe Asn Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115

<210> 309
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 309
ggattctcac tcagtaatgt tagaatgggt 30

<210> 310
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 310
Gly Phe Ser Leu Ser Asn Val Arg Met Gly
1 5 10

[0097]

<210> 311
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 311
atttttcga atgacgaaaa a 21

<210> 312
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 312
Ile Phe Ser Asn Asp Glu Lys
1 5

<210> 313
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 313
gcacggatac ggaatttggc ctttaattac 30

<210> 314
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 314
Ala Arg Ile Arg Asn Leu Ala Phe Asn Tyr
1 5 10

[0098]

<210> 315
<211> 339
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 315
gacttcgtga tgacccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgca agtccagcca gagtgtgtta cacaggtcca gcaataagaa ctacttagct 120
tggtatcagc agaagccagg acagcctcct aacctgctca tttactgggc atctacccgg 180
gaatccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatggtact 300
ctatttactt tcggccctgg gaccaaagtg gatatcaaa 339

<210> 316
<211> 112
<212> PRT
<213> 人工序列

<220>

<223> 合成的

<400> 316

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Asp Phe Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu His Arg
            20                  25                  30
Ser Ser Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45
Pro Pro Asn Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95
Tyr Tyr Gly Thr Leu Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
            100                 105                 110
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<210> 317
<211> 36
<212> DNA
<213> 人工序列

[0099]

<220>
<223> 合成的

<400> 317

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cagagtgtgt tacacaggtc cagcaataag aactac                              36
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<210> 318
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 318

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Gln Ser Val Leu His Arg Ser Ser Asn Lys Asn Tyr
 1               5                  10
```

<210> 319
<211> 9
<212> DNA
<213> 人工序列

<220>
<223> 合成的

<400> 319
tgggcatct 9

<210> 320
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 320
Trp Ala Ser
1

<210> 321
<211> 27
<212> DNA
<213> 人工序列

[0100]

<220>
<223> 合成的

<400> 321
cagcaatatt atggtactct atttact 27

<210> 322
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 322
Gln Gln Tyr Tyr Gly Thr Leu Phe Thr
1 5

<210> 323
<211> 537
<212> PRT

<213> 人工序列

<220>
<223> 合成的

<400> 323

Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val
1 5 10 15
Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr
20 25 30
Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe
35 40 45
Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Ala Val Ala Asp Ser
50 55 60
Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly
65 70 75 80
Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro
85 90 95
Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys
100 105 110
Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu
115 120 125
Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His
[0101] 130 135 140
Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp
145 150 155 160
Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val
165 170 175
Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu
180 185 190
Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu
195 200 205
Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly
210 215 220
Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile
225 230 235 240
Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln
245 250 255
Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala
260 265 270
Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu
275 280 285
Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn
290 295 300
Pro Ile Asp His His Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
305 310 315 320
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys

325 330 335
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
340 345 350
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
355 360 365
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
370 375 380
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
385 390 395 400
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
405 410 415
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
420 425 430
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
435 440 445
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
450 455 460
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
465 470 475 480
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
485 490 495
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
500 505 510
[0102] Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
515 520 525
Lys Ser Leu Ser Leu Ser Pro Gly Lys
530 535

<210> 324
<211> 543
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 324
Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val
1 5 10 15
Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr
20 25 30
Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe
35 40 45
Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Ala Val Ala Asp Ser
50 55 60
Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly

65 70 75 80
Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro
85 90 95
Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys
100 105 110
Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu
115 120 125
Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His
130 135 140
Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp
145 150 155 160
Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val
165 170 175
Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu
180 185 190
Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu
195 200 205
Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly
210 215 220
Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile
225 230 235 240
Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln
245 250 255

[0103]
Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala
260 265 270
Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu
275 280 285
Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn
290 295 300
Pro Ile Asp His His Ser Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
305 310 315 320
Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
325 330 335
Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
340 345 350
Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
355 360 365
Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
370 375 380
Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
385 390 395 400
Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
405 410 415
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
420 425 430
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
435 440 445

Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
450 455 460
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
465 470 475 480
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
485 490 495
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
500 505 510
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
515 520 525
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
530 535 540

<210> 325
<211> 884
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 325
[0104] Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val
1 5 10 15
Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr
20 25 30
Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe
35 40 45
Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Ala Val Ala Asp Ser
50 55 60
Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly
65 70 75 80
Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro
85 90 95
Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys
100 105 110
Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu
115 120 125
Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His
130 135 140
Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp
145 150 155 160
Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val
165 170 175
Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu
180 185 190

Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu
195 200 205
Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly
210 215 220
Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile
225 230 235 240
Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln
245 250 255
Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala
260 265 270
Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu
275 280 285
Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn
290 295 300
Pro Ile Asp His His Ser Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp
305 310 315 320
Thr Met Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys
325 330 335
Cys Pro Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His
340 345 350
Ser Ala Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp
355 360 365
Leu Glu Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys
[0105] 370 375 380
Glu Lys Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly
385 390 395 400
Asn Tyr Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala
405 410 415
Phe Pro Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met
420 425 430
Lys Leu Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile
435 440 445
Thr Cys Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr
450 455 460
Ile Thr Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val
465 470 475 480
Ile Pro Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn
485 490 495
Asn Gly Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr
500 505 510
Phe His Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys
515 520 525
Asn Ala Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr
530 535 540
Glu Lys Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe
545 550 555 560
Ser Phe Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly

565 570 575
Lys Lys Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile
580 585 590
Ser His Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile
595 600 605
Lys Lys Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala
610 615 620
Arg Ser Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys
625 630 635 640
Val Pro Ala Pro Arg Tyr Thr Val Glu Ser Gly Glu Pro Arg Gly Pro
645 650 655
Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
660 665 670
Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
675 680 685
Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
690 695 700
Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
705 710 715 720
Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
725 730 735
Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
740 745 750

[0106] Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
755 760 765
Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
770 775 780
Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
785 790 795 800
Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
805 810 815
Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu
820 825 830
Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
835 840 845
Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
850 855 860
Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
865 870 875 880
Thr Pro Gly Lys

<210> 326
<211> 880
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 326

[0107]

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Ser Lys Ser Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val Arg Cys
 1               5                  10                  15
Pro Gln Arg Gly Arg Ser Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp
            20                  25                  30
Thr Asn Glu Ser Ile Pro Thr Gln Lys Arg Asn Arg Ile Phe Val Ser
        35                  40                  45
Arg Asp Arg Leu Lys Phe Leu Pro Ala Arg Val Glu Asp Ser Gly Ile
    50                  55                  60
Tyr Ala Cys Val Ile Arg Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu
65                  70                  75                  80
Asn Val Thr Ile His Lys Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr
                85                  90                  95
Leu Met Tyr Ser Thr Val Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr
            100                 105                 110
Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe
        115                 120                 125
Lys Asn Cys Lys Ala Leu Gln Glu Pro Arg Phe Arg Ala His Arg Ser
    130                 135                 140
Tyr Leu Phe Ile Asp Asn Val Thr His Asp Asp Glu Gly Asp Tyr Thr
145                 150                 155                 160
Cys Gln Phe Thr His Ala Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala
                165                 170                 175
Thr Arg Ser Phe Thr Val Glu Glu Lys Gly Phe Ser Met Phe Pro Val
            180                 185                 190
Ile Thr Asn Pro Pro Tyr Asn His Thr Met Glu Val Glu Ile Gly Lys
        195                 200                 205
Pro Ala Ser Ile Ala Cys Ser Ala Cys Phe Gly Lys Gly Ser His Phe
    210                 215                 220
Leu Ala Asp Val Leu Trp Gln Ile Asn Lys Thr Val Val Gly Asn Phe
225                 230                 235                 240
Gly Glu Ala Arg Ile Gln Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser
                245                 250                 255
Asn Asp Met Asp Cys Leu Thr Ser Val Leu Arg Ile Thr Gly Val Thr
            260                 265                 270
Glu Lys Asp Leu Ser Leu Glu Tyr Asp Cys Leu Ala Leu Asn Leu His
        275                 280                 285
Gly Met Ile Arg His Thr Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp
    290                 295                 300
His Arg Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met Arg Gln
305                 310                 315                 320
Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro Leu Phe
                325                 330                 335
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Glu His Phe Leu Lys Tyr Asn Tyr Ser Thr Ala His Ser Ser Gly Leu
340 345 350
Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu Glu Pro
355 360 365
Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys Asp Val
370 375 380
Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr Thr Cys
385 390 395 400
Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro Leu Glu
405 410 415
Val Val Gln Lys Asp Ser Cys Phe Asn Ser Ala Met Arg Phe Pro Val
420 425 430
His Lys Met Tyr Ile Glu His Gly Ile His Lys Ile Thr Cys Pro Asn
435 440 445
Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Ser Val Thr Trp Tyr
450 455 460
Lys Gly Cys Thr Glu Ile Val Asp Phe His Asn Val Leu Pro Glu Gly
465 470 475 480
Met Asn Leu Ser Phe Phe Ile Pro Leu Val Ser Asn Asn Gly Asn Tyr
485 490 495
Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Leu Phe His Leu Thr
500 505 510
Arg Thr Val Thr Val Lys Val Val Gly Ser Pro Lys Asp Ala Leu Pro
[0108] 515 520 525
Pro Gln Ile Tyr Ser Pro Asn Asp Arg Val Val Tyr Glu Lys Glu Pro
530 535 540
Gly Glu Glu Leu Val Ile Pro Cys Lys Val Tyr Phe Ser Phe Ile Met
545 550 555 560
Asp Ser His Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys Pro Asp
565 570 575
Asp Val Thr Val Asp Ile Thr Ile Asn Glu Ser Val Ser Tyr Ser Ser
580 585 590
Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys Val Thr
595 600 605
Pro Glu Asp Leu Arg Arg Asn Tyr Val Cys His Ala Arg Asn Thr Lys
610 615 620
Gly Glu Ala Glu Gln Ala Ala Lys Val Lys Gln Lys Val Ile Pro Pro
625 630 635 640
Arg Tyr Thr Val Glu Ser Gly Glu Pro Arg Gly Pro Thr Ile Lys Pro
645 650 655
Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser
660 665 670
Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu
675 680 685
Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro
690 695 700
Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala

705 710 715 720
Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val
725 730 735
Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe
740 745 750
Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr
755 760 765
Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu
770 775 780
Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys
785 790 795 800
Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn
805 810 815
Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp
820 825 830
Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys
835 840 845
Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly
850 855 860
Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
865 870 875 880

[0109] <210> 327
<211> 310
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 327
Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val
1 5 10 15
Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr
20 25 30
Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe
35 40 45
Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Ala Val Ala Asp Ser
50 55 60
Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly
65 70 75 80
Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro
85 90 95
Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys
100 105 110
Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu

```
          115                 120                 125
Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His
      130                 135                 140
Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp
145                 150                 155                 160
Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val
                  165                 170                 175
Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu
              180                 185                 190
Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu
          195                 200                 205
Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly
      210                 215                 220
Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile
225                 230                 235                 240
Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln
                  245                 250                 255
Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala
              260                 265                 270
Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu
          275                 280                 285
Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn
      290                 295                 300
Pro Ile Asp His His Ser
305                 310
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[0110]

<210> 328
<211> 306
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 328

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Ser Lys Ser Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val Arg Cys
 1               5                  10                  15
Pro Gln Arg Gly Arg Ser Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp
            20                  25                  30
Thr Asn Glu Ser Ile Pro Thr Gln Lys Arg Asn Arg Ile Phe Val Ser
        35                  40                  45
Arg Asp Arg Leu Lys Phe Leu Pro Ala Arg Val Glu Asp Ser Gly Ile
    50                  55                  60
Tyr Ala Cys Val Ile Arg Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu
65                  70                  75                  80
Asn Val Thr Ile His Lys Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr
```

85 90 95
Leu Met Tyr Ser Thr Val Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr
100 105 110
Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe
115 120 125
Lys Asn Cys Lys Ala Leu Gln Glu Pro Arg Phe Arg Ala His Arg Ser
130 135 140
Tyr Leu Phe Ile Asp Asn Val Thr His Asp Asp Glu Gly Asp Tyr Thr
145 150 155 160
Cys Gln Phe Thr His Ala Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala
165 170 175
Thr Arg Ser Phe Thr Val Glu Glu Lys Gly Phe Ser Met Phe Pro Val
180 185 190
Ile Thr Asn Pro Pro Tyr Asn His Thr Met Glu Val Glu Ile Gly Lys
195 200 205
Pro Ala Ser Ile Ala Cys Ser Ala Cys Phe Gly Lys Gly Ser His Phe
210 215 220
Leu Ala Asp Val Leu Trp Gln Ile Asn Lys Thr Val Val Gly Asn Phe
225 230 235 240
Gly Glu Ala Arg Ile Gln Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser
245 250 255
Asn Asp Met Asp Cys Leu Thr Ser Val Leu Arg Ile Thr Gly Val Thr
260 265 270
[0111] Glu Lys Asp Leu Ser Leu Glu Tyr Asp Cys Leu Ala Leu Asn Leu His
275 280 285
Gly Met Ile Arg His Thr Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp
290 295 300
His Arg
305

<210> 329
<211> 339
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 329
Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met Arg Gln Ile Gln
1 5 10 15
Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro Leu Phe Glu His
20 25 30
Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala Gly Leu Thr Leu
35 40 45
Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu Glu Pro Ile Asn

50 55 60

Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys Asp Val Leu Trp
65 70 75 80
Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr Thr Cys Met Leu
85 90 95
Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro Leu Glu Val Val
100 105 110
Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu Pro Val His Lys
115 120 125
Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys Pro Asn Val Asp
130 135 140
Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr Trp Tyr Met Gly
145 150 155 160
Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro Glu Gly Met Asn
165 170 175
Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly Asn Tyr Thr Cys
180 185 190
Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His Leu Thr Arg Thr
195 200 205
Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala Val Pro Pro Val
210 215 220
Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys Glu Pro Gly Glu
225 230 235 240

[0112]

Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe Leu Met Asp Ser
245 250 255
Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys Pro Asp Asp Ile
260 265 270
Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His Ser Arg Thr Glu
275 280 285
Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys Val Thr Ser Glu
290 295 300
Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser Ala Lys Gly Glu
305 310 315 320
Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro Ala Pro Arg Tyr
325 330 335
Thr Val Glu

<210> 330
<211> 339
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 330

Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met Arg Gln Ile Gln
1 5 10 15
Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro Leu Phe Glu His
20 25 30
Phe Leu Lys Tyr Asn Tyr Ser Thr Ala His Ser Ser Gly Leu Thr Leu
35 40 45
Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu Glu Pro Ile Asn
50 55 60
Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys Asp Val Leu Trp
65 70 75 80
Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr Thr Cys Met Leu
85 90 95
Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro Leu Glu Val Val
100 105 110
Gln Lys Asp Ser Cys Phe Asn Ser Ala Met Arg Phe Pro Val His Lys
115 120 125
Met Tyr Ile Glu His Gly Ile His Lys Ile Thr Cys Pro Asn Val Asp
130 135 140
Gly Tyr Phe Pro Ser Ser Val Lys Pro Ser Val Thr Trp Tyr Lys Gly
145 150 155 160
[0113] Cys Thr Glu Ile Val Asp Phe His Asn Val Leu Pro Glu Gly Met Asn
165 170 175
Leu Ser Phe Phe Ile Pro Leu Val Ser Asn Asn Gly Asn Tyr Thr Cys
180 185 190
Val Val Thr Tyr Pro Glu Asn Gly Arg Leu Phe His Leu Thr Arg Thr
195 200 205
Val Thr Val Lys Val Val Gly Ser Pro Lys Asp Ala Leu Pro Pro Gln
210 215 220
Ile Tyr Ser Pro Asn Asp Arg Val Val Tyr Glu Lys Glu Pro Gly Glu
225 230 235 240
Glu Leu Val Ile Pro Cys Lys Val Tyr Phe Ser Phe Ile Met Asp Ser
245 250 255
His Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys Pro Asp Asp Val
260 265 270
Thr Val Asp Ile Thr Ile Asn Glu Ser Val Ser Tyr Ser Ser Thr Glu
275 280 285
Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys Val Thr Pro Glu
290 295 300
Asp Leu Arg Arg Asn Tyr Val Cys His Ala Arg Asn Thr Lys Gly Glu
305 310 315 320
Ala Glu Gln Ala Ala Lys Val Lys Gln Lys Val Ile Pro Pro Arg Tyr
325 330 335
Thr Val Glu

<210> 331
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 331
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110
[0114] Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220
Pro Gly Lys
225

<210> 332
<211> 233
<212> PRT
<213> 人工序列

<220>

<223> 合成的

<400> 332

Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
1 5 10 15
Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
20 25 30
Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
35 40 45
Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
50 55 60
Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
65 70 75 80
Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
85 90 95
Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
100 105 110
Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
115 120 125
Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
130 135 140
Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
145 150 155 160

[0115] Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn
165 170 175
Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
180 185 190
Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
195 200 205
Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
210 215 220
Lys Ser Phe Ser Arg Thr Pro Gly Lys
225 230

<210> 333
<211> 167
<212> PRT
<213> 人工序列

<220>
<223> 合成的

<400> 333

Ser Ile Thr Gly Ile Ser Pro Ile Thr Glu Ser Leu Ala Ser Leu Ser
1 5 10 15
Thr Tyr Asn Asp Gln Ser Ile Thr Phe Ala Leu Glu Asp Glu Ser Tyr

```
                20                  25                  30
Glu Ile Tyr Val Glu Asp Leu Lys Lys Asp Lys Lys Lys Asp Lys Val
        35                  40                  45
Leu Leu Ser Tyr Tyr Glu Ser Gln His Pro Ser Ser Glu Ser Gly Asp
    50                  55                  60
Gly Val Asp Gly Lys Met Leu Met Val Thr Leu Ser Pro Thr Lys Asp
65                  70                  75                  80
Phe Trp Leu Gln Ala Asn Asn Lys Glu His Ser Val Glu Leu His Lys
                85                  90                  95
Cys Glu Lys Pro Leu Pro Asp Gln Ala Phe Phe Val Leu His Asn Arg
            100                 105                 110
Ser Phe Asn Cys Val Ser Phe Glu Cys Lys Thr Asp Pro Gly Val Phe
        115                 120                 125
Ile Gly Val Lys Asp Asn His Leu Ala Leu Ile Lys Val Asp Tyr Ser
    130                 135                 140
Glu Asn Leu Gly Ser Glu Asn Ile Leu Phe Lys Leu Ser Glu Ile Leu
145                 150                 155                 160
Glu His His His His His His
                165
```

<210> 334
<211> 167
[0116] <212> PRT
<213> 人工序列

<220>
<223> 氨基酸 1-159：成束猴 IL-33（在 E269 之后具有额外的 I 的 EHH57404.1 的 S112-E269）
氨基酸 160-161：接头
氨基酸 162-167：六组氨酸标签

<400> 334

