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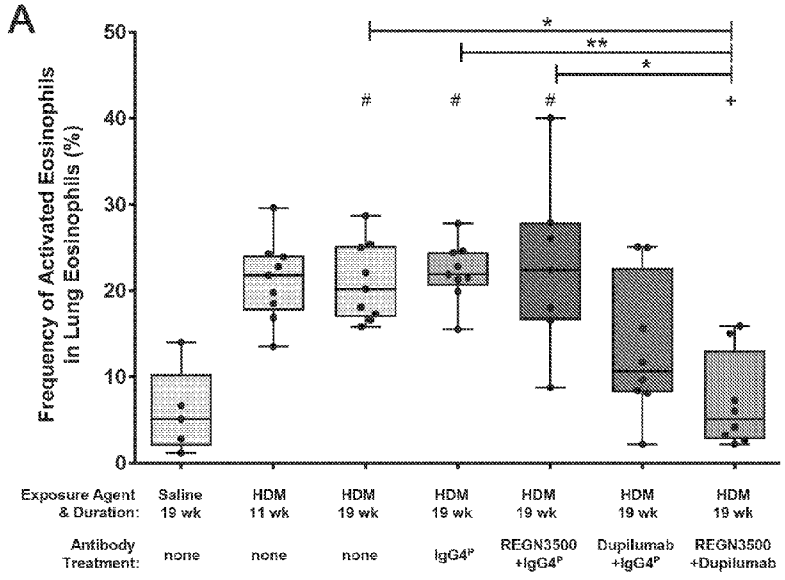

Figure 4A

(57) Abstract: The present invention provides methods for treating inflammatory diseases, or conditions associated with, or resulting in part from, elevated levels of IL-33 and IL-4, in particular inflammatory lung disorders. The methods of the present invention comprise administering to a subject in need thereof one or more therapeutically effective doses of an IL-33 antagonist alone or in combination with one or more therapeutically effective doses of an IL-4R antagonist. In certain embodiments, the methods of the present invention include use of the antagonists to treat any inflammatory disease or condition mediated in part by enhanced IL-33-mediated signaling and IL-4-mediated signaling.

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[Continued on next page]

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METHODS OF TREATING INFLAMMATORY CONDITIONS

FIELD OF THE INVENTION

[0001] The present invention relates to methods for treating an inflammatory condition comprising administering to a subject in need thereof a therapeutically effective amount of an interleukin-33 (IL-33) antagonist alone or in combination with an interleukin-4 (IL-4) antagonist. More specifically, the present invention relates to treating inflammatory or obstructive lung diseases or disorders by administering a therapeutically effective amount of an interleukin-33 (IL-33) antibody alone or in combination with an interleukin-4R (IL-4R) antibody.

BACKGROUND

[0002] Inflammation is initiated as a protective response by the host, but it can often result in systemic pathologies. Inflammatory lung diseases such as asthma, allergy and chronic obstructive pulmonary disease (COPD) are increasing in developed countries, resulting in great consequences to healthcare costs. Several inflammatory cells and their mediators participate in the development and progression of these diseases. In certain cases, these diseases reflect the outcome of type 2 immunity, which is characterized by the infiltration of tissues with eosinophils, basophils, mast cells, CD4+ T helper 2 (Th2) cells, group 2 innate lymphoid cells (ILC2s), interleukin-4 (IL-4) and/or IL-13 induced macrophages, as well as by an elevation in serum IgE and by an increase in the cytokines IL-4, IL-5, IL-9 and IL-13.

[0003] One cytokine believed to play a role in inflammatory lung diseases is interleukin-33 (IL-33), a proinflammatory cytokine released by damaged epithelial tissue in response to insults such as allergens, viruses, or smoke. IL-33 is a member of the interleukin-1 (IL-1) family that potently drives production of T helper-2 (Th2)-associated cytokines (e.g., IL-4). IL-33 is expressed by a wide variety of cell types, including fibroblasts, mast cells, dendritic cells, macrophages, osteoblasts, endothelial cells, and epithelial cells. Interleukin-33 (IL-33) is a ligand for ST2 (sometimes referred to as "suppression of tumorigenicity 2"), a toll-like/interleukin-1 receptor super-family member, which associates with an accessory protein, IL-1RAcP ("Interleukin-1 receptor accessory protein", 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. IL-33 signaling has been implicated as a factor in a variety of diseases and disorders, including the inflammatory lung diseases disclosed herein. (Liew et al., *Nature Reviews – Immunology 10*:103-110 (2010)). Inhibitors of IL-33 signaling have been described in, for

example, US9,453,072; US8187596; US2013/17373761; US2014/0212412; US2014/0271658; US2014/0271642; US2014/0004107;WO2015/099175; WO2015/106080; WO2011/031600; WO2014/164959; WO2014/152195; WO2013/165894; WO2013/173761; EP1725261; EP10815921A1; and EP2850103A2.

[0004] Interleukin-4 (IL-4, also known as B cell stimulating factor or BSF-1) has also been implicated as a key cytokine that drives allergic and T helper cell type 2 (Th2) polarized inflammatory processes. IL-4 has been shown to possess a broad spectrum of biological activities, including growth stimulation of T cells, mast cells, granulocytes, megakaryocytes and erythrocytes. IL-4 induces the expression of class II major histocompatibility complex molecules in resting B cells, and enhances the secretion of IgE and IgG1 isotypes by stimulated B cells. The biological activities of IL-4 are mediated by specific cell surface receptors for IL-4. Human IL-4 receptor alpha (hIL-4R) (SEQ ID NO: 347) is described in, for example, U.S. Pat. Nos. 5,599,905, 5,767,065, and 5,840,869. Antibodies to hIL-4R are described in U.S. Pat. Nos. 5,717,072, 7,186,809 and 7,605,237. Methods for using antibodies to hIL-4R are described in U.S. Pat. Nos. 5,714,146; 5,985,280; 6,716,587 and 9,290,574.

[0005] Current therapies for treating inflammatory diseases of the lung leave significant room for improvement of safety and efficacy in patients suffering from, for example, asthma and chronic obstructive pulmonary disease (COPD), particularly in reducing exacerbations. Despite the availability of numerous inhaled combinations of anti-inflammatory and bronchodilator drugs, many patients continue to experience exacerbations. Exacerbations may require the use of systemic corticosteroids, which are efficacious due to their broad immune neutralizing capacity, but laden with undesirable side effects, including bone loss and infection. Several biologic therapies, most of which target single immune mediators, are in late stage development for asthma and COPD. However, due to the complexity of the inflammatory milieu, these agents will likely be limited in their use.

[0006] Accordingly, an unmet need exists in the art for novel combinations of therapies for the treatment and/or prevention of inflammatory lung diseases, such as those described herein.

BRIEF SUMMARY OF THE INVENTION

[0007] According to certain aspects of the present invention, methods are provided for treating an inflammatory disease or disorder, or at least one symptom associated with the inflammatory disease or disorder, the method comprising administering to a subject in need thereof one or more doses of a therapeutically effective amount of an interleukin-33 (IL-33) antagonist alone, or in combination with one or more doses of a therapeutically effective amount of an interleukin-4 (IL-4) antagonist, or a pharmaceutical composition comprising an IL-33 antagonist and an IL-4α antagonist, to a patient in need thereof. In one embodiment, the administration of the IL-33 antagonist in combination with the IL-4 antagonist results in enhanced therapeutic efficacy as

compared to that observed with administration of the IL-33 antagonist alone or the IL-4 antagonist alone.

[0008] In certain embodiments, the IL-33 antagonist is any agent that is capable of blocking, attenuating, or otherwise interfering with IL-33 signaling and/or the interaction between IL-33 and a cell receptor (*e.g.* ST2) or a co-receptor (*e.g.* IL-1RAcP) or a complex thereof. Any of the above may block or inhibit at least one biological activity of IL-33.

[0009] In certain embodiments, the IL-33 antagonist is an antibody that binds to or interacts with IL-33 and blocks the interaction of IL-33 with its receptor, ST2 and prevents or inhibits the interaction of ST2 with the co-receptor, IL1-RAcP, or prevents the formation of the signaling complex. In one embodiment, the IL-33 antagonist is a monoclonal antibody that binds to, or interacts specifically with human IL-33. In one embodiment, the IL-33 antagonist is a receptor-based trap that binds to, or interacts specifically with human IL-33.

[0010] In one embodiment, the IL-4 antagonist is an interleukin-4 receptor (IL-4R) antagonist.

[0011] In one embodiment, the IL-4R antagonist is any agent, which binds to or interacts with IL-4Rα or an IL-4R ligand, and inhibits or attenuates the normal biological signaling function of a type 1 and/or a type 2 IL-4 receptor. In one embodiment, the IL-4R antagonist is a monoclonal antibody that binds specifically to human IL-4Rα. In one embodiment, the IL-4R antagonist is a monoclonal antibody that binds IL-4Rα and blocks both IL-4 and IL-13 mediated signaling through either the type I or type II receptor. In one embodiment, the monoclonal antibody that binds specifically to human IL-4Rα and blocks both IL-4 and IL-13 mediated signaling is dupilumab, or a bioequivalent thereof. In one embodiment, the method of treating an inflammatory disorder or condition is accomplished through use of a combination of REGN3500 having a heavy chain variable region/light chain variable region (HCVR/LCVR) amino acid sequence pair of SEQ ID NOs: 274/282 and dupilumab having an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 337/338.

[0012] In one embodiment, the inflammatory disease or disorder treatable by the methods of the invention is selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), asthma and COPD overlap syndrome (ACOS), atopic dermatitis, nasal polyps, an allergic response, chronic bronchitis, emphysema, chronic rhinosinusitis with or without nasal polyps, inflammatory bowel disease, Crohn's disease, ulcerative colitis, hypersensitivity pneumonitis, multiple sclerosis, arthritis (including osteoarthritis, rheumatoid arthritis, or psoriatic arthritis), allergic rhinitis, fibrosis, eosinophilic esophagitis, vasculitis, urticaria, Churg Strauss syndrome, inflammatory pain and psoriasis.

[0013] In one embodiment, the asthma is eosinophilic asthma.

[0014] In one embodiment, the asthma is non-eosinophilic asthma.

[0015] In one embodiment, the asthma is allergic asthma.

[0016] In one embodiment, the asthma is non-allergic asthma.

[0017] In one embodiment, the asthma is severe refractory asthma.

[0018] In one embodiment, the asthma is steroid resistant asthma.

[0019] In one embodiment, the asthma is steroid sensitive asthma.

[0020] In one embodiment, the asthma is steroid refractory asthma.

[0021] In one embodiment, the asthma is an asthma exacerbation.

[0022] In one embodiment, the inflammatory disease or disorder is alleviated, or reduced in severity, duration or frequency of occurrence, or at least one symptom associated with the inflammatory disease or disorder is alleviated, or reduced in severity, duration, or frequency of occurrence.

[0023] In one embodiment, the administering of a therapeutically effective amount of one or more doses of an IL-33 antagonist to a subject in need thereof alone, or in combination with one or more doses of a therapeutically effective amount of an IL-4R antagonist results in enhanced therapeutic efficacy as measured by any one or more of the following parameters:

a) a reduction in the frequency of one or more of the following: eosinophils, activated B cells, activated CD8 T cells, or CD4/CD8 T cell ratio in a tissue sample;

b) a reduction in one or more of the following: interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-13 (IL-13), monocyte chemoattractant protein-1 (MCP-1), or tumor necrosis factor alpha (TNFα) levels in a tissue sample; or

c) a reduction in the gene expression level of one or more of the following: *Il4, Il5, Il6*, *Il9, Il13*, *Il1rl1*, *Il13ra2*, *tnf, Tgfb1, Ccl2, Ccl11, Ccl24*, *Col15a1* or *Col24a1* in a tissue sample.

[0024] In one embodiment, the administering of a therapeutically effective amount of one or more doses of an IL-33 antagonist to a subject in need thereof alone, or in combination with one or more doses of a therapeutically effective amount of an IL-4R antagonist results in enhanced therapeutic efficacy as further measured by one or more of the following:

d) a reduction in serum IgE levels

e) a reduction in goblet cell metaplasia in the lung;

f) an improvement in lung consolidation; or

g) a decrease in sub-epithelial fibrosis in the lung.

[0025] In one embodiment, the tissue sample is obtained from the lung.

[0026] In one embodiment, the tissue sample is selected from the group consisting of liver, kidney, heart or whole blood. In certain embodiments, blood cells, serum or plasma may be used for measuring one or more of the parameters described above.

[0027] In one embodiment, the chronic obstructive pulmonary disease that is treatable by the methods of the invention is exacerbated by one or more of the following: asthma, a viral

disease, a bacterial infection, an exposure to an allergen, an exposure to a chemical or chemical fumes, or an exposure to an environmental irritant or air pollution.