```
Ser Ile Thr Gly Ile Ser Pro Ile Thr Glu Ser Leu Ala Ser Leu Ser
 1               5                  10                  15
Thr Tyr Asn Asp Gln Ser Ile Thr Phe Ala Leu Glu Asp Glu Ser Tyr
            20                  25                  30
Glu Ile Tyr Val Glu Asp Leu Lys Lys Asp Lys Lys Lys Asp Lys Val
        35                  40                  45
Leu Leu Ser Tyr Tyr Glu Ser Gln His Pro Ser Ser Glu Ser Gly Asp
    50                  55                  60
Gly Val Asp Gly Lys Met Leu Met Val Thr Leu Ser Pro Thr Lys Asp
65                  70                  75                  80
Phe Trp Leu Gln Ala Asn Asn Lys Glu His Ser Val Glu Leu His Lys
                85                  90                  95
Cys Glu Lys Pro Leu Pro Asp Gln Ala Phe Phe Val Leu His Asn Arg
            100                 105                 110
```

Ser Phe Asn Cys Val Ser Phe Glu Cys Lys Thr Asp Pro Gly Val Phe
115 120 125
Ile Gly Val Lys Asp Asn His Leu Ala Leu Ile Lys Val Asp Tyr Ser
130 135 140
Glu Asn Leu Gly Ser Glu Asn Ile Leu Phe Lys Leu Ser Glu Ile Leu
145 150 155 160
Glu His His His His His His
165

<210> 335
<211> 876
<212> PRT
<213> 人工序列

<220>
<223> 氨基酸 1-310：人 ST2 (NP_057316.3 的 K19-S328)
氨基酸 311-649：人 IL1RacP (Q9NPH3 的 S21-E359)
氨基酸 650-876：hFc 标签(P01857 的 D104-K330)

<400> 335
Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val