[0028] In a related embodiment, the asthma that is treatable by the methods of the invention is exacerbated by one or more of the following: a viral disease, a bacterial infection, an exposure to an allergen, an exposure to a chemical or chemical fumes, or an exposure to an environmental irritant or air pollution.

[0029] In a certain embodiment, the asthma that is treatable by the methods of the invention is selected from the group consisting of eosinophilic asthma, non-eosinophilic asthma, steroid resistant asthma and steroid sensitive asthma.

[0030] In one embodiment, the chronic obstructive pulmonary disease that is treatable by the methods of the invention results from, or is exacerbated in part by cigarette smoke.

[0031] In one embodiment, the patients suffering from chronic obstructive pulmonary disease that is treatable by the methods of the invention may or may not exhibit an increase in the number of eosinophils.

[0032] In one embodiment, the patients suffering from asthma and COPD overlap syndrome (ACOS) that is treatable by the methods of the invention may or may not exhibit an increase in the number of eosinophils.

[0033] A second aspect of the invention provides for treating an inflammatory disease or disorder, or at least one symptom associated with the inflammatory disease or disorder, by administering an effective amount of one or more additional therapeutic agents useful for alleviating the inflammatory disease or disorder, or at least one symptom of the inflammatory disease or disorder in combination with a therapeutically effective amount of an interleukin-33 (IL-33) antagonist, *e.g.* an IL-33 antibody or an IL-33 trap, and a therapeutically effective amount of an interleukin-4 (IL-4) antagonist, *e.g.* an IL-4R antibody such as dupilumab, or a therapeutic equivalent thereof.

[0034] In one embodiment, the one or more additional therapeutic agents are selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid (*e.g.* an inhaled corticosteroid or ICS), a long acting β2 adrenergic agonist (LABA), a long acting muscarinic antagonist (LAMA), a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-1 antagonist, an IL-8 antagonist, an IL-13 antagonist, a different IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-33/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, a TNF inhibitor, an IgE inhibitor, a leukotriene inhibitor, an oral PDE4 inhibitor, a methylxanthine, nedocromil sodium, cromolyn sodium, a long-acting beta 2 agonist and another IL-33 antagonist(*e.g.* a different antibody to IL-33, a different IL-33 receptor based trap, an ST2 antagonist, including an antibody to ST2, or a soluble ST2 receptor, or an antagonist to another IL-33 receptor other than ST2, or an IL-

1RAcP antagonist, including an antibody to IL-1RAcP, or an antibody that interacts with an IL-33/ST2 complex).

[0035] In some embodiments, the present invention provides methods for treating moderate-to-severe chronic obstructive pulmonary disease (COPD) comprising concomitant administration of an IL-4R antagonist (*e.g.* dupilumab) and an IL-33 antagonist (*e.g.* REGN3500), in addition to background therapy, including for example, an inhaled corticosteroid (ICS) and/or a long acting β2 adrenergic agonist (LABA) and/or a long acting muscarinic antagonist (LAMA).

[0036] In certain embodiments, the present invention provides methods for reducing the incidence of "loss of asthma control" (LOAC) events comprising treating patients suffering from asthma with an IL-4R antagonist (*e.g.* dupilumab) in combination with an IL-33 antagonist (*e.g.* REGN3500). In certain embodiments, the combined use of an IL-4R antagonist in combination with an IL-33 antagonist provides a more effective outcome than administration with either the IL-4R antagonist alone or the IL-33 antagonist alone.

[0037] In a related embodiment, the administration of the IL-33 antagonist in combination with the IL-4R antagonist results in an increase in a type 1 immune response, and/or a reduction in a type 2 immune response elicited by the disease, or by the causative agent of the disease or allergy.

[0038] A third aspect of the invention provides a method for treating a fibrotic disease or disorder, or at least one symptom associated with the fibrotic disease or disorder, the method comprising administering a combination of an IL-33 antagonist (an IL-33 antibody or IL-33 trap) that binds specifically to IL-33, and an antibody that binds specifically to IL-4Rα or an antigen-binding fragment thereof, or a pharmaceutical composition comprising an IL-33 antagonist and an IL-4α antagonist, to a patient in need thereof, wherein the fibrotic disease or disorder is alleviated, or reduced in severity, or duration, or at least one symptom associated with the fibrotic disease or disorder is alleviated, or reduced in severity, duration, or frequency of occurrence. In one embodiment, treatment of the fibrotic disease with an IL-33 antagonist in combination with an IL-4R antagonist may result in restoring the fibrotic tissue to its normal state.

[0039] In one embodiment, the fibrotic diseases or disorders that are treatable by administering the anti-IL-33 and IL-4R antagonists of the invention, such as the IL-33 antibodies or IL-33 traps in combination with the IL-4Rα antibodies described herein, include pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis, bleomycin-induced pulmonary fibrosis, asbestos-induced pulmonary fibrosis, and bronchiolitis obliterans syndrome), chronic asthma, fibrosis associated with acute lung injury and acute respiratory distress (*e.g.*, bacterial pneumonia induced fibrosis, trauma induced fibrosis, viral pneumonia induced fibrosis, ventilator induced fibrosis, non-pulmonary sepsis induced fibrosis and aspiration induced fibrosis), silicosis, radiation-induced fibrosis, chronic obstructive pulmonary disease (COPD, which may or may not be related to,

caused in part by, or resulting from, exposure to first or second hand cigarette smoke), scleroderma, ocular fibrosis, skin fibrosis (*e.g.*, scleroderma), hepatic fibrosis (*e.g.*, cirrhosis, alcohol-induced liver fibrosis, non-alcoholic steatohepatitis (NASH), biliary duct injury, primary biliary cirrhosis, infection- or viral-induced liver fibrosis, autoimmune hepatitis, kidney (renal) fibrosis, cardiac fibrosis, atherosclerosis, stent restenosis, and myelofibrosis.

[0040] A fourth aspect of the invention provides a method for preventing or reducing the severity of an allergic response, the method comprising administering one or more doses of a therapeutically effective amount of an IL-33 antagonist in combination with one or more doses of a therapeutically effective amount of an IL-4R antagonist to a subject in need thereof, wherein the administration of the combination results in enhanced therapeutic efficacy for preventing or reducing the severity of an allergic response as compared to that observed with administration of the IL-33 antagonist alone or the IL-4R antagonist alone. The subject treated with the IL-33 antagonist in combination with the IL-4R antagonist may demonstrate a reduced sensitivity to, or a diminished allergic reaction against the allergen, or may not experience any sensitivity or allergic reaction to, or anaphylactic response to the allergen following administration of the combination of the IL-33 antagonist and the IL-4R antagonist or a composition comprising the antagonists.

[0041] In one embodiment, the IL-33 antagonist for use in the methods of the invention is a monoclonal antibody, or an antigen-binding fragment thereof that binds to, or interacts specifically with, human IL-33. The IL-33 antibody or antigen-binding fragment thereof may block the interaction of IL-33 and ST2, or it may allow for low affinity binding of IL-33 to the ST2 receptor. In so doing, ST2 may be prohibited from interacting with IL-1RAcP. As such, the IL-33 antibodies of the invention are useful, *inter alia*, for inhibiting IL-33-mediated signaling and for treating diseases and disorders caused by or related to IL-33 activity and/or signaling.

[0042] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof reduces the frequency of one or more of the following: eosinophils, CD4+ T cells, B cells, ST2+/CD4+ cells in the T cell population or reduces the CD4/CD8 T cell ratio in the lungs when administered to a mammal having allergen-induced lung inflammation.

[0043] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof reduces the expression level of one or more of the following: IL-4, IL-5, IL-6, IL-9, IL-13, Ccl2, Ccl11, Ccl24 or MCP-1 in the lungs when administered to a mammal having allergen-induced lung inflammation.

[0044] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof reduces serum IgE levels, goblet cell metaplasia, or epithelial collagen thickness in the lungs when administered to a mammal having allergen-induced lung inflammation.

[0045] In certain embodiments, the IL-33 antibodies that bind specifically to human IL-33, or antigen-binding fragments thereof that may be used in the methods of the invention comprise

three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and comprises three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) 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.

[0046] In certain embodiments, the IL-33 antibodies that bind specifically to human IL-33, or antigen-binding fragments thereof that may be used in the methods of the invention comprise a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0047] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention comprise a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0048] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention comprise a HCVR and LCVR (HCVR/LCVR) sequence pair selected from the group consisting of 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, and 308/316.

[0049] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention comprise a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, and 314, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, and 322, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0050] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention comprise a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 24/32, 40/48, 56/64,

72/80, 88/96, 104/112, 120/128, 136/144, 152/160, 168/176, 184/192, 200/208, 216/224, 232/240, 248/256, 264/272, 280/288, 296/304 and 314/322.

[0051] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention further comprise a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, and 310, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, and 312, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, and 318, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, and 320, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0052] Certain non-limiting, exemplary anti-IL-33 antibodies and antigen-binding fragments that may be used in the methods of the invention comprise HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16 (*e.g.* H1M9559N); 20-22-24-28-30-32 (*e.g.* H1M9566N); 36-38-40-44-46-48 (*e.g.* H1M9568N); 52-54-56-60-62-64 (*e.g.* H4H9629P); 68-70-72-76-78-80 (*e.g.* H4H9633P); 84-86-88-92-94-96 (*e.g.* H4H9640P); 100-102-104-108-110-112 (*e.g.* H4H9659P); 116-118-120-124-126-128 (*e.g.* H4H9660P); 132-134-136-140-142-144 (*e.g.* H4H9662P); 148-150-152-156-158-160 (*e.g.*, H4H9663P); 164-166-168-172-174-176 (*e.g.* H4H9664P); 180-182-184-188-190-192 (*e.g.*, H4H9665P); 196-198-200-204-206-208 (*e.g.* H4H9666P); 212-214-216-220-222-224 (*e.g.* H4H9667P); 228-230-232-236-238-240 (*e.g.* H4H9670P); 244-246-248-252-254-256 (*e.g.* H4H9671P); 260-262-264-268-270-272 (*e.g.* H4H9672P); 276-278-280-284-286-288 (*e.g.* H4H9675P); 292-294-296-300-302-304 (*e.g.* H4H9676P); and 310-312-314-318-320-322 (H1M9565N).

[0053] According to certain embodiments, the anti-IL-33 antibodies, or antigen-binding fragments thereof for use in the methods of the invention, *e.g.* for treating inflammatory conditions, comprise the heavy and light chain CDR domains contained within heavy and light chain variable region (HCVR/LCVR) sequences selected from the group consisting of 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, and 308/316. 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.

[0054] In one embodiment, the IL-33 antibody or antigen-binding fragment for use in the methods of the invention comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0055] In a related embodiment, the IL-33 antibody or antigen-binding fragment for use in the methods of the invention comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, of SEQ ID NOs: 276-278-280-284-286-288.

[0056] In one embodiment, the antibody that specifically binds human interleukin-33 (IL-33), or an antigen-binding fragment for use in the methods of the invention comprises: (a) a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 274; and (b) a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:282.

[0057] In one embodiment, the IL-33 antibody or antigen-binding fragment for use in the methods of the invention comprises the HCVR/LCVR amino acid sequence pair of: SEQ ID NOs: 274/282.

[0058] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof for use in the methods of the invention interacts with an amino acid sequence ranging from about position 1 to about position 12 of SEQ ID NO: 349, and/or with an amino acid sequence ranging from about position 50 to about position 94 of SEQ ID NO: 349 as determined by hydrogen/deuterium exchange.

[0059] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof for use in the methods of the invention interacts with an amino acid sequence ranging from about position 112 to about position 123 of SEQ ID NO: 348, and/or with an amino acid sequence ranging from about position 161 to about position 205 of SEQ ID NO: 348 as determined by hydrogen/deuterium exchange.

[0060] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof for use in the methods of the invention interacts with either the amino acid sequence of SEQ ID NO: 350, or

with the amino acid sequence of SEQ ID NO: 351, or interacts with both SEQ ID NOs: 350 and 351 as determined by hydrogen/deuterium exchange.

[0061] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof for use in the methods of the invention competes for binding to IL-33 with a reference antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0062] In one embodiment, the IL-33 antibody or antigen-binding fragment thereof for use in the methods of the invention binds to the same epitope on IL-33 as a reference antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0063] In a fifth aspect, the invention provides nucleic acid molecules encoding the anti-IL-33 antibodies or antigen-binding fragments thereof to be used in the methods of the invention. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0064] In one embodiment, the invention provides methods of using an antibody or fragment thereof that binds specifically to human IL-33 comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, and 307, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0065] The present invention also provides methods of using an antibody or fragment thereof that binds specifically to human IL-33 comprising a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, and 315, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0066] The present invention also provides methods for using an antibody or antigen-binding fragment of an antibody that binds specifically to human IL-33 comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, and 313, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, and 321, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0067] The present invention also provides methods of using an antibody or fragment thereof that binds specifically to human IL-33, which further comprises a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, and 309, or a substantially

identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, and 311, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, and 317, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, and 319, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0068] According to certain embodiments, the methods of the invention provide for use of an antibody or fragment thereof that binds specifically to human IL-33, which comprises the heavy and light chain CDR sequences encoded by the nucleic acid sequences of SEQ ID NOs: 1 and 9 (*e.g.* H1M9559N), 17 and 25 (*e.g.* H1M9566N), 33 and 41 (*e.g.* H1M9568N), 49 and 57 (*e.g.* H4H9629P), 65 and 73 (*e.g.* H4H9633P), 81 and 89 (*e.g.* H4H9640P), 97 and 105 (*e.g.* H4H9659P), 113 and 121 (*e.g.* H4H9660P), 129 and 137 (*e.g.* H4H9662P), 145 and 153 (*e.g.* H4H9663P), 161 and 169 (*e.g.* H4H9664P), 177 and 185 (*e.g.* H4H9665P), 193 and 201 (*e.g.* H4H9666P), 209 and 217 (*e.g.* H4H9667P), 225 and 233 (*e.g.* H4H9670P), 241 and 249 (*e.g.* H4H9671P), 257 and 265 (*e.g.* H4H9672P), 273 and 281 (*e.g.* H4H9675P), 289 and 297 (*e.g.* H4H9676P), or 307 and 315 (H1M9565N).

[0069] In one embodiment, the IL-33 antagonist for use in the methods of the invention is an IL-33 receptor based trap, such as those described herein (See Figure 1).

[0070] In one embodiment, the IL-33 receptor based trap 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.

[0071] In one embodiment, the IL-33 trap for use in the methods of the invention further comprises a second IL-33 binding domain (D2) attached to D1 and/or M, wherein D2 comprises an extracellular portion of an IL-1RAcP protein. In one embodiment, D1 is attached to the N-terminus of M. In one embodiment, D1 is attached to the C-terminus of M. In one embodiment, D2 is attached to the N-terminus of M. In one embodiment, D2 is attached to the C-terminus of M. In one embodiment, D1 is attached to the N-terminus of D2, and wherein D2 is attached to the N-terminus of M.

[0072] In one embodiment, D1 comprises the amino acid sequence of SEQ ID NO: 328 or 329, or an amino acid sequence having at least 90% identity thereto. In one embodiment, D2 comprises the amino acid sequence of SEQ ID NO: 330 or 331, or an amino acid sequence

having at least 90% identity thereto.

[0073] In one embodiment, the IL-33 antagonist for use in the methods of the invention comprises a first IL-33 binding domain (D1) attached to a first multimerizing domain (M1), and a second IL-33 binding domain (D2) attached to a second multimerizing domain (M2), wherein the D1 and/or D2 domains comprise an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

[0074] In one embodiment, the IL-33 antagonist for use in the methods of the invention comprises a third IL-33 binding domain (D3) that is attached to either D1 or M1, and wherein D3 comprises an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

[0075] In one embodiment, the IL-33 antagonist for use in the methods of the invention comprises a fourth IL-33 binding domain (D4) that is attached to either D2 or M2, and wherein D4 comprises an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

[0076] In one embodiment, D1 is attached to the N-terminus of M1, and D2 is attached to the N-terminus of M2.

[0077] In one embodiment, D3 is attached to the N-terminus of D1.

[0078] In one embodiment, D3 is attached to the C-terminus of M1.

[0079] In one embodiment, D4 is attached to the N-terminus of D2.

[0080] In one embodiment, D4 is attached to the C-terminus of M2.

[0081] In one embodiment, D3 is attached to the N-terminus of D1, and D1 is attached to the N-terminus of M1; and wherein D4 is attached to the N-terminus of D2, and D2 is attached to the N-terminus of M2.

[0082] In one embodiment, D3 is identical or substantially identical to D4 and wherein D1 is identical or substantially identical to D2.

[0083] In one embodiment, D3 and D4 each comprise an IL-33-binding portion of an ST2 protein; and wherein D1 and D2 each comprise an extracellular portion of an IL-1RAcP protein.

[0084] In one embodiment, the IL-33 trap for use in the methods of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 327.

[0085] In one embodiment, the IL-4 antagonist for use in the methods of the invention is an interleukin-4 receptor (IL-4R) antagonist.

[0086] In one embodiment, the IL-4R antagonist for use in the methods of the invention is an antibody or antigen-binding fragment thereof that binds IL-4Rα and prevents the interaction of IL-4 and/or IL-13 with a type 1 or a type 2 IL-4 receptor.

[0087] In a related embodiment, the IL-4R antibody or antigen-binding fragment thereof for use in the methods of the invention prevents the interaction of IL-4 and/or IL-13 with both type 1 and type 2 IL-4 receptors.

[0088] In one embodiment, the IL-4R antagonist for use in the methods of the invention is a monoclonal antibody that binds specifically to human IL-4Rα.

[0089] In one embodiment, the monoclonal antibody that binds specifically to human IL-4Rα for use in the methods of the invention is dupilumab, or a bioequivalent thereof.

[0090] In a certain embodiment, the IL-4R antibody or antigen-binding fragment thereof for use in the methods of the invention comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:335 or SEQ ID NO: 337 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:336 or SEQ ID NO: 338.

[0091] In a related embodiment, the IL-4R antibody or antigen-binding fragment thereof for use in the methods of the invention comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 339, the HCDR2 comprises the amino acid sequence of SEQ ID NO:340; the HCDR3 comprises the amino acid sequence of SEQ ID NO:341; the LCDR1 comprises the amino acid sequence of SEQ ID NO:342; the LCDR2 comprises the amino acid sequence of SEQ ID NO:343; and the LCDR3 comprises the amino acid sequence of SEQ ID NO:344.

[0092] In one embodiment, the IL-4R antibody or antigen-binding fragment thereof for use in the methods of the invention comprises an HCVR comprising the amino acid sequence of SEQ ID NO: 335 or SEQ ID NO: 337 and an LCVR comprising the amino acid sequence of SEQ ID NO: 336 or SEQ ID NO: 338.

[0093] In one embodiment, the IL-4R antibody or antigen-binding fragment thereof for use in the methods of the invention comprises an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 335/336 or SEQ ID NOs: 337/338.

[0094] In a related embodiment, the IL-4R antagonist for use in the methods of the invention is dupilumab (SEQ ID NOs: 337/338), or a bioequivalent thereof.

[0095] In certain embodiments, the IL-33 antagonist and the IL-4R antagonist are administered in separate formulations.

[0096] In certain embodiments, the IL-33 antagonist and the IL-4R antagonist are co-formulated for administration to a patient in need thereof.

[0097] In certain embodiments, the IL-33 antagonist and the IL-4R antagonist are administered to the subject subcutaneously, intravenously, intramuscularly, or intranasally.

[0098] The IL-33 and IL-4R antibodies of the invention can be full-length (for example, an IgG1

or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933).

[0099] In one embodiment, the antibodies that bind specifically to human interleukin-33, or human IL-4R are isolated fully human monoclonal antibodies.

[0100] In a sixth aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof, or a trap, which specifically binds IL-33, or an antibody that specifically binds IL-4R and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition, which is a combination of an anti-IL-33 antibody or an IL-33 trap, or an antibody that specifically binds IL-4R and one or more additional therapeutic agents. In one embodiment, the one or more additional therapeutic agents is any agent that is advantageously combined with either, or both, the IL-33 antagonist and/or the IL-4R antagonist. Exemplary agents that may be advantageously combined with an IL-33 antagonist and/or an IL-4R antagonist include, without limitation, other agents that inhibit IL-33 activity and/or IL-4 activity (including other antibodies or antigen-binding fragments thereof, peptide inhibitors, small molecule antagonists, etc.) and/or agents, which do not directly bind IL-33, or IL-4 or IL-4R, but nonetheless interfere with, block or attenuate IL-33 or IL-4 mediated signaling. In one embodiment the one or more additional therapeutic agents may be selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid (*e.g.* an inhaled corticosteroid), a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-1 antagonist, an IL-8 antagonist, an IL-13 antagonist, a different IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-33/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, a TNF inhibitor, an IgE inhibitor, a leukotriene inhibitor, an oral PDE4 inhibitor, a methylxanthine, nedocromil sodium, cromolyn sodium, a long-acting beta 2 agonist (LABA), a long acting muscarinic antagonist (LAMA), an inhaled corticosteroid (ICS) and another IL-33 or IL-4 antagonist or a different antibody to IL-33 or IL-4 or IL-4R, and another IL-33 antagonist.

[0101] In certain embodiments, the cytokine antagonist may be a small molecule inhibitor (synthetic or naturally derived), or a protein (*e.g.* an antibody) that interacts with either the cytokine itself, or to a receptor for the cytokine, or to a complex comprising both the cytokine and its receptor(s). Additional combination therapies and co-formulations involving the anti-IL-33 antibodies of the present invention and/or the IL-4R antibodies are disclosed elsewhere herein.

[0102] In yet another aspect, the invention provides therapeutic methods for inhibiting IL-33, and/or IL-4 signaling activity using an anti-IL-33 antagonist (such as an IL-33 antibody or an IL-33 trap), and an IL-4R antibody or antigen-binding portion of one or more antibodies of the invention, wherein the therapeutic methods comprise administering a therapeutically effective

amount of a pharmaceutical composition comprising an IL-33 antibody or an IL-33 trap, either alone, or in combination with an IL-4R antibody or antigen-binding fragment of one or more antibodies of the invention. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of IL-33 and/or IL-4 signaling. The anti-IL-33 and/or IL-4R antagonists of the invention, when used together, may function to block the interaction between IL-33 and an IL-33 binding partner, and IL-4 and an IL-4 binding partner or otherwise inhibit the signaling activity of both IL-33 and IL-4. In one embodiment, the IL-4R antagonist is an antibody that binds to IL-4Rα and in so doing prevents both IL-4 and IL-13 signaling through either the type I or type II receptors. In one embodiment, the IL-4Rα antagonist is dupilumab or a bioequivalent thereof. Given the dual blocking activity of dupilumab for both IL-4 and IL-13, it is believed that when used in combination with the IL-33 antagonists of the invention, the combined treatment regimen will result in enhanced inhibition of unwanted inflammatory activity resulting in part from signaling through the IL-4, IL-13 and IL-33 signaling pathways, which may occur during inflammation.

[0103] In one embodiment, the IL-33 antagonist is an antibody or antigen-binding fragment thereof that binds specifically to IL-33 and blocks the interaction of IL-33 and its receptor ST2 (also known as IL1RL1).

[0104] In one embodiment, the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and comprises three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) 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.

[0105] In one embodiment, the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290 and 308.

[0106] In one embodiment, the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises 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.

[0107] In one embodiment, the antibody or antigen-binding fragment thereof that binds specifically to IL-33, comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292 and 310;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294 and 312;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296 and 314;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284 and 318;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286 and 320;

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288 and 322.

[0108] In one embodiment, the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 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 and 308/316.

[0109] The present invention also includes the use of an IL-33 antagonist alone, or in combination with an IL-4R antagonist in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by IL-33 and/or IL-4 activity or signaling in a patient. In one embodiment, the disease or disorder related to, or caused by IL-33 activity and/or IL-4 activity in a patient is an inflammatory disease or disorder, wherein the inflammatory disease or disorder is selected from the group consisting of asthma (eosinophilic or non-eosinophilic), chronic obstructive pulmonary disease (COPD), asthma and COPD overlap syndrome (ACOS), atopic dermatitis, nasal polyps, an allergic response, chronic bronchitis, emphysema, chronic rhinosinusitis with or without nasal polyps, inflammatory bowel disease, Crohn's disease, ulcerative colitis, hypersensitivity pneumonitis, multiple sclerosis, arthritis (including osteoarthritis, rheumatoid arthritis and psoriatic arthritis), allergic rhinitis, fibrosis, eosinophilic esophagitis, vasculitis, urticaria, Churg Strauss syndrome, inflammatory pain, and psoriasis. The present invention also includes a therapeutically effective amount of an IL-33 antagonist combined with a therapeutically effective amount of an IL-4R antagonist for use in treating an

inflammatory disease or disorder, or at least one symptom of an inflammatory disease or disorder, wherein the administration of the IL-33 antagonist in combination with the IL-4R antagonist results in enhanced therapeutic efficacy as compared to that observed with administration of the IL-33 antagonist alone or the IL-4R antagonist alone. Any of the methods discussed herein also encompass use of the IL-33 and/or IL-4R antagonists (*e.g.*, antibodies) to treat, or for treating, the diseases, disorders and/or symptoms discussed in connection with the methods.