1 5 10 15
[0117] Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr
20 25 30
Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe
35 40 45
Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Ala Val Ala Asp Ser
50 55 60
Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly
65 70 75 80
Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro
85 90 95
Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys
100 105 110
Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu
115 120 125
Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His
130 135 140
Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp
145 150 155 160
Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val
165 170 175
Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu
180 185 190
Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu
195 200 205

Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly
210 215 220
Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile
225 230 235 240
Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln
245 250 255
Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala
260 265 270
Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu
275 280 285
Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn
290 295 300
Pro Ile Asp His His Ser Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp
305 310 315 320
Thr Met Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys
325 330 335
Cys Pro Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His
340 345 350
Ser Ala Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp
355 360 365
Leu Glu Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys
370 375 380
Glu Lys Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly
[0118] 385 390 395 400
Asn Tyr Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala
405 410 415
Phe Pro Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met
420 425 430
Lys Leu Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile
435 440 445
Thr Cys Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr
450 455 460
Ile Thr Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val
465 470 475 480
Ile Pro Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn
485 490 495
Asn Gly Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr
500 505 510
Phe His Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys
515 520 525
Asn Ala Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr
530 535 540
Glu Lys Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe
545 550 555 560
Ser Phe Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly
565 570 575
Lys Lys Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile

580 585 590
Ser His Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile
595 600 605
Lys Lys Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala
610 615 620
Arg Ser Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys
625 630 635 640
Val Pro Ala Pro Arg Tyr Thr Val Glu Asp Lys Thr His Thr Cys Pro
645 650 655
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
660 665 670
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
675 680 685
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
690 695 700
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
705 710 715 720
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
[0119] 725 730 735
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
740 745 750
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
755 760 765
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
770 775 780
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
785 790 795 800
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
805 810 815
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
820 825 830
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
835 840 845
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
850 855 860
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
865 870 875

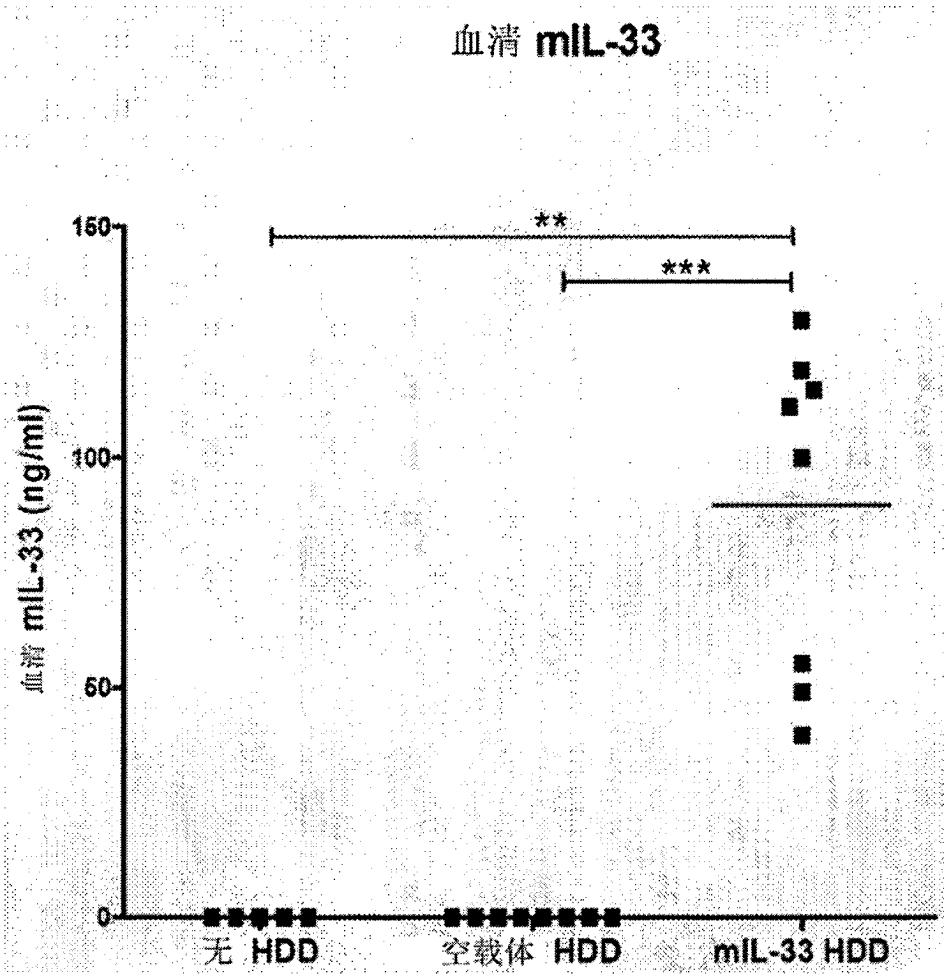

图1A

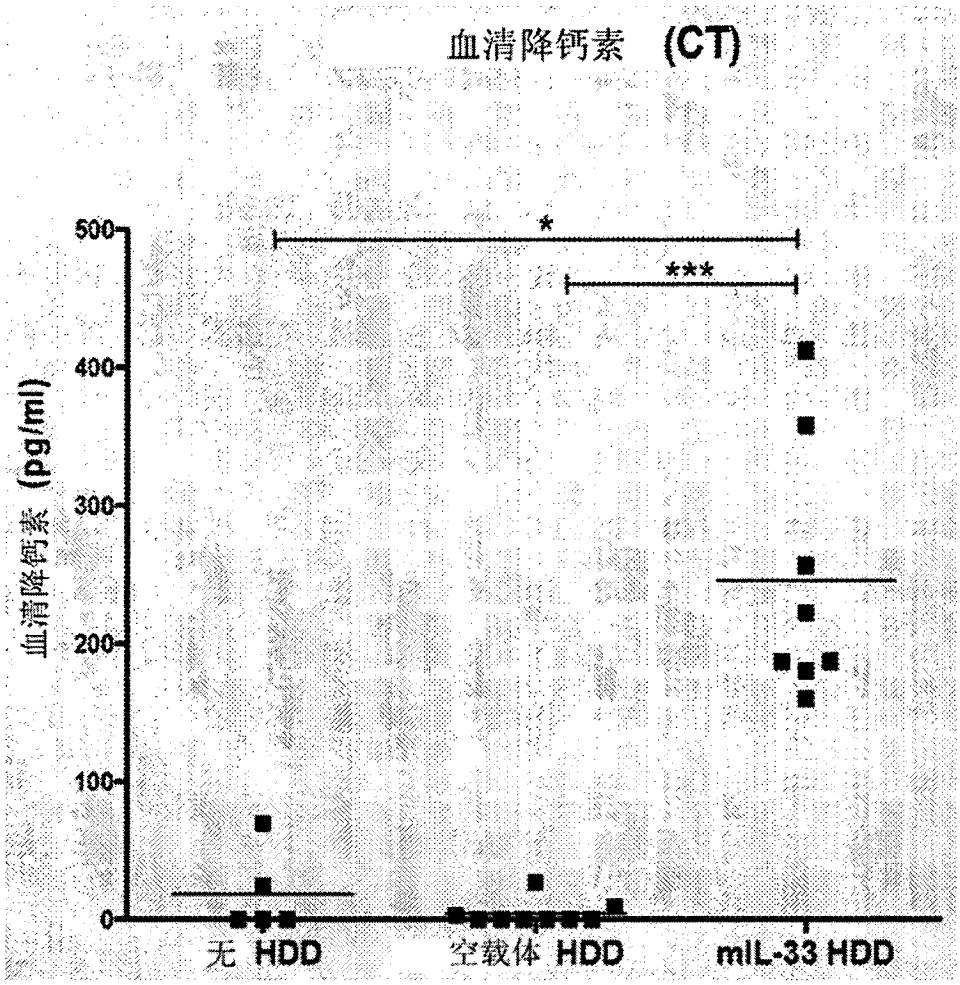
血清降钙素 (CT)
血清降钙素 (pg/ml)
500
400
300
200
100
0
*
***
无 HDD
空载体 HDD
mIL-33 HDD

图1B

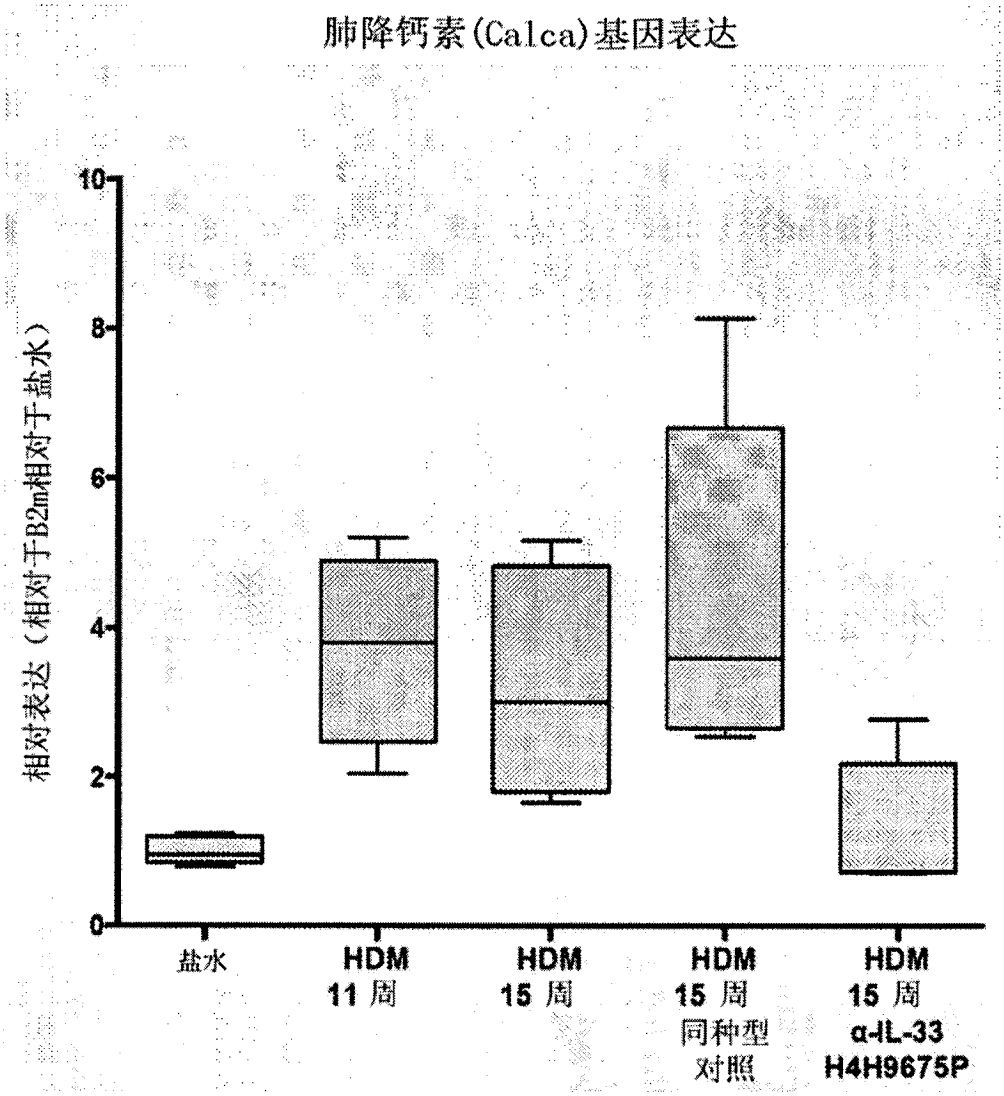
肺降钙素(Calca)基因表达
相对表达（相对于B2m相对于盐水）
10
8
6
4
2
0
盐水
HDM 11 周
HDM 15 周
HDM 15 周 同种型 对照
HDM 15 周 α-IL-33 H4H9675P

图2

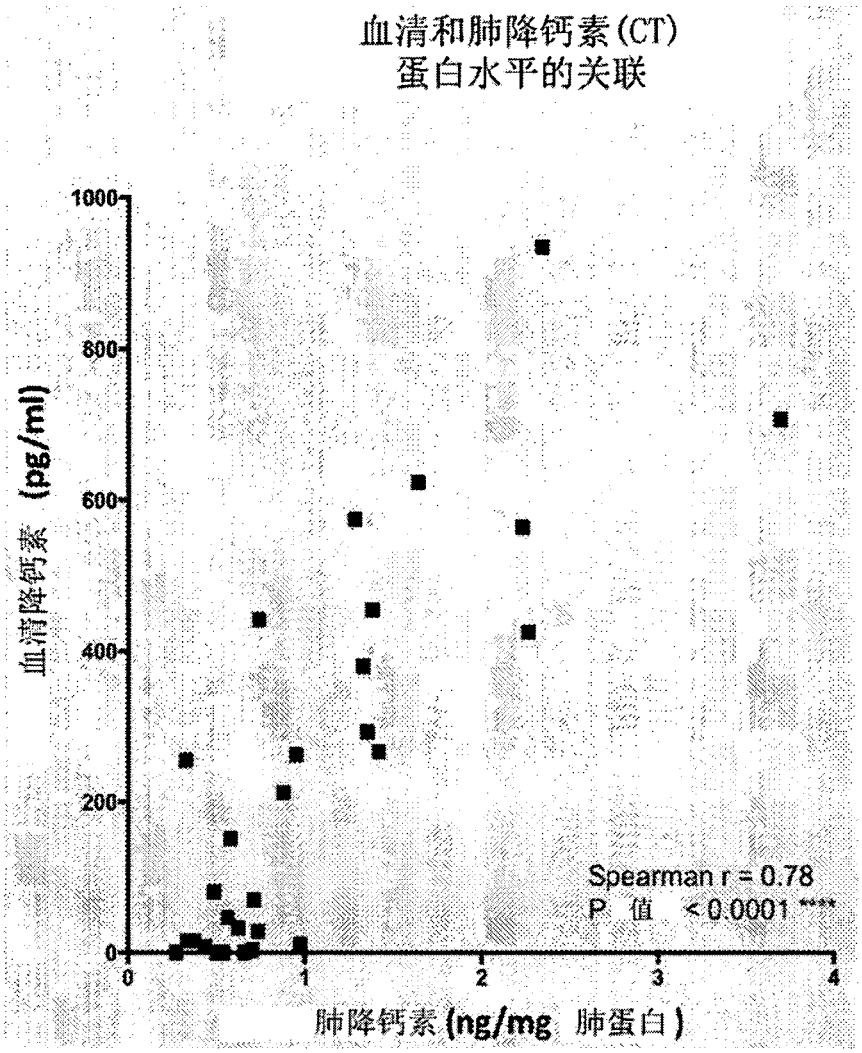
血清和肺降钙素(CT)
蛋白水平的关联
血清降钙素 (pg/ml)
1000
800
600
400
200
0
0
1
2
3
4
肺降钙素(ng/mg 肺蛋白)
Spearman r = 0.78
P 值 < 0.0001 ****

图3

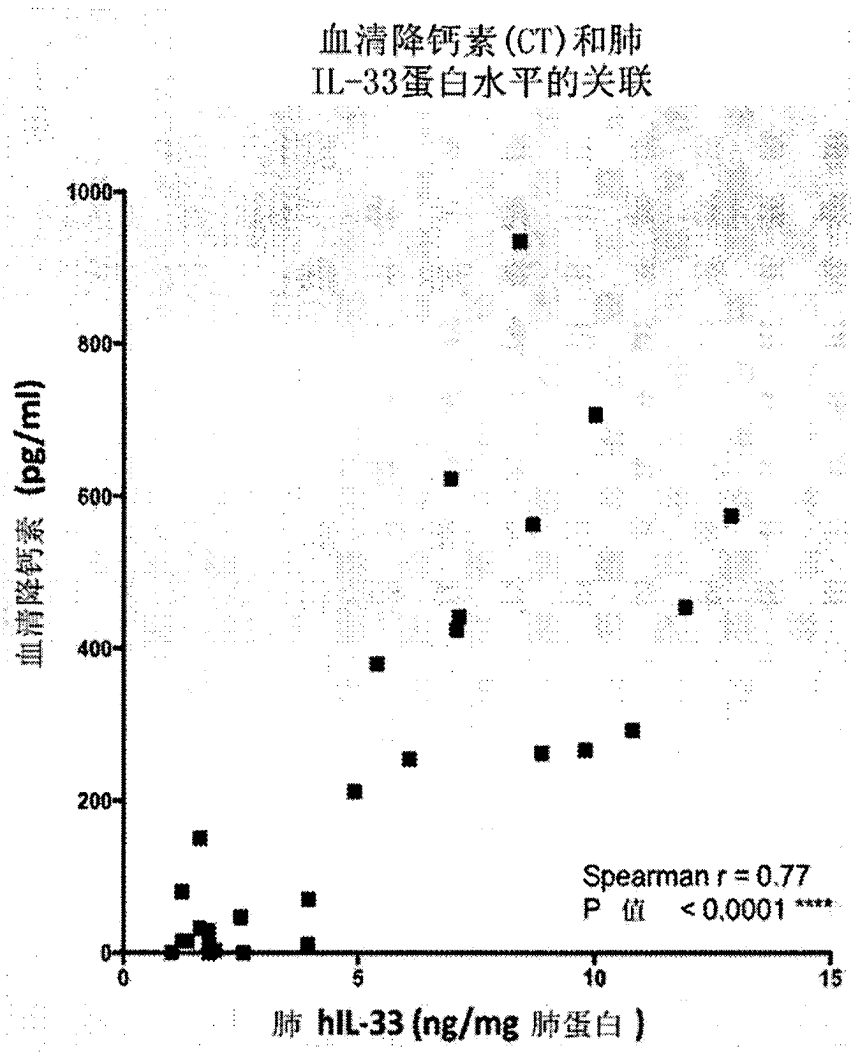
血清降钙素(CT)和肺
IL-33蛋白水平的关联
血清降钙素 (pg/ml)
1000
800
600
400
200
0
0
5
10
15
Spearman r = 0.77
P 值 < 0.0001 ****
肺 hIL-33 (ng/mg 肺蛋白)

图4

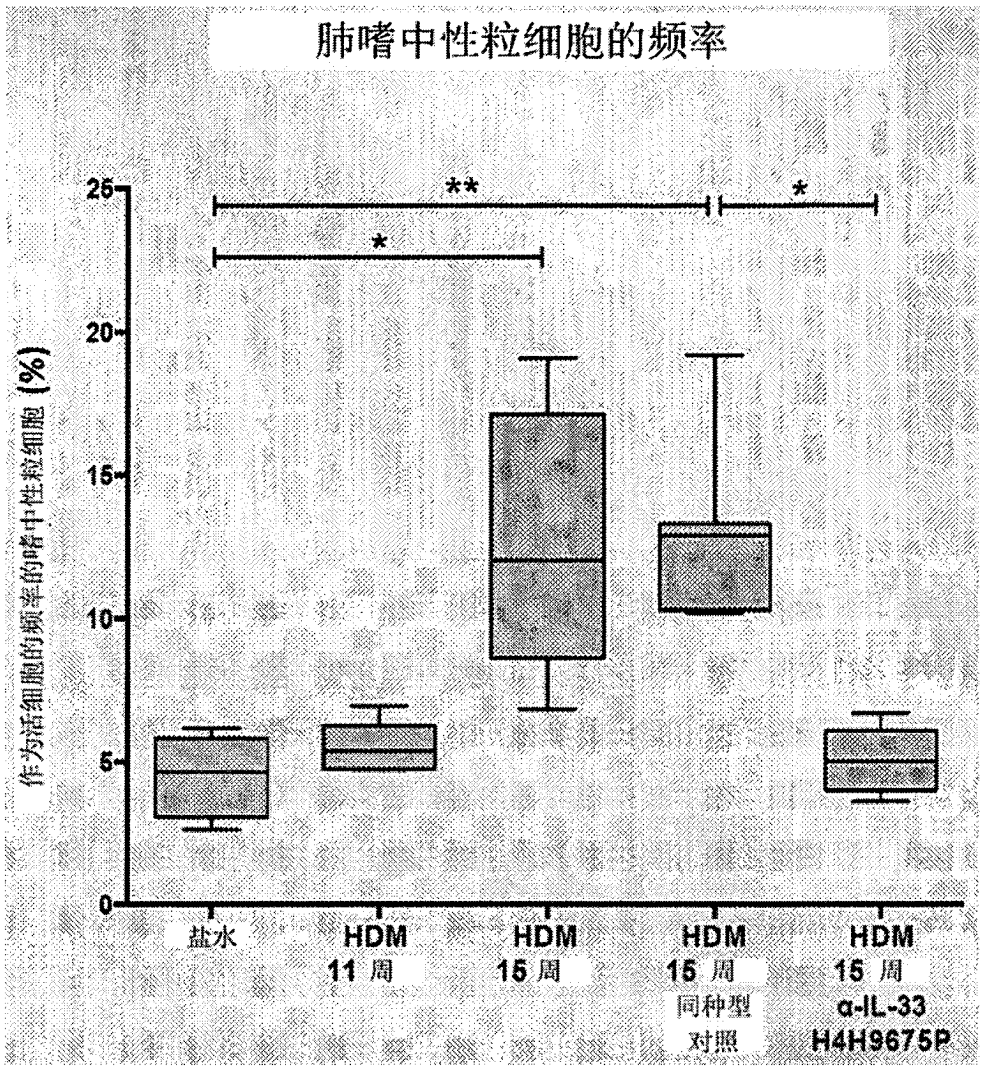
肺嗜中性粒细胞的频率
作为活细胞的肺中性粒细胞的频率 (%)
25
20
15
10
5
0
**
*
*
盐水
HDM 11 周
HDM 15 周
HDM 15 周 同种型对照
HDM 15 周 α-IL-33 H4H9675P

图5A

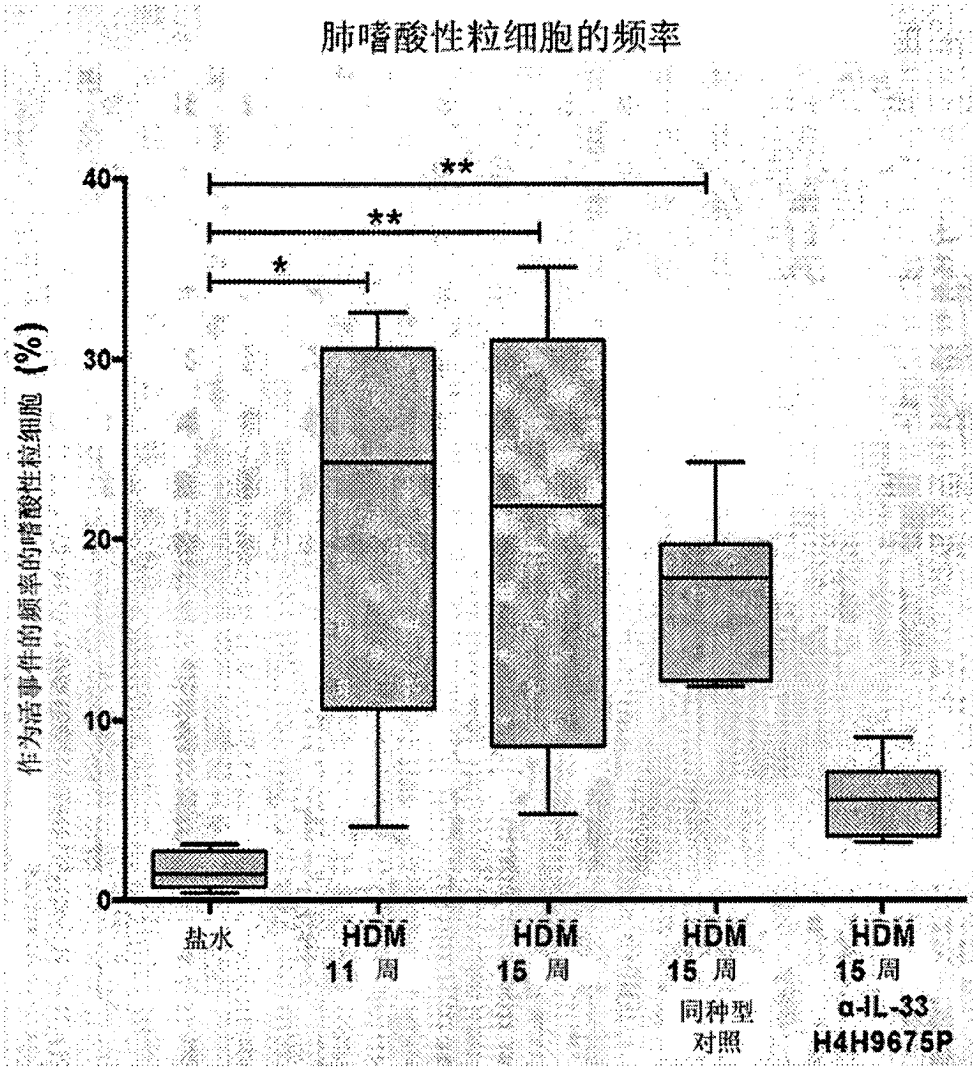
肺嗜酸性粒细胞的频率
作为活事件的频率的嗜酸性粒细胞 (%)
40
30
20
10
0
**
**
*
盐水
HDM 11 周
HDM 15 周
HDM 15 周 同种型对照
HDM 15 周 α-IL-33 H4H9675P

图5B

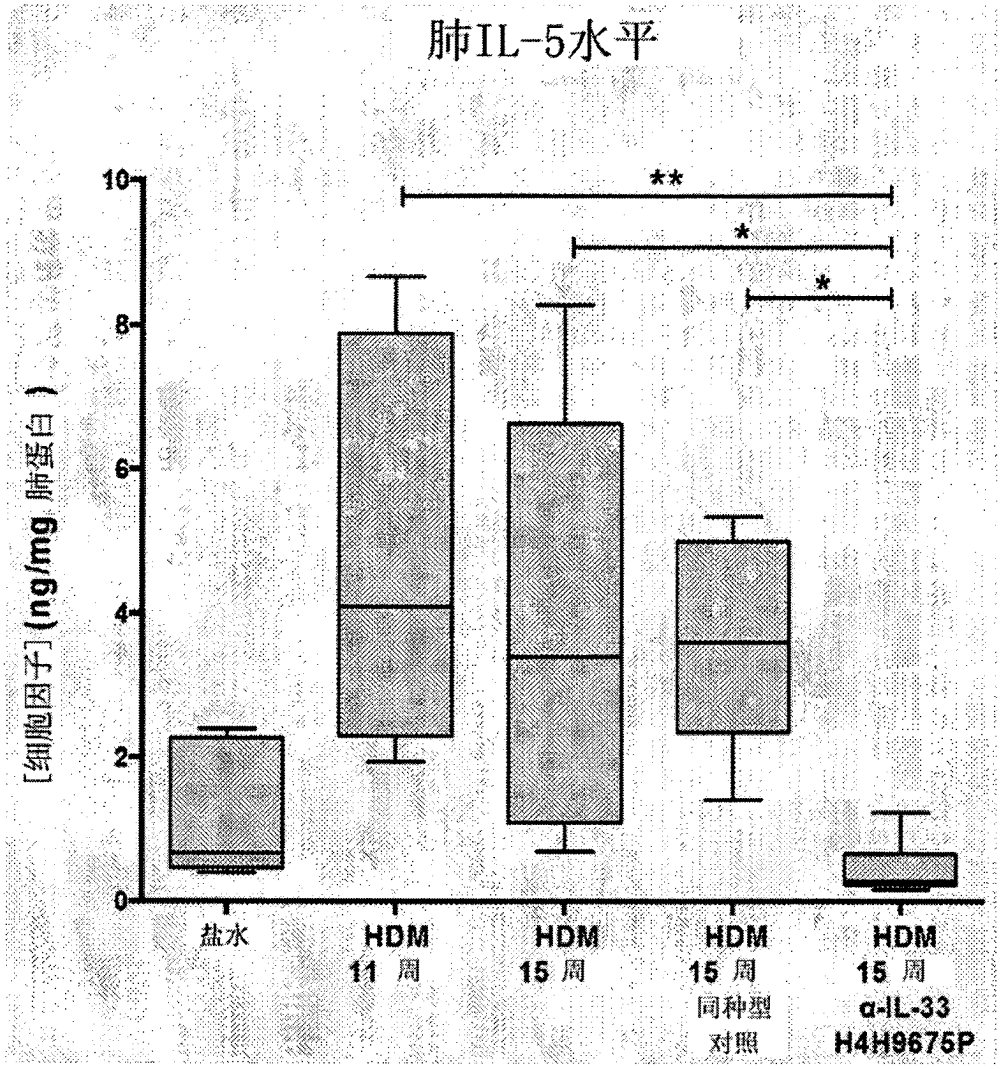
肺IL-5水平
[细胞因子] (ng/mg 肺蛋白)
10
8
6
4
2
0
**
*
*
盐水
HDM 11 周
HDM 15 周
HDM 15 周 同种型 对照
HDM 15 周 α-IL-33 H4H9675P

图5C

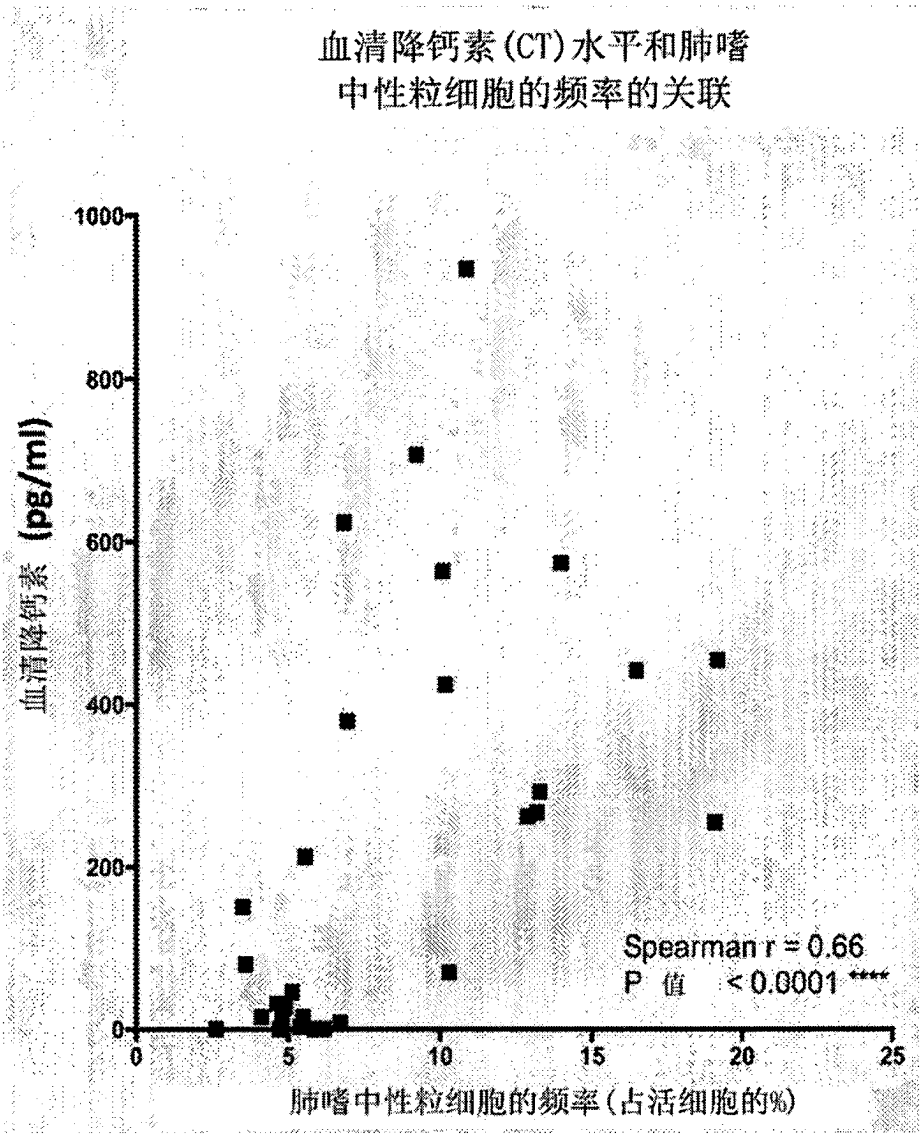
血清降钙素(CT)水平和肺嗜
中性粒细胞的频率的关联
血清降钙素 (pg/ml)
1000
800
600
400
200
0
0
5
10
15
20
25
肺嗜中性粒细胞的频率(占活细胞的%)
Spearman r = 0.66
P 值 < 0.0001 ****

图6A

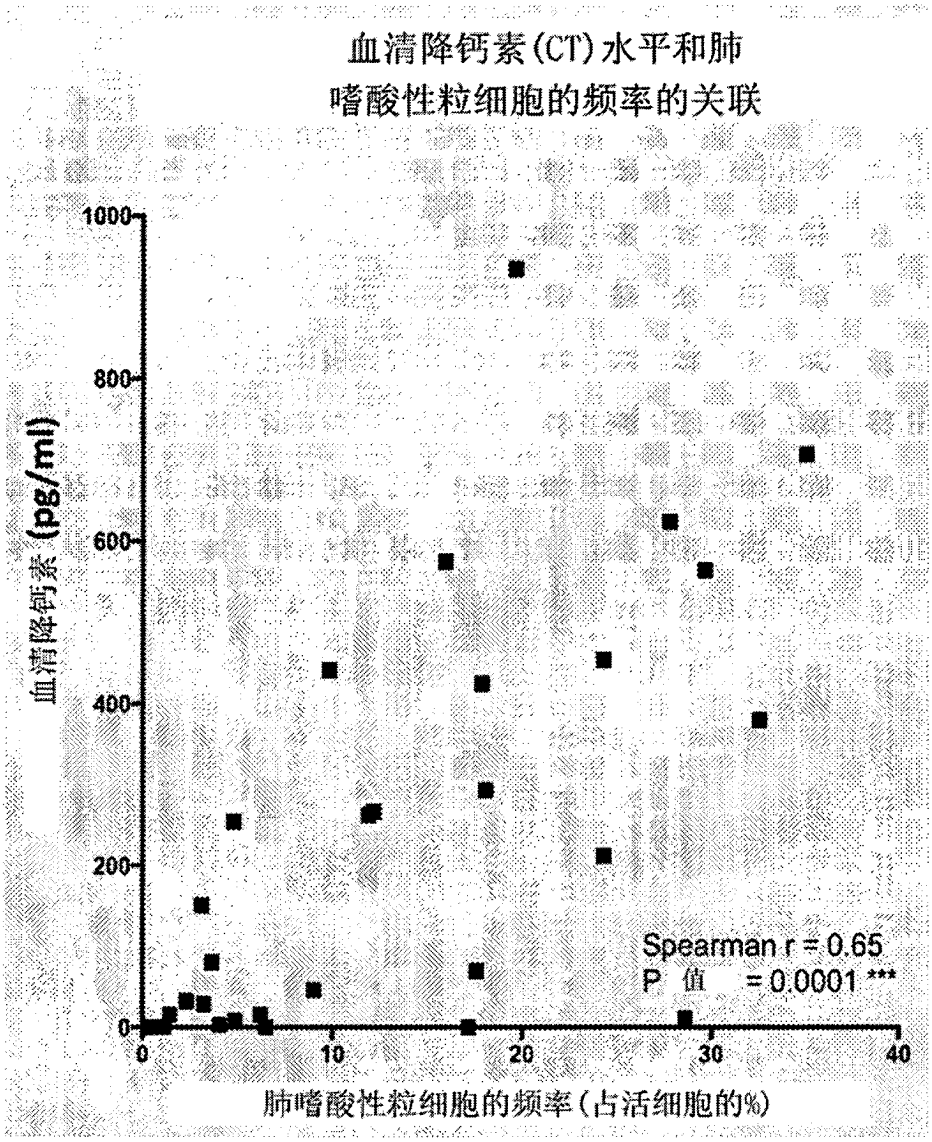
血清降钙素(CT)水平和肺
嗜酸性粒细胞的频率的关联
血清降钙素 (pg/ml)