[0110] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0111] Figure 1 shows four exemplary arrangements of the individual components of the IL-33 antagonists relative to one another. **Panel A** shows an arrangement in which a first IL-33-binding domain (D1) is attached to the N-terminus of a first multimerizing domain (M1), and a second IL-33-binding domain (D2) is attached to the N-terminus of a second multimerizing domain (M2). D1 is shown as a white box and D2 is shown as a black box to indicate that D1 and D2 are derived from different IL-33 binding proteins. **Panel B** shows an arrangement in which a first IL-33-binding domain (D1) is attached to the N-terminus of a first multimerizing domain (M1), and a second IL-33-binding domain (D2) is attached to the C-terminus of a second multimerizing domain (M2). D1 is shown as a white box and D2 is shown as a black box to indicate that D1 and D2 are derived from different IL-33 binding proteins. **Panels C** and **D** show arrangements comprising four IL-33-binding domains, D1, D2, D3 and D4. In these arrangements, D3-D1-M1 and D4-D2-M2 are attached in tandem, wherein D3 is attached to the N-terminus of D1, and D1 is attached to the N-terminus of M1; and D4 is attached to the N-terminus of D2, and D2 is attached to the N-terminus of M2. In Panel **C**, D3 and D4 are identical or substantially identical to one another, and D1 and D2 are identical or substantially identical to one another. In Panel **D**, D1 and D4 are identical or substantially identical to one another, and D3 and D2 are identical or substantially identical to one another.

[0112] Figure 2 Shows that HDM exposure induces similar increases in activated eosinophils in lungs of IL-33-, IL-4-, and IL-4Rα-triple humanized and wild-type mice. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons between saline- and HDM-exposed mice of the same genotype; "&"ampersand symbols mark comparisons with the respective saline- or HDM-exposed wildtype group of mice. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001; 4x=p≤0.0001. Abbreviations: WT = *wild type*. All mice were of a mixed C57BL/6NTac / 129S6SvEvTac background.

[0113] Figure 3 shows that combined administration of REGN3500 and dupilumab blocks HDM exposure-induced increases in relative lung weight. Relative lung weight is expressed as the ratio of lung wet weight (in mg) to body weight (in g). Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; and "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0114] Figures 4A and 4B show the effect of REGN3500 and dupilumab, alone and in combination, on HDM exposure-induced pulmonary eosinophilic infiltration. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups; and "+" plus signs mark comparison of 11-week HDM-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001; 4x=p≤0.0001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0115] Figure 5 shows that REGN3500, alone or in combination with dupilumab, blocks HDM exposure-induced lung infiltration by $ST2^+$ $CD4^+$ T cells. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups; and "+" plus signs mark comparison of 11-week HDM-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001; 4x=p≤0.0001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0116] Figure 6 shows that combined administration of REGN3500 and dupilumab blocks HDM exposure-induced increases in levels of MPO lung protein, a marker of neutrophilic infiltration. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; and "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0117] Figures 7A and 7B show the effect of REGN3500 and dupilumab, alone or in

combination, on HDM exposure-induced increases in lung IL-5 and IL-6 protein levels. Lungs (cranial and middle lobes of the right lung) were harvested and IL-5 (A) and IL-6 (B) protein levels were measured by multiplexed immunoassay. Lung tissue IL-5 and IL-6 protein levels are expressed as protein amount (pg) per lung lobe. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; and "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0118] Figure 8 shows that REGN3500 alone or in combination with dupilumab blocks HDM exposure-induced increases in circulating SAA protein levels. Four days after the last antibody injection whole blood was collected by cardiac puncture and serum was isolated. Circulating SAA protein levels were measured using a commercially available ELISA kit. Circulating SAA protein levels are expressed as SAA protein amount (µg) per mL of serum. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; and "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

[0119] Figure 9 shows that circulating IgE protein levels are increased in response to HDM exposure. Whole blood was collected by cardiac puncture and serum was isolated. Circulating IgE protein levels were measured using a commercially available ELISA kit. Circulating IgE protein levels are expressed as IgE protein amount (µg) per mL of serum. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test. The following symbols were used to indicate statistically significant differences: "*" asterisks mark comparisons among all 19-week HDM-exposure groups; and "#" hashtags mark comparison of saline-exposed, untreated animals with all other groups. Increasing numbers of symbols indicate increasing significance: 1x=p≤0.05; 2x= p≤0.01; 3x=p≤0.001. Abbreviations: wk = week; $IgG4^P$ = isotype control antibody, REGN1945.

DETAILED DESCRIPTION

[0120] 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.

[0121] 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.).

[0122] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[0123] The terms "interleukin-33," "IL-33," and the like, as used herein, refer to a human IL-33 protein and encompasses the 270 amino acid, full-length, unprocessed IL-33 (See, for example, SEQ ID NO: 348, or UniProtKB accession number 095760), as well as any form of IL-33 that results from processing in the cell (See, for example, SEQ ID NO: 349, which contains amino acid residues 112-270 of the full length protein). Other processed forms of IL-33 are described in Lefrançais, *et.al.* (Lefrançais, *et al.*, (2012), Proc. Natl. Acad. Sci. 109(5):1693-1678). The term also encompasses naturally occurring variants of IL-33, for example, splice variants (See for example, Hong, *et. al.,* (2011), J. Biol. Chem. 286(22):20078-20086, or), or allelic variants, or any other isoform of IL-33, such as that described in WO2016/156440. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species.

[0124] As used herein, the expression "IL-33 antagonist" means any agent that is capable of blocking, attenuating or otherwise interfering with IL-33 signaling and/or the interaction between IL-33 and a cell surface receptor (*e.g.*, ST2, also known as IL1RL1) or a co-receptor (*e.g.* IL1-RAcP), or a complex thereof. For example, an "IL-33 antagonist", also referred to as an "IL-33 inhibitor", or an "IL-33 blocker" includes any of the following: (1) an agent that binds to, or interacts with IL-33; or (2) an agent that binds to, or interacts with the IL-33 receptor (sometimes referred to as "suppression of tumorigenicity" or "ST2"; also known as "IL1RL1"); or (3) an agent that binds to or interacts with the IL-33 co-receptor (interleukin-1 receptor accessory protein, or IL1-RAcP); or (4) an agent that binds to a complex of IL-33/ST2; or (5) an agent that binds to or interacts with ST2/IL-1RAcP. Any of the above may result in inhibition, or attenuation of at least one biological activity of IL-33, such as, but not limited to, the biological signaling function that occurs upon binding of IL-33 to its receptor/co-receptor complex.

[0125] In one embodiment, an "IL-33 antagonist" is an antibody that specifically binds to or interacts with IL-33 and prevents IL-33 binding to ST2 and in so doing prevents the interaction of ST2 with the co-receptor IL-1RAcP. In one embodiment, an "IL-33 antagonist" is an antibody that specifically binds to either ST2, or to the ST2/IL-1RAcP complex and prevents binding of IL-33 to ST2, or to the ST2/IL1-RAcP receptor complex. In one embodiment, an "IL-33 antagonist" is an antibody that binds to the IL-33/ST2 complex and then prevents interaction of ST2 with the IL-1RAcP co-receptor. In one embodiment, an "IL-33 antagonist" is an antibody that binds to IL-33 and may allow for low affinity binding to ST2, but at the same time, such low affinity binding may prevent subsequent interaction of ST2 with the co-receptor IL-1RAcP.

[0126] An "IL-33 antagonist" may also be an agent such as a soluble ST2 receptor, or an IL-33 receptor based trap, such as those described herein and disclosed in US2014/0271642. Any agent that blocks IL-33-mediated signaling is considered an "IL-33 antagonist". The "IL-33 antagonist" may be a small organic molecule, a protein, such as an antibody or fragment thereof, or a soluble IL-33 receptor based trap (as described herein), or a nucleic acid, such as an anti-sense molecule or an siRNA. As used herein, "an antibody that binds IL-33" or an "anti-IL-33 antibody" includes antibodies, and antigen-binding fragments thereof, that bind a human IL-33 protein or a biologically active fragment thereof, (*e.g.,* See SEQ ID NOs: 348, 349, 350 and 351).

[0127] The expression "interleukin-4 receptor", or "IL-4R" as used herein, refers to a human IL-4Rα receptor having an amino acid sequence of SEQ ID NO: 347.

[0128] As used herein, an “IL-4R antagonist” (also referred to herein as an “IL-4R inhibitor,” an “IL-4Rα antagonist,” an “IL-4R blocker,” an “IL-4Rα blocker,” etc.) is any agent, which binds to or interacts with IL-4Rα or an IL-4R ligand, and inhibits or attenuates the normal biological signaling function of a type 1 and/or a type 2 IL-4 receptor. A type 1 IL-4 receptor is a dimeric receptor comprising an IL-4Rα chain and a γc chain. A type 2 IL-4 receptor is a dimeric receptor comprising an IL-4Rα chain and an IL-13Rα1 chain. Type 1 IL-4 receptors interact with and are stimulated by IL-4, while type 2 IL-4 receptors interact with and are stimulated by both IL-4 and IL-13. Thus, the IL-4R antagonists that can be used in the methods of the present invention may function by blocking IL-4-mediated signaling, IL-13-mediated signaling, or both IL-4- and IL-13-mediated signaling. The IL-4R antagonists of the present invention may thus prevent the interaction of IL-4 and/or IL-13 with a type 1 or type 2 receptor. Non-limiting examples of categories of IL-4R antagonists include small molecule IL-4R inhibitors, anti-IL-4R aptamers, peptide-based IL-4R inhibitors (*e.g.*, “peptibody” molecules), “receptor-bodies” (*e.g.*, engineered molecules comprising the ligand-binding domain of an IL-4R component), and antibodies or antigen-binding fragments of antibodies that specifically bind human IL-4Rα. As used herein, IL-4R antagonists also include antigen-binding proteins that specifically bind IL-4 and/or IL-13.

[0129] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, IL-33 or IL-4R). The term "antibody" includes 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), or the anti-IL-4R antibody 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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)).

[0134] 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, including the exemplary bispecific antibody formats disclosed herein, 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. For example, the present invention includes methods comprising the use of bispecific antibodies wherein one arm of an immunoglobulin is specific for IL-4Rα or a fragment thereof, or an immunoglobulin specific

for IL-33 or a fragment thereof and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. Exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED) body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

[0135] In certain embodiments of the invention, the anti-IL-33 and the IL-4R antibodies of the invention are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The term includes antibodies 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.

[0136] The antibodies of the invention may, in some embodiments, be recombinant human antibodies. 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*.

[0137] 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.

[0138] 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.

[0139] The antibodies of the invention may be isolated antibodies. 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.

[0140] The present invention includes neutralizing and/or blocking anti-IL-33 antibodies and IL-4R antibodies. A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to the target molecule, *e.g.* either IL-33 or IL-4R: (i) interferes with the interaction between the target molecule and either its receptor (in the case of IL-33 antibodies), or its ligand (in the case of IL-4R antibodies); and/or (ii) results in inhibition of at least one biological function of the target molecule, *e.g.* signaling. The inhibition caused by an IL-33 or IL-4R neutralizing or blocking antibody need not be complete so long as it is detectable using an appropriate assay.

[0141] The antibodies disclosed herein 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 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. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0142] The present invention also includes 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 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.

[0143] 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.

[0144] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0145] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a

positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0146] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402, each herein incorporated by reference.

[0147] A "disease or disorder", as used herein, is any condition that is treatable with the IL-33 and IL-4 antagonists of the invention. An "inflammatory disease or disorder", as used herein, refers to a disease, disorder or pathological condition where the pathology results, in whole or in part, from, *e.g.*, a change in number, change in rate of migration, or change in activation, of cells of the immune system. Cells of the immune system include, e.g., T cells, B cells, monocytes or macrophages, innate lymphoid cells, antigen presenting cells (APCs), dendritic cells, microglia, NK cells, neutrophils, eosinophils, mast cells, or any other cell specifically associated with the immunology, for example, cytokine-producing endothelial or epithelial cells. As used herein, in one embodiment, the "inflammatory disease or disorder" is an immune disorder or condition selected from the group consisting of asthma, (including steroid resistant asthma, steroid sensitive asthma, eosinophilic asthma or non-eosinophilic asthma), allergy, anaphylaxis, multiple sclerosis, inflammatory bowel disorder (*e.g.* Crohn's disease or ulcerative colitis), chronic obstructive pulmonary disease (COPD, which may or may not be related to, caused in part by, or resulting from, exposure to first or second hand cigarette smoke), asthma and COPD overlap syndrome (ACOS), eosinophilic esophagitis, chronic bronchitis, emphysema, chronic rhinosinusitis with or without nasal polyps, lupus, atopic dermatitis, psoriasis, scleroderma and other fibrotic diseases, sjogren's syndrome, vasculitis (behcet's disease, Giant cell arteritis, Henoch-Schonlein purpura and Churg Strauss syndrome), inflammatory pain and arthritis. In

one embodiment, the arthritis is selected from the group consisting of rheumatoid arthritis, osteoarthritis, and psoriatic arthritis.

[0148] In one embodiment, the "inflammatory disease or disorder" is an immune disorder or condition that comprises a Type 1 response and/or a Type 2 response.

[0149] A "type 1 immune response" is defined by T helper 1 (T_H1) cells and T_H17 cells, cytotoxic T cells, group 1 and group 3 innate lymphoid cells (ILCs) and immunoglobulin M (IgM), IgA and specific IgG antibody classes and the cytokines including, for example, TNF, IL-1β and IL-6. This effector response mediates immunity to many microorganisms including bacteria, viruses, fungi, and protozoa. Elements of Type 1 immunity also help to maintain tumor immune surveillance.

[0150] A "type 2 immune response" is characterized by CD4+ T helper 2 (T_H2) cells, group 2 innate lymphoid cells (ILCs), eosinophils, basophils, mast cells, IL-4 and/or IL-13 activated macrophages, the IgE antibody subclass, and the cytokines including, for example, IL-4, IL-5, IL-9, IL-13, thymic stromal lymphopoietin, IL-25 and IL-33. Type 2 immunity provides protection against large extracellular parasites by boosting barrier defenses. Elements of the type 2 immune response also help to maintain metabolic homeostasis and to promote tissue remodeling following injury. This type of response can also be initiated in response to allergens.

[0151] The phrase "inhibits or attenuates IL-33-mediated signaling", as used herein, refers to the degree to which IL-33 stimulates signal transduction through its receptor, ST2 and the co-receptor, IL-1RAcP, which is diminished in the presence of an antagonist, such as an IL-33 antibody, or an IL-33 trap, as described herein, relative to the degree to which IL-33 stimulates signal transduction through ST2 and IL-1RAcP in the absence of the antagonist such as an IL-33 antibody, or IL-33 trap as described herein. The phrase "Inhibits or attenuates IL-4R-mediated signaling", as used herein, refers to the degree to which IL-4 stimulates signal transduction through a type 1 and/or a type 2 IL-4 receptor, which is diminished in the presence of an antagonist, such as an IL-4 or IL-4R antibody, as described herein, relative to the degree to which IL-4 stimulates signal transduction through a type 1 and/or type 2 IL-4 receptor in the absence of the antagonist such as an IL-4 or IL-4R antibody, as described herein. To examine the extent of inhibition, a sample is treated with a potential inhibitor/antagonist and is compared to a control sample without the inhibitor/antagonist. Control samples, *i.e.*, not treated with antagonist, are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, or 20% or less. An endpoint in inhibition may comprise a predetermined quantity or percentage of, *e.g.*, an indicator of inflammation, or cell degranulation, secretion or activation, such as the release of a cytokine. Inhibition of IL-33 signal transduction through ST2 and IL-1RAcP can be determined by assaying for IL-33 signal transduction in an *in vitro* assay, such as those known to one skilled in the art. In addition, *in*

vivo assays can be used to determine whether a molecule is an antagonist of IL-33. For example, an *in vivo* assay may be used to assess the effect of an antibody to IL-33 on lung inflammation in allergen-sensitized animals that are homozygous for expression of human IL-33. Following sensitization of the animals with allergen, a subset of the animals is treated with either an anti-IL-33 antibody of the invention or a negative isotype control antibody. Afterwards, the animals are sacrificed and the lungs are harvested for assessment of cellular infiltrates, as well as cytokine measurements (IL-4 and IL-5). An IL-33 antibody that is effective as an antagonist should demonstrate a trend towards reduction in inflammatory cells in the lung, as well as a trend towards reduction in cytokines such as IL-4 and IL-5. Similar assays may be done to assess the ability of an IL-4R antagonist to block signal transduction following binding of IL-4 to a type 1 and/or a type 2 receptor *in vitro* or *in vivo*. Moreover, any of the above-noted assays may be modified in order to compare the effects of using either an IL-33 antagonist alone, an IL-4 or IL-4R antagonist alone, or the effect of using a combination of both the IL-33 antagonist and the IL-4 or IL-4R antagonist together.

[0152] In another aspect, the invention provides methods for reducing the incidence or recurrence of asthma or COPD, or an asthma or COPD exacerbation, in a subject in need thereof comprising administering a pharmaceutical composition comprising an interleukin-4 receptor (IL-4R) antagonist to the subject in combination with a pharmaceutical composition comprising an IL-33 antagonist. As used herein, the expression "asthma or COPD exacerbation" means an increase in the severity and/or frequency and/or duration of one or more symptoms or indicia of asthma or COPD. An "asthma or COPD exacerbation" also includes any deterioration in the respiratory health of a subject that requires and or is treatable by a therapeutic intervention for asthma or COPD (such as, e.g., steroid treatment, inhaled corticosteroid treatment, hospitalization, etc.).

[0153] A "reduction in the incidence or recurrence" of an asthma or COPD exacerbation means that a subject who has received the pharmaceutical compositions of the present invention experiences fewer asthma or COPD exacerbations (i.e., at least one fewer exacerbation) after treatment than before treatment, or experiences no asthma or COPD exacerbations for at least 4 weeks (e.g., 4, 6, 8, 12, 14, or more weeks) following initiation of treatment with a pharmaceutical composition of the present invention. A "reduction in the incidence or recurrence" of an asthma or COPD exacerbation alternatively means that, following administration of a pharmaceutical composition of the present invention, the likelihood that a subject experiences an asthma or COPD exacerbation is decreased by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more) as compared to a subject who has not received a pharmaceutical composition of the present invention.

[0154] A "fibrotic disease or disorder" as used herein refers to conditions that involve an excess of fibrous connective tissue in a tissue or organ. "Fibrosis" refers to a pathologic process, which

includes scar formation and overproduction of extracellular matrix by the connective tissue as a response to tissue damage. As used herein, exemplary "fibrotic diseases or disorders" that are treatable by administering the anti-IL-33 and IL-4R antagonists of the invention include pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis, bleomycin-induced pulmonary fibrosis, asbestos-induced pulmonary fibrosis, and bronchiolitis obliterans syndrome), chronic asthma, fibrosis associated with acute lung injury and acute respiratory distress (*e.g.*, bacterial pneumonia induced fibrosis, trauma induced fibrosis, viral pneumonia induced fibrosis, ventilator induced fibrosis, non-pulmonary sepsis induced fibrosis and aspiration induced fibrosis), silicosis, radiation-induced fibrosis, chronic obstructive pulmonary disease (COPD, which may or may not be related to, caused in part by, or resulting from, exposure to first or second hand cigarette smoke), scleroderma, ocular fibrosis, skin fibrosis (*e.g.*, scleroderma), hepatic fibrosis (*e.g.*, cirrhosis, alcohol-induced liver fibrosis, non-alcoholic steatohepatitis (NASH), biliary duct injury, primary biliary cirrhosis, infection- or viral-induced liver fibrosis, autoimmune hepatitis, kidney (renal) fibrosis, cardiac fibrosis, atherosclerosis, stent restenosis, and myelofibrosis. While asthma and COPD are generally considered to be inflammatory conditions, each is also known to exhibit properties of fibrotic disorders.

[0155] A "response" of a patient or a patient's "responsiveness" to treatment or therapy, for example treatment comprising an IL-33 antagonist (e.g., an IL-33 or ST2 binding antagonist), or an IL-4 antagonist, refers to the clinical or therapeutic benefit imparted to a patient at risk for or having an IL-33-mediated disorder (e.g., asthma, COPD, ACOS, nasal polyps, or pulmonary fibrosis, e.g., idiopathic pulmonary fibrosis) from or as a result of the treatment. Such benefit may include cellular or biological responses, a complete response, a partial response, a stable disease (without progression or relapse), or a response with a later relapse of the patient from or as a result of the treatment with the antagonist. A skilled person will readily be in position to determine whether a patient is responsive. For example, a patient suffering from asthma who is responsive to treatment comprising an IL-33 antagonist and/or an IL-4 antagonist may show observable and/or measurable reduction in or absence of one or more of the following exemplary symptoms: recurrent wheezing, coughing, trouble breathing, chest tightness, symptoms that occur or worsen at night, symptoms that are triggered by cold air, exercise or exposure to allergens.

[0156] Furthermore, "enhanced therapeutic efficacy" may be determined by assessing whether treating with the combination of an IL-33 antagonist plus an IL-4R antagonist results in marked improvement in at least one symptom of the disease or disorder, or an improvement in at least one of the biological parameters, as measured herein (e.g. lung inflammation, cytokine release, etc.) when compared to the results achieved when the IL-33 antagonist or the IL-4R antagonist is used alone. Any of the biological measures of efficacy, as described in the present application, may be used to determine therapeutic efficacy, or enhancement thereof.

IL-33 Antagonists and IL-4R Antagonists

[0157] The methods of the present invention comprise administering to a patient suffering from an inflammatory disease or disorder a therapeutically effective amount of an IL-33 antagonist in combination with a therapeutically effective amount of an IL-4R antagonist.

IL-33 Antagonists

[0158] The term "human interleukin-33" or "human IL-33" or "hIL-33", or "IL-33" refers to the 270 amino acid, full-length, unprocessed IL-33 (See, for example, SEQ ID NO: 348, or UniProtKB accession number O95760), or a biologically active fragment thereof, as well as any form of IL-33 that results from processing in the cell (See, for example, SEQ ID NO: 349, which contains amino acid residues 112-270 of the full length protein). The term also encompasses naturally occurring variants of IL-33, for example, splice variants (See for example, Hong, *et. al.,* (2011), J. Biol. Chem. 286(22):20078-20086), or allelic variants, or any other isoform of IL-33, such as the oxidized or reduced forms of IL-33 described in WO2016/156440. The activity of IL-33 that can be neutralized, inhibited, blocked, abrogated, attenuated, reduced or interfered with, by an antibody or antigen-binding fragment thereof of the invention, or by an IL-33 trap of the invention, includes, but is not limited to, inhibition of IL-33 receptor-mediated signaling, or inhibition of IL-33-mediated inflammation.

[0159] As used herein, an “IL-33 antagonist” (also referred to herein as an “IL-33 inhibitor,” or an “IL-33 blocker,” etc.) is any agent, which inhibits the interaction of IL-33 with one or more of its binding partners and in so doing may inhibit IL-33-mediated signaling. For example, an "IL-33 antagonist" may bind to and/or interact with IL-33, or with the IL-33 receptor referred to as "suppression of tumorigenicity" ("ST2"), or with the IL-33 co-receptor referred to as "Interleukin-1 Receptor Accessory Protein ("IL-1RAcP"), or with a complex of any of the following: IL-33/ST2, or ST2/IL-1RAcP and in so doing, may inhibit IL-33-mediated signaling.

[0160] Non-limiting examples of categories of IL-33 antagonists include small molecule IL-33 inhibitors, or receptor antagonists, or nucleic acids that hybridize under stringent conditions to nucleic acid sequences encoding either IL-33, or an IL-33 receptor or co-receptor (e.g., short interfering RNAs (siRNA) or clustered regularly interspaced short palindromic repeat RNAs (CRISPR-RNA or crRNA), including single guide RNAs (sgRNAs) having a crRNA and tracrRNA sequence as described in Mali et al. (Science. 339: 823-26, 2013), which is incorporated herein by reference in its entirety). Other IL-33 antagonists include proteins comprising a ligand-binding portion of an IL-33 receptor (*e.g.* ST2), IL-33-binding scaffold molecules (*e.g.*, DARPins, HEAT repeat proteins, ARM repeat proteins, tetratricopeptide repeat proteins, fibronectin-based scaffold constructs, and other scaffolds based on naturally occurring repeat proteins, etc., [*see, e.g.*, Boersma and Pluckthun, 2011, *Curr. Opin. Biotechnol. 22*:849-857, and references cited therein]), and anti-IL-33 aptamers or portions thereof.

IL-33 Antibodies

[0161] According to certain embodiments, IL-33 antagonists or inhibitors that can be used in the context of the present invention are anti-IL-33 antibodies or antigen-binding fragments of antibodies that specifically bind human IL-33. The amino acid sequence identifiers for exemplary anti-IL-33 antibodies for use in the methods described herein are shown in Table 1 and the nucleic acid sequence identifiers encoding these IL-33 antibodies are shown in Table 2.

[0162] In one embodiment, the anti-IL-33 antibodies described herein for use in the methods of the invention are disclosed in US9,453,072, which is incorporated by reference in its entirety.

[0163] According to certain embodiments, the anti-IL-33 antibodies used in the methods of the present invention specifically bind to IL-33. 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 to IL-33 or a biologically active 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- IL-33 molecules from other (non-human) species.

[0164] According to certain exemplary embodiments of the present invention, the IL-33 antagonist is an anti-IL-33 antibody, or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR), light chain variable region (LCVR), and/or complementarity determining regions (CDRs) comprising any of the amino acid sequences of the anti-IL-33 antibodies as set forth in US Patent No. 9,453,072 and in Table 1 disclosed herein. In certain embodiments, the IL-33 antagonist is an anti-IL-33 antibody having the binding characteristics of the reference antibody described in US9,453,072. In certain exemplary embodiments, the anti-IL-33 antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present invention comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 274 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 282. According to certain embodiments, the anti-IL-33 antibody or antigen-binding fragment thereof comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and

LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 276; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 278; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 280; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 284; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 286; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 288. In yet other embodiments, the anti-IL-33 antibody or antigen-binding fragment thereof comprises an HCVR comprising SEQ ID NO: 274 and an LCVR comprising SEQ ID NO: 282.

[0165] In one embodiment, the IL-33 antagonist is an IL-33 antibody referred to as REGN3500, which comprises a HCVR having the amino acid sequence of SEQ ID NO: 274 and a LCVR having the amino acid sequence of SEQ ID NO: 282 and heavy chain complementarity determining regions (HCDR1-HCDR2-HCDR3) having the amino acid sequences of SEQ ID NOS: 276-278-280, respectively and light chain complementarity determining regions (LCDR1-LCDR2-LCDR3) having the amino acid sequences of SEQ ID NOS: 284-286-288, respectively.

[0166] Other anti-IL-33 antibodies and antigen-binding fragments thereof that may be used in the methods described herein are disclosed in EP1725261, US8187596, WO2011031600, WO2015099175, WO2015106080 (ANB020), US2016/0168242, WO2016/077381, WO2016/077366, or WO2016/156440, which are each incorporated herein by reference in their entirety.

IL-33 Traps

[0167] According to certain embodiments, IL-33 antagonists or inhibitors that can be used in the context of the present invention are receptor based IL-33 traps, such as those described herein.

[0168] The IL-33 traps described herein comprise at least one IL-33 binding domain, which comprises an IL-33 binding portion of an IL-33 receptor protein, designated ST2. In certain embodiments an IL-33 trap further comprises an extracellular portion of an IL-33 co-receptor, designated IL-1 receptor accessory protein, or IL-1RAcP. The IL-33 trap may also contain at least one multimerizing component, which functions to connect the various components of the trap with one another. The various components of the IL-33 traps are described below and shown in Figure 1.

[0169] In one embodiment, the IL-33 traps described herein for use in the methods of the invention are disclosed in US2014/0271642 and WO2014/152195, incorporated herein by reference in their entirety.

[0170] Briefly, the IL-33 traps comprise a first IL-33 binding domain (D1) attached to a multimerizing domain (M). In certain embodiments, the IL-33 antagonists of the invention comprise a second IL-33 binding domain (D2) attached to D1 and/or M. According to certain embodiments, D1 comprises an IL-33-binding portion of an ST2 protein. According to certain embodiments, D2 comprises an extracellular portion of an IL-1RAcP protein.

[0171] The individual components of the IL-33 traps may be arranged relative to one another in a variety of ways that result in functional antagonist molecules capable of binding IL-33. For example, D1 and/or D2 may be attached to the N-terminus of M. In other embodiments D1 and/or D2 is attached to the C-terminus of M. In yet other embodiments, D1 is attached to the N-terminus of D2, and D2 is attached to the N-terminus of M, resulting in an in-line fusion, from N- to C-terminus, of an antagonist molecule represented by the formula D1-D2-M. Other orientations of the individual components are disclosed elsewhere herein in Figure 1.

[0172] Non-limiting examples of IL-33 traps for use in the methods of the invention are shown in Tables 3a and 3b, and include the IL-33 traps designated "hST2-hFc," "hST2-mFc," "hST2-hIL1RAcP-mFc," "hST2-hIL1RAcP-hFc" and "mST2-mIL1RAcP-mFc". These correspond to SEQ ID NOs: 323, 324, 325, 326 and 327, respectively. The present invention includes IL-33 receptor based traps having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any of the exemplary IL-33 receptor based traps set forth herein (e.g. SEQ ID NOs: 323, 324, 325, 326 and 327).

[0173] Standard molecular biological techniques (*e.g.*, recombinant DNA technology) may be used to construct any of the IL-33 traps of the invention or variants thereof.

[0174] The IL-33 traps for use in the methods of the invention comprise at least one IL-33 binding domain (sometimes referred to herein by the designation "D," or "D1," "D2," etc.). In certain embodiments, the IL-33 binding domain comprises an IL-33-binding portion of an ST2 protein. An IL-33-binding portion of an ST2 protein can comprise or consist of all or part of the extracellular domain of an ST2 protein. In certain embodiments, an ST2 protein is a human ST2 protein. A "human ST2 protein," as used herein, refers to an ST2 protein as shown in amino acids 1-556 of accession number NP_057316.3, shown also as SEQ ID NO: 352. In certain embodiments, the ST2 protein is an ST2 protein from a non-human species (*e.g.*, mouse ST2, monkey ST2, etc). An exemplary IL-33-binding portion of an ST2 protein is set forth herein as the amino acid sequence of SEQ ID NO: 328 (corresponding to the extracellular domain of human ST2 [K19-S328 of NCBI Accession No. NP_057316.3]). Other examples of an IL-33-binding portion of an ST2 protein is set forth herein as the amino acid sequence of SEQ ID NO:329 (corresponding to the extracellular domain of mouse ST2 [S27-R332 of NCBI Accession No. P14719]).

[0175] In certain embodiments, the IL-33 binding domain comprises an extracellular portion of an IL-1RAcP protein. In certain embodiments, an IL-1RAcP protein is a human IL-1RAcP protein. A "human IL-1RAcP protein," as used herein, refers to an IL-1RAcP protein having the amino acid sequence of SEQ ID NO:353. In certain embodiments, the IL-1RAcP protein is an IL-1RAcP protein from a non-human species (*e.g.*, mouse IL-1RAcP, monkey IL-1RAcP, etc). An exemplary extracellular portion of an IL-1RAcP protein is set forth herein as the amino acid sequence of SEQ ID NO:330 (corresponding to the extracellular domain of human IL-1RAcP

[S21-E359 of NCBI Accession No. Q9NPH3]). Another example of an extracellular portion of an IL-1RAcP protein is set forth herein as the amino acid sequence of SEQ ID NO:331 (corresponding to the extracellular domain of mouse IL-1RAcP [S21-E359 of NCBI Accession No. Q61730]).

[0176] The present invention includes IL-33 traps comprising D1 and/or D2 components having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any of the exemplary IL-33 binding domain component amino acid sequences set forth herein (*e.g.*, SEQ ID NOs: 328, 329, 330 and 331).

[0177] The IL-33 antagonists of the present invention also comprise at least one multimerizing domain (sometimes referred to herein by the abbreviation "M," "M1", "M2", etc.). In general terms, the multimerizing domain(s) of the present invention function to connect the various components of the IL-33 antagonists (*e.g.*, the IL-33-binding domain(s)) with one another. As used herein, a "multimerizing domain" is any macromolecule that has the ability to associate (covalently or non-covalently) with a second macromolecule of the same or similar structure or constitution. For example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing domain is an Fc portion of an immunoglobulin, *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

[0178] Non-limiting exemplary multimerizing domains that can be used in the IL-33 antagonists of the present invention include human IgG1 Fc (SEQ ID NO:332) or mouse IgG2a Fc (SEQ ID NO:333). The present invention includes IL-33 antagonists comprising M components having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any of the exemplary M component amino acid sequences set forth herein (*e.g.*, SEQ ID NOs:332 or 333).

[0179] In certain embodiments, the IL-33 antagonists of the present invention comprise two multimerizing domains, M1 and M2, wherein M1 and M2 are identical to one another. For example, M1 can be an Fc domain having a particular amino acid sequence, and M2 is an Fc domain with the same amino acid sequence as M1.

[0180] The individual components of the IL-33 antagonists of the present invention (*e.g.*, D1, D2, M, etc.) can be arranged relative to one another in a variety of ways. Non-limiting examples of all of the above noted arrangements are illustrated schematically in Figure 1.

[0181] Non-limiting illustrative examples of IL-33 traps for use in the methods of the invention comprising two multimerizing domains (M1 and M2) and four IL-33 binding domains (D1, D2, D3 and D4) are also shown in Figure 1, arrangements C and D).

[0182] The individual components of the IL-33 traps of the present invention (*e.g.*, D1, D2, M1, M2, etc.) may be attached to one another directly (*e.g.*, D1 and/or D2 may be directly attached to M, etc.); alternatively, the individual components may be attached to one another via a linker

component (*e.g.*, D1 and/or D2 may be attached to M via a linker oriented between the individual components; D1 may be attached to D2 via a linker; etc.). In any of the arrangements disclosed herein, wherein one component is described as being "attached" to another component, the attachment may be through a linker (even if not specifically designated as such). As used herein, a "linker" is any molecule that joins two polypeptide components together.

[0183] The biological characteristics of the IL-33 traps for use in the methods of the invention are described in US2014/0271642 and in WO2014/152195, incorporated by reference herein in their entirety.

Other IL-33 Antagonists

[0184] Polypeptides that bind IL-33 and/or its receptor (ST2 and/or IL-1 RAcP) and block ligand-receptor interaction are considered as IL-33 antagonists and are disclosed in WO2014/152195, which is incorporated by reference in its entirety. Other agents that may act as IL-33 antagonists and which may be used in the methods of the invention include immunoadhesins, peptibodies, and soluble ST2, or derivatives thereof; anti-IL-33 receptor antibodies (e.g., anti-ST2 antibodies, for example, AMG-282 (Amgen) or STLM15 (Janssen) or any of the anti-ST2 antibodies described in WO2012/113813, WO 2013/173761, WO 2013/165894, US8,444,987, or US7,452,980, which are each incorporated herein by reference in their entirety. Other IL-33 antagonists for use in the methods of the invention include ST2-Fc proteins, such as those described in WO2013/173761, or WO 2013/165894, which are each incorporated herein by reference in their entirety.

IL-4R Antagonists

[0185] As used herein, an “IL-4R antagonist” (also referred to herein as an “IL-4R inhibitor,” an “IL-4Rα antagonist,” an “IL-4R blocker,” an “IL-4Rα blocker,” etc.) is any agent, which binds to or interacts with IL-4Rα or an IL-4R ligand, and inhibits or attenuates the normal biological signaling function of a type 1 and/or a type 2 IL-4 receptor. The term "human IL-4R" or "hIL-4R", as used herein, refers to IL-4R having the amino acid sequence of SEQ ID NO: 347, or a biologically active fragment thereof. A type 1 IL-4 receptor is a dimeric receptor comprising an IL-4Rα chain and a γc chain. A type 2 IL-4 receptor is a dimeric receptor comprising an IL-4Rα chain and an IL-13Rα1 chain. Type 1 IL-4 receptors interact with and are stimulated by IL-4, while type 2 IL-4 receptors interact with and are stimulated by both IL-4 and IL-13. Thus, the IL-4R antagonists that can be used in the methods of the present invention may function by blocking IL-4-mediated signaling, IL-13-mediated signaling, or both IL-4- and IL-13-mediated signaling. The IL-4R antagonists of the present invention may thus prevent the interaction of IL-4 and/or IL-13 with a type 1 or type 2 receptor.

[0186] Non-limiting examples of categories of IL-4R antagonists include small molecule IL-4R antagonists, nucleic acid-based inhibitors of IL-4R expression or activity (*e.g.*, siRNA or antisense), peptide-based molecules that specifically interact with IL-4R (*e.g.*, peptibodies), "receptor-bodies" (*e.g.*, engineered molecules comprising the ligand-binding domain of an IL-4R component), IL-4R-binding scaffold molecules (*e.g.*, DARPins, HEAT repeat proteins, ARM repeat proteins, tetratricopeptide repeat proteins, fibronectin-based scaffold constructs, and other scaffolds based on naturally occurring repeat proteins, etc., [*see*, *e.g.*, Boersma and Pluckthun, 2011, *Curr. Opin. Biotechnol. 22*:849-857, and references cited therein]), and anti-IL-4R aptamers or portions thereof. According to certain embodiments, IL-4R antagonists that can be used in the context of the present invention are anti-IL-4R antibodies or antigen-binding fragments of antibodies that specifically bind human IL-4R.

[0187] In one embodiment, the anti-IL-4R antibody that is disclosed herein for use in the methods of the invention is dupilumab (See also US Patent Numbers 7,605,237; 7,608,693 and 9,290,574).

Anti-IL-4R Antibodies

[0188] According to certain exemplary embodiments of the present invention, the IL-4R antagonist is an anti-IL-4Rα antibody or an antigen-binding fragment thereof, which specifically binds to IL-4Rα. 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-4Rα as used in the context of the present invention, includes antibodies that bind IL-4Rα or a biologically active 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-4Rα may, however, have cross-reactivity to other antigens, such as IL-4Rα molecules from other (non-human) species.

[0189] According to certain exemplary embodiments of the present invention, the IL-4R antagonist is an anti-IL-4Rα antibody, or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR), light chain variable region (LCVR), and/or complementarity determining regions (CDRs) comprising any of the amino acid sequences of the anti-IL-4R antibodies as set forth in US Patent Nos. 7,605,237 and 7,608,693. In certain embodiments, the

IL-4R antagonist is an anti-IL-4R antibody having the binding characteristics of the reference antibody referred to herein as dupilumab (See US7,605,237 and US7,608,693). In certain exemplary embodiments, the anti-IL-4Rα antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present invention comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 337 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 338. According to certain embodiments, the anti-IL-4Rα antibody or antigen-binding fragment thereof comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 339; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 340; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 341; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 342; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 343; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 344. In yet other embodiments, the anti-IL-4R antibody or antigen-binding fragment thereof comprises an HCVR comprising SEQ ID NO: 337 and an LCVR comprising SEQ ID NO: 338. In yet other embodiments, the anti-IL-4R antibody or antigen-binding fragment thereof comprises an HCVR comprising SEQ ID NO: 335 and an LCVR comprising SEQ ID NO: 336. In yet other embodiments, the anti-IL-4R antibody or antigen-binding fragment thereof comprises a heavy chain (HC) amino acid sequence as set forth in SEQ ID NO: 345 and a light chain (LC) amino acid sequence as set forth in SEQ ID NO: 346. According to certain exemplary embodiments, the methods of the present invention comprise the use of the anti-IL-4Rα antibody referred to and known in the art as dupilumab, or a bioequivalent thereof. Dupilumab comprises a HCVR having the amino acid sequence of SEQ ID NO: 337 and a LCVR having the amino acid sequence of SEQ ID NO: 338 and heavy chain complementarity determining regions (HCDR1-HCDR2-HCDR3) having the amino acid sequences of SEQ ID NOS: 339-340-341, respectively and light chain complementarity determining regions (LCDR1-LCDR2-LCDR3) having the amino acid sequences of SEQ ID NOS: 342-343-344, respectively.

[0190] Other anti-IL-4Rα antibodies that can be used in the context of the methods of the present invention include, *e.g.*, the antibody referred to and known in the art as AMG317 (Corren *et al.*, 2010, *Am J Respir Crit Care Med., 181*(8):788-796), or MEDI 9314, or any of the anti-IL-4Rα antibodies as set forth in US Patent No. 7,186,809, US Patent No. 7,605,237, US Patent No. 7,638,606, US Patent No. 8,092,804, US Patent No. 8,679,487, or US Patent No. 8,877,189.

pH Dependent Characteristics of the Anti-IL-4 and/or Anti-IL-33 Antibodies

[0191] The anti-IL-4Rα and the IL-33 antibodies used in the context of the methods of the

present invention may have pH-dependent binding characteristics. For example, an anti-IL-4Rα antibody or an anti-IL-33 antibody for use in the methods of the present invention may exhibit reduced binding to IL-4Rα, or to IL-33 respectively, at acidic pH as compared to neutral pH. Alternatively, an anti-IL-4Rα antibody of the invention, or an anti-IL-33 antibody of the invention may exhibit enhanced binding to its antigen at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, *e.g.*, about 6.0, 5.95, 5.9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[0192] In certain instances, “reduced binding to IL-4Rα at acidic pH as compared to neutral pH”, or “reduced binding to IL-33 at acidic pH as compared to neutral pH” is expressed in terms of a ratio of the K_D value of the antibody binding to IL-4Rα, or IL-33, respectively at acidic pH to the K_D value of the antibody binding to IL-4Rα, or IL-33, respectively at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to IL-4Rα at acidic pH as compared to neutral pH", or "reduced binding to IL-33 at acidic pH as compared to neutral pH", for purposes of the present invention if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment of the present invention can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0, or greater.

[0193] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained. As used herein, the expression "acidic pH" means a pH of 6.0 or less.

Biological Effects of the IL-33 and IL-4R Antagonists Used in Combination Therapy

[0194] The present invention includes the use of an IL-33 antagonist in combination with an IL-4R antagonist for treating an inflammatory condition. In one embodiment, the use of an anti-IL-33 antibody in combination with an anti-IL4R antibody in an animal model of fibrosis and lung inflammation, demonstrates enhanced efficacy, as compared to the results obtained when each antibody is used alone as monotherapy.

[0195] For example, in the animal model described herein (referred to as a House Dust Mite (HDM) model of lung inflammation and fibrosis), the level of certain cytokines in the lungs is

significantly elevated. This includes an elevation of IL-4, IL-5, IL-6, IL-1β, and MCP-1. There was also a trend for increased levels of IL-13 and TNFα in the lungs of mice following administration of house dust mite allergen. However, when tested in this model, the combined use of the IL-33 antibody and the IL-4R antibody resulted in reduced levels of the cytokines IL-4, IL-5, IL-6, IL-13, IL-1β, MCP-1 and TNFα in the lungs of treated mice. The effect on lung cytokine levels observed with the combination of anti-IL-33 and anti-IL-4R antibodies was greater than treatment with either individual antibody when used alone, as shown in Example 4.

[0196] In addition, the levels of cytokine genes, chemokine genes and collagen genes, including *Il4, Il13, Il6, Ccl2, Tgfb1, Il13ra2* and *Col24a1,* were elevated in mice receiving a house dust mice allergen. There was a trend towards increased levels of *Il5, Il9, Ccl11, Ccl24, Tnf, Il1rl1*, and *Col15a1* in this model. Upon treatment with the combination of the anti-IL-33 antibody and the anti-IL-4R antibody, there was significant reduction in expression of *Il6, Ccl2, Ccl11* and *Ccl24,* as compared to the levels observed with treatment with either antibody alone. There was also a trend towards reduced *Il4, Il5, Il13, Il9, Tnf, Tgfb1, Il1rl1, Il13ra2, Col15a1* and *Col24a1* when the mice were treated with the anti-IL-33 antibody plus the anti-IL-4R antibody, compared to treatment with either antibody alone.

[0197] Another biological effect associated with combined use of the anti-IL-33 plus the anti-IL-4R antibodies was observed when an analysis was done on pulmonary cell infiltrates in the house dust mite model. As shown in Example 4, the frequency of eosinophils, activated B cells, activated CD8 cells, ST2+CD4+ T cells and CD4/CD8 T cell ratios were significantly higher in mice receiving house dust mite allergen. There was also a trend towards an increased frequency of activated CD4+ T cells in the lungs of mice given the house dust mite allergen. There was a trend towards reduced frequency of eosinophils, activated B cells, activated CD8 cells, ST2+CD4+ T cells and CD4/CD8 T cell ratios in mice treated with both the anti-IL-33 antibody plus the anti-IL-4R antibody as compared to that observed when either antibody was used alone.

[0198] Furthermore, mice receiving house dust mite allergen also show an increase in goblet cell metaplasia in their lungs. Similarly, there was also an increase in lung consolidation (the accumulation of solid or liquid material in the alveolar space), and sub-epithelial fibrosis (an excess of interstitial collagen deposition beneath the pulmonary epithelium) in this mouse model. Treatment of these mice with an anti-IL-33 antibody in combination with an anti-IL-4R antibody resulted in a reduction in goblet cell metaplasia and sub-epithelial collagen thickness and a significant reduction in lung consolidation, as compared to the results observed when either of the two antibodies was used alone.

[0199] The mice receiving house dust mite allergen also demonstrated an increase in circulating levels of IgE, as well as a trend towards an increase in house dust mite (HDM) specific IgG1. Administration of both an anti-IL-33 antibody and an IL-4R antibody resulted in a

significant decrease in serum IgE levels and a trend towards a decrease in HDM specific IgG1 as compared to the levels of IgE and HDM-specific IgG1 observed when either of the antibodies was used alone.

[0200] An IL-33 antagonist and an IL-4R antagonist, such as the antibodies described herein for use as combination therapy to treat inflammatory lung disorders or conditions, may inhibit or attenuate IL-33-mediated signaling and IL-4R-mediated signaling and they may exhibit one or more of the biological properties observed in the HDM model described herein, for example, (1) a reduction in cytokine levels that are elevated in a mammal as a result of exposure to an allergen, *e.g.* IL-4 or IL-5; (2) inhibition of lung inflammation resulting from acute or chronic exposure to an allergen (*e.g.* house dust mites (HDM)); (3) a decrease in cellular lung infiltration resulting from acute or chronic exposure to an allergen (*e.g.* house dust mites (HDM)); (4) an improvement in composite lung gross pathology.

[0201] Inhibition of IL-33-mediated signaling or IL-4R-mediated signaling may be measured in a cell-based bioassay and means that an anti-IL-33 antibody or antigen-binding fragment thereof, or an anti-IL-4R antibody or antigen-binding fragment thereof inhibits or reduces the signal produced in cells that express an IL-33 receptor or an IL-4 receptor and a reporter element that produces a detectable signal in response to IL-33 binding, or IL-4 binding. For example, the present invention includes antibodies and antigen-binding fragments thereof that block IL-33-mediated signaling, or IL-4 mediated signaling in cells expressing human ST2, or in cells expressing an IL-4 receptor, respectively, with an IC_{50} of less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 350 pM, less than about 300 pM, less than about 250 pM, less than about 200 pM, less than about 150 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, or less than about 10 pM, as measured in a cell-based blocking bioassay.

[0202] The antibodies of the present invention may demonstrate one or more of the aforementioned biological effects, or any combination thereof. Other biological effects of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein. The use of other IL-33 antagonists in combination with an IL-4 antagonist may demonstrate similar effects.

Pharmaceutical Compositions and Administration

[0203] The invention provides pharmaceutical compositions comprising the IL-33 antagonists, and/or the IL-4R antagonists of the present invention. The IL-33 antagonists and the IL-4R antagonists may be formulated in separate compositions, or they may be co-formulated in one composition. The pharmaceutical compositions of the invention are formulated with suitable

carriers, excipients, and other agents that provide improved 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™, Life Technologies, Carlsbad, CA), 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.

[0204] The dose of antibody 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 antibody of the present invention is used for treating a condition or disease associated with IL-33 activity and/or IL-4 in an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single 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. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering 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).

[0205] 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 introduction 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. Administration can be systemic or local.

[0206] 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.

[0207] 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 about 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.

[0208] 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.

[0209] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. 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 is preferably filled in an appropriate ampoule.

[0210] 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. The amount of the aforesaid antagonists contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antagonists are contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Dosage

[0211] The amount of IL-33 and IL-4R antagonist 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 and IL-4R antagonist that, when used in combination, results in a significant change in one or more of the following: (a) prevention of inflammation; (b) treatment of or reduction in the severity of inflammation; (c) a reduction in the frequency of one or more of the following: eosinophils, activated B cells, activated CD8 T cells, or CD4/CD8 T cell ratio in the lungs; (d) a reduction in one or more of the following: interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-13 (IL-13), monocyte chemoattractant protein-1 (MCP-1) or tumor necrosis factor alpha (TNFα) levels in the lung; (e) a reduction in the gene expression level of one or more of the following: *Il4, Il5, Il6*, *Il9, Il13*, *Il1rl1*, *Il13ra2*, *tnf, Tgfb1, Ccl2, Ccl11, Ccl24*, *Col15a1* or *Col24a1* in the lung; (f) a reduction in serum IgE levels; (g) a reduction in goblet cell metaplasia in the lung; or (h) a reduction in lung consolidation, as described herein. While the administration of either the IL-33 antagonist alone, or the IL-4R antagonist alone may result in a positive therapeutic effect as measured using one or more of the above-noted parameters, the use of the IL-33 and the IL-4R antagonists in combination will show a significant improvement (*e.g.* an additive or a synergistic effect) in any one or more of the parameters compared to that observed using monotherapy with either the IL-33 antagonist alone or the IL-4R antagonist alone.

[0212] In the case of an IL-33 antagonist, or an IL-4R antagonist, 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. In certain embodiments, 75 mg, 150 mg, 200 mg, or 300 mg of an IL-4R antagonist is administered to a subject in combination with an IL-33 antagonist. In certain embodiments, 75 mg, 150 mg, 200 mg, or 300 mg of an IL-33 antagonist is administered to a subject in combination with an IL-4R antagonist.

[0213] The amount of IL-33 antagonist or IL-4R 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 or the IL-4R antagonist may be administered to a patient at a dose of about 0.0001 mg/kg to about 25 mg/kg of patient body weight. In certain embodiments, each of the IL-4R and the IL-33 antagonists may be administered at doses of about 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg, or 10 mg/kg.

[0214] The combination of the IL-33 antagonist and the IL-4R antagonist may be administered to the subject subcutaneously, intravenously, intramuscularly, or intranasally. They may be administered concurrently or sequentially.

Therapeutic Uses of the Antibodies

[0215] Experiments using a mouse model system, conducted by the present inventors, have contributed to the identification of various diseases and conditions that can be treated, prevented and/or ameliorated by combined IL-33 and IL-4R antagonism. For example, in a house dust mite model of lung inflammation and fibrosis, treatment with a combination of an IL-33 antibody and an IL-4R antibody resulted in a reduction of cytokine levels in the lungs, a reduction in pulmonary cell infiltrates in the lungs (eosinophils, activated B cells, activated CD8 positive cells, ST2+CD4+ T cells and CD4/CD8 T cell ratio), as well as an improvement in lung consolidation and sub-epithelial fibrosis, as compared to the results obtained when each antibody was used alone as monotherapy.

[0216] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with, or mediated by IL-33 expression and IL-4 expression, signaling, or activity, or treatable by blocking the interaction between IL-33 and

an IL-33 receptor (*e.g.*, ST2), or blocking the interaction between IL-4 and an IL-4 receptor, or otherwise inhibiting IL-33 and IL-4 activity and/or signaling. In certain embodiments, the IL-4R antagonist is an antibody that binds to, or interacts with IL-4Rα and in so doing, blocks both the IL-4 and IL-13 signaling pathways through the IL-4R type 1 and type 2 receptors. As such, the use of this dual IL-4 and IL-13 antagonist in combination with an IL-33 antagonist may provide for additional clinical benefits when administered to patients having an inflammatory condition mediated in part by all three signaling pathways. For example, the present invention provides methods for treating asthma (allergic asthma, non-allergic asthma, severe refractory asthma, asthma exacerbations, steroid resistant or steroid refractory asthma, steroid sensitive asthma, eosinophilic asthma or non-eosinophilic asthma, etc.), chronic obstructive pulmonary disease (COPD) and COPD exacerbations, asthma and COPD overlap syndrome (ACOS), chronic bronchitis, emphysema, hypersensitivity pneumonitis, atopic dermatitis, urticaria, psoriasis, allergy, allergic rhinitis, chronic rhinosinusitis with or without nasal polyps, eosinophilic esophagitis, anaphylaxis, cardiovascular disease, central nervous system disease, pain (including inflammatory pain), arthritis (*e.g.*, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, etc.), giant cell arteritis, vasculitis (behcet's disease and Churg Strauss syndrome), Henoch-Schonlein purpura, multiple sclerosis, inflammatory bowel disorder (*e.g.* Crohn's disease or ulcerative colitis), lupus, sjogren's syndrome and other inflammatory diseases or disorders mediated in part by IL-33 and/or IL-4 signaling.

[0217] The antibodies of the present invention are also useful for the treatment, prevention and/or amelioration of one or more fibrotic diseases or disorders. Exemplary fibrotic diseases or disorders that are treatable by administering the anti-IL-33 and IL-4R antagonists of the invention include pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis, bleomycin-induced pulmonary fibrosis, asbestos-induced pulmonary fibrosis, and bronchiolitis obliterans syndrome), fibrosis associated with acute lung injury and acute respiratory distress (*e.g.*, bacterial pneumonia induced fibrosis, trauma induced fibrosis, viral pneumonia induced fibrosis, ventilator induced fibrosis, non-pulmonary sepsis induced fibrosis and aspiration induced fibrosis), silicosis, radiation-induced fibrosis, scleroderma, ocular fibrosis, skin fibrosis (*e.g.*, scleroderma), hepatic fibrosis (*e.g.*, cirrhosis, alcohol-induced liver fibrosis, non-alcoholic steatohepatitis (NASH), biliary duct injury, primary biliary cirrhosis, infection- or viral-induced liver fibrosis, autoimmune hepatitis, kidney (renal) fibrosis, cardiac fibrosis, atherosclerosis, stent restenosis, and myelofibrosis.

[0218] In the context of the methods of treatment described herein, the anti-IL-33 antibody and the IL-4R antibody may be administered together (*i.e.*, as the only therapeutic regimen) or in combination with one or more additional therapeutic agents (examples of which are described elsewhere herein).

Combination Therapies

[0219] The present invention includes the use of compositions and therapeutic formulations comprising any of the anti-IL-33 antagonists and IL-4R antagonists described herein in combination with one or more additional therapeutically active components, and methods of treatment comprising administering such combinations to subjects in need thereof. 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 and the IL-4R antagonist. The term “in combination with” also includes sequential or concomitant administration of an IL-4R antagonist and an IL-33 antagonist and one or more additional therapeutic agents. The present invention includes pharmaceutical compositions in which an IL-33 antagonist and an IL-4R antagonist of the present invention is co-formulated with one or more of the additional therapeutically active component(s).

[0220] For example, when administered "before" the pharmaceutical compositions comprising the IL-33 antagonist and the IL-4R 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 compositions comprising the IL-33 antagonist and the IL-4R antagonist. When administered "after" the pharmaceutical compositions comprising the IL-33 antagonist and the IL-4R 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 compositions comprising the IL-33 antagonist and the IL-4R antagonist. Administration "concurrent" or with the pharmaceutical compositions comprising the IL-33 antagonist and the IL-4R 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 compositions comprising the IL-33 antagonist and the IL-4R antagonist, or administered to the subject as a single combined dosage formulation comprising both the additional therapeutic agent, the IL-33 antagonist and the IL-4R antagonist.

[0221] The additional therapeutic agent may be, *e.g.*, another IL-33 antagonist, another IL-4R antagonist, an IL-1 antagonist (including, *e.g.*, an IL-1 antagonist as set forth in US 6,927,044), an IL-6 antagonist, an IL-6R antagonist (including, *e.g.*, an anti-IL-6R antibody as set forth in US 7,582,298), an IL-13 antagonist, a TNF antagonist, an IL-8 antagonist, an IL-9 antagonist, an IL-17 antagonist, an IL-5 antagonist (*e.g.* mepolizumab, or NUCALA®), an IgE antagonist (*e.g.* omalizumab or XOLAIR®), a CD48 antagonist, an IL-31 antagonist (including, e.g., as set forth in US7,531,637), a thymic stromal lymphopoietin (TSLP) antagonist (including, e.g., as set forth

in US 2011/027468), interferon-gamma (IFNγ), antibiotics, corticosteroids (including inhaled corticosteroids, or ICS), long acting β2 adrenergic agonists (LABA), long acting muscarinic antagonists (LAMA), tacrolimus, pimecrolimus, cyclosporine, azathioprine, methotrexate, cromolyn sodium, proteinase inhibitors, anti-histamines, or combinations thereof.

Administration Regimens

[0222] According to certain embodiments of the present invention, multiple doses of an IL-33 antagonist and an IL-4R antagonist (or a pharmaceutical composition comprising a combination of an IL-33 antagonist, an IL-4R antagonist and any of the additional therapeutically active agents mentioned herein) 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 and an IL-4R antagonist of the invention. As used herein, "sequentially administering" means that each dose of the IL-33 antagonist and the IL-4R 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 and an IL-4R antagonist, followed by one or more secondary doses of the IL-33 antagonist and the IL-4R antagonist, and optionally followed by one or more tertiary doses of the IL-33 antagonist and the IL-4R antagonist.

[0223] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the IL-33 antagonist and the IL-4R antagonist of the invention. 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 and IL-4R antagonist, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of IL-33 antagonist and IL-4R 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 are administered 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").

[0224] In certain exemplary embodiments of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*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, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, 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 and IL-4R antagonist, which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0225] 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 and an IL-4R 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.

[0226] 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 or 1 to 2 months 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 12 weeks after the immediately preceding dose. In certain embodiments of the invention, 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.

[0227] The present invention includes administration regimens in which 2 to 6 loading doses are administered to a patient a first frequency (*e.g.*, once a week, once every two weeks, once every three weeks, once a month, once every two months, etc.), followed by administration of two or more maintenance doses to the patient on a less frequent basis. For example, according to this aspect of the invention, if the loading doses are administered at a frequency of once a month, then the maintenance doses may be administered to the patient once every six weeks, once every two months, once every three months, etc.).

EXAMPLES

[0228] 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

[0229] Human anti-IL-33 antibodies were generated as described in US Patent Number 9,453,072. 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 2 sets forth the nucleic acid sequences encoding 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 Amino Acid Sequence Identifiers

	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

Table 2 Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1M9559N	1	3	5	7	9	11	13	15
H1M9566N	17	19	21	23	25	27	29	31
H1M9568N	33	35	37	39	41	43	45	47
H4H9629P	49	51	53	55	57	59	61	63
H4H9633P	65	67	69	71	73	75	77	79
H4H9640P	81	83	85	87	89	91	93	95
H4H9659P	97	99	101	103	105	107	109	111
H4H9660P	113	115	117	119	121	123	125	127
H4H9662P	129	131	133	135	137	139	141	143
H4H9663P	145	147	149	151	153	155	157	159
H4H9664P	161	163	165	167	169	171	173	175
H4H9665P	177	179	181	183	185	187	189	191
H4H9666P	193	195	197	199	201	203	205	207
H4H9667P	209	211	213	215	217	219	221	223
H4H9670P	225	227	229	231	233	235	237	239
H4H9671P	241	243	245	247	249	251	253	255
H4H9672P	257	259	261	263	265	267	269	271
H4H9675P	273	275	277	279	281	283	285	287
H4H9676P	289	291	293	295	297	299	301	303
H1M9565N	307	309	311	313	315	317	319	321

[0230] 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 2: Construction of IL-33 Antagonists (IL-33 traps)

[0231] Human anti-IL-33 traps were generated as described in US Patent Publication Number 2014/0271642. Table 3a sets forth a summary of the amino acid sequence identifiers for the various components of the IL-33 traps and Table 3b sets forth the full length amino acid sequences of the traps.

[0232] Five different exemplary IL-33 antagonists 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:328) fused at its C-terminus to the N-terminus of a human IgG1 Fc region (SEQ ID NO:332). The second IL-33 antagonist (hST2-mFc, SEQ ID NO:324) consists of the soluble extracellular region of human ST2 (SEQ ID NO:328) fused at its C-terminus to the N-terminus of a mouse IgG2a Fc region (SEQ ID NO:333). The third IL-33 antagonist (hST2-hIL1RAcP-mFc, SEQ ID NO: 325) consists of an in-line fusion having human ST2 (SEQ ID NO:328) at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO:330), followed by a mouse IgG2a Fc (SEQ ID NO:333) 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:329) at its N-terminus, followed by the extracellular region of mouse IL-1RAcP (SEQ ID NO:331), followed by a mouse IgG2a Fc (SEQ ID NO:333) at its C-terminus. The fifth IL-33 antagonist (hST2-hIL1RAcP-hFc, SEQ ID NO:327) consists of an in line fusion having human ST2 of SEQ ID NO: 328 at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO: 330) followed by a human IgG1 Fc (SEQ ID NO: 332) at its C terminus. Table 3a sets forth a summary description of the different IL-33 antagonists and their component parts. Table 3b sets forth the amino acid sequences of the IL-33 antagonists and their component parts.

Table 3a: Summary of IL-33 Antagonists and the Component Parts

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:328)	Absent	human IgG1 Fc (SEQ ID NO:332)
hST2-mFc	SEQ ID NO:324	human ST2 extracellular (SEQ ID NO:328)	Absent	mouse IgG2a Fc (SEQ ID NO:333)

hST2-hIL1RAcP-mFc	SEQ ID NO:325	human ST2 extracellular (SEQ ID NO:328)	human IL-1RAcP extracellular (SEQ ID NO:330)	mouse IgG2a Fc (SEQ ID NO:333)
mST2-mIL1RAcP-mFc	SEQ ID NO:326	mouse ST2 extracellular (SEQ ID NO:329)	mouse IL-1RAcP extracellular (SEQ ID NO:331)	mouse IgG