1000
800
600
400
200
0
0
10
20
30
40
Spearman r = 0.65
P 值 = 0.0001 ***
肺嗜酸性粒细胞的频率(占活细胞的%)

图6B

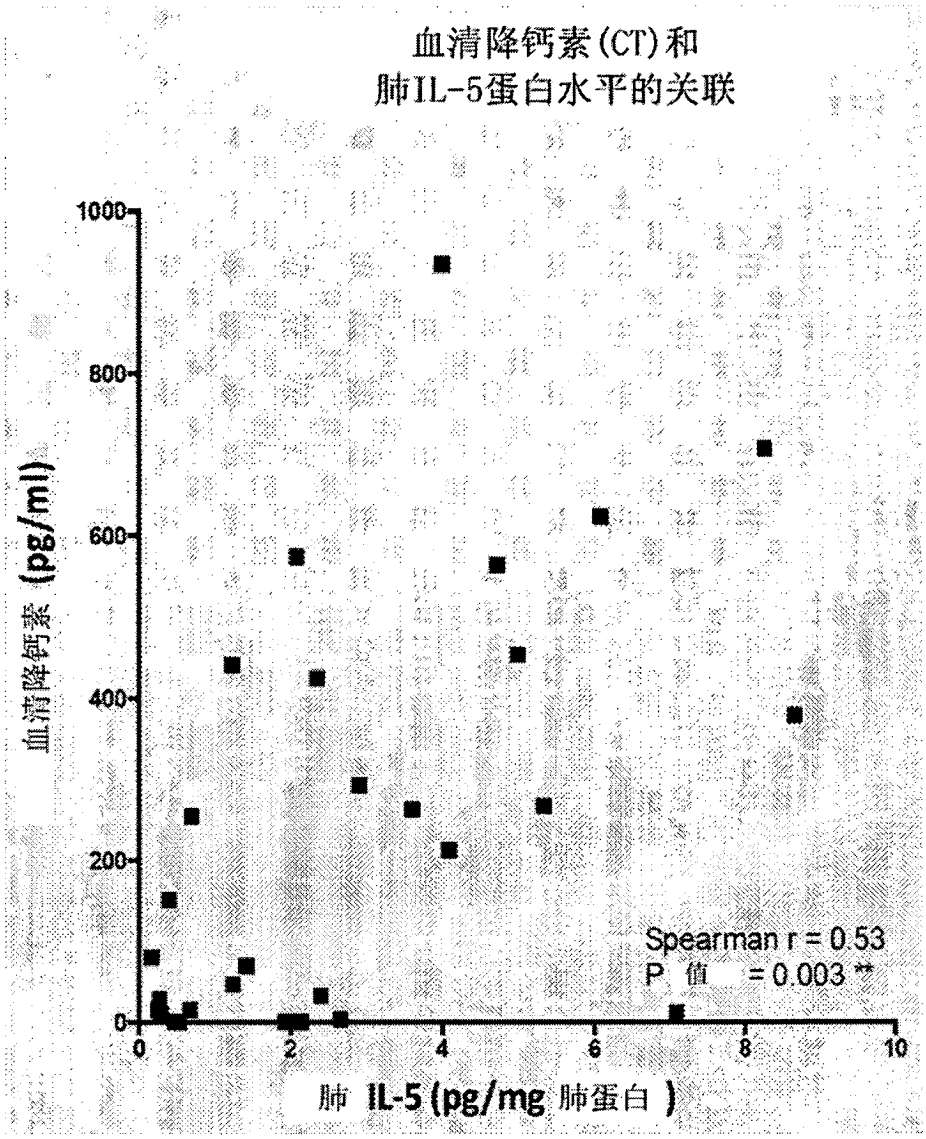
血清降钙素(CT)和
肺IL-5蛋白水平的关联
血清降钙素 (pg/ml)
1000
800
600
400
200
0
0
2
4
6
8
10
肺 IL-5 (pg/mg 肺蛋白)
Spearman r = 0.53
P 值 = 0.003 **

图6C

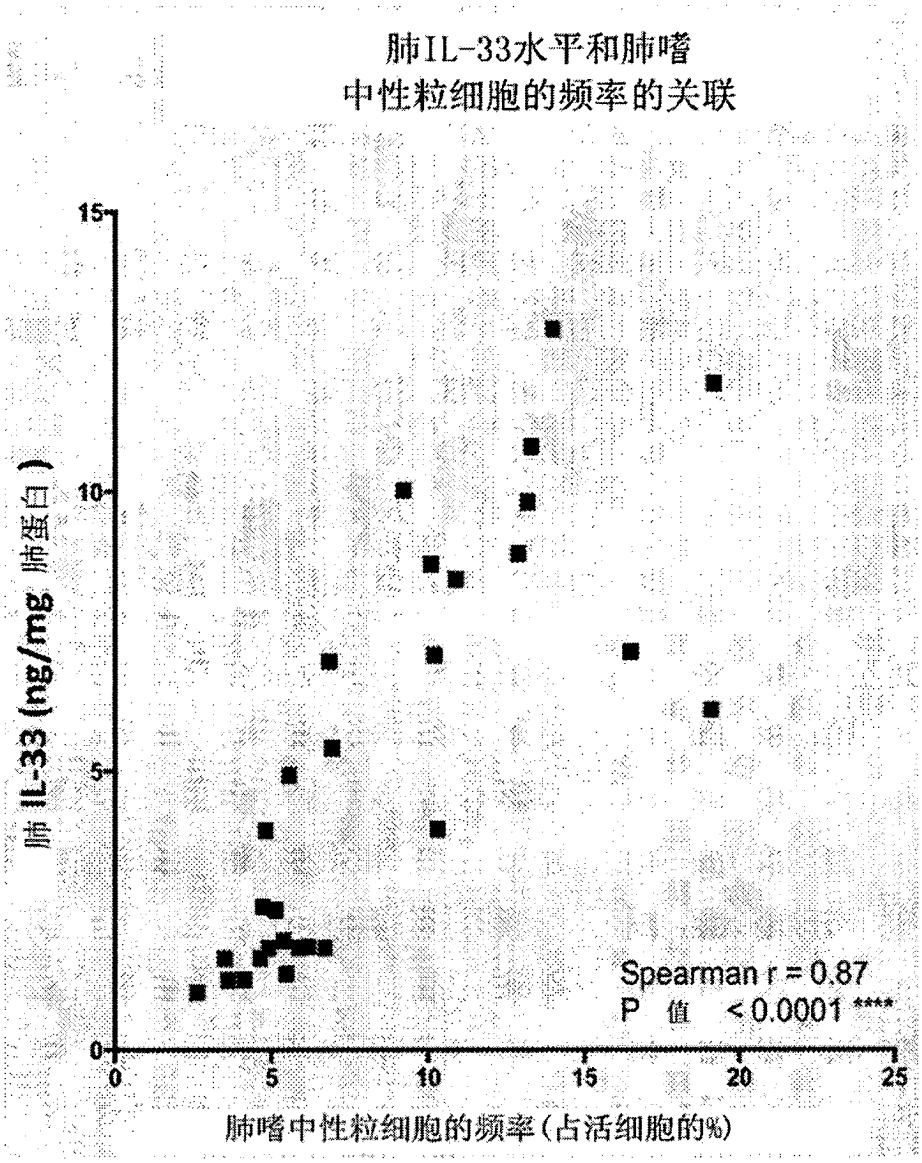
肺IL-33水平和肺嗜
中性粒细胞的频率的关联
肺 IL-33 (ng/mg 肺蛋白)
15
10
5
0
0
5
10
15
20
25
Spearman r = 0.87
P 值 < 0.0001 ****
肺嗜中性粒细胞的频率(占活细胞的%)

图7A

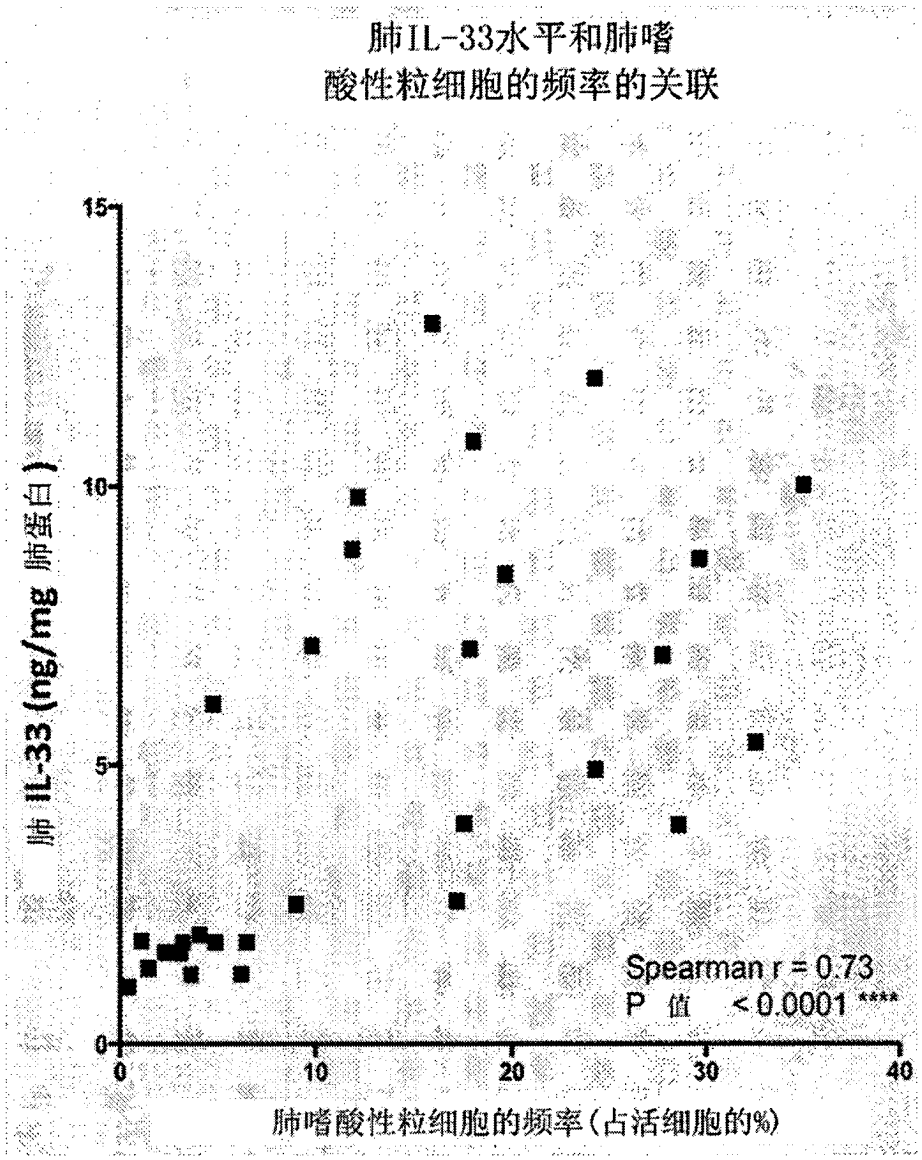
肺IL-33水平和肺嗜
酸性粒细胞的频率的关联
肺 IL-33 (ng/mg 肺蛋白)
15
10
5
0
0
10
20
30
40
肺嗜酸性粒细胞的频率(占活细胞的%)
Spearman r = 0.73
P 值 < 0.0001 ****

图7B

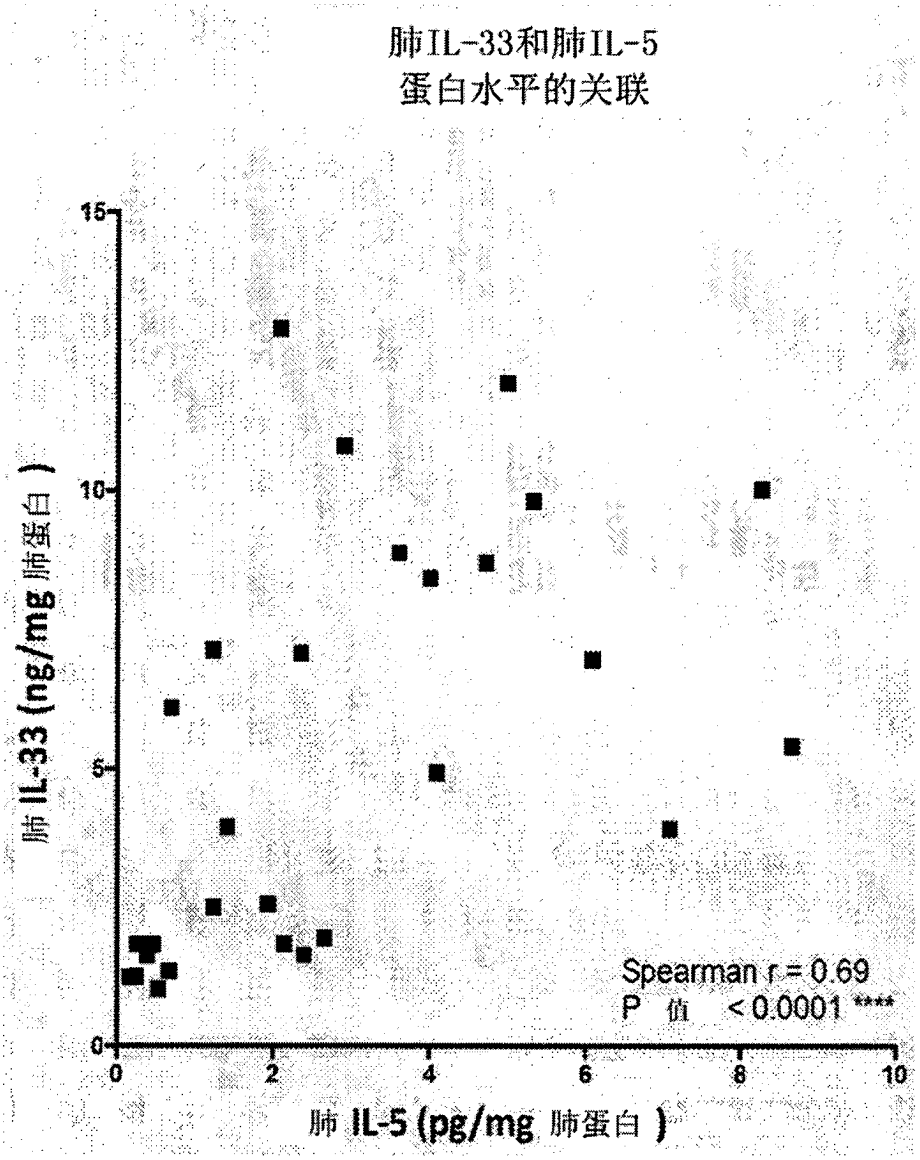
肺IL-33和肺IL-5
蛋白水平的关联
肺 IL-33 (ng/mg 肺蛋白)
肺 IL-5 (pg/mg 肺蛋白)
Spearman r = 0.69
P 值 < 0.0001 ****
0
5
10
15
0
2
4
6
8
10

图7C

Abstract

The present invention relates to the identification of certain biomarkers for use in identifying patients who have, or are likely to develop an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein. Also provided are methods for decreasing the level of at least one biomarker in a subject suffering from an IL-33-mediated disease or disorder, and methods for treating such diseases or disorders according to the expression levels of one or more biomarkers. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising an interleukin-33 antagonist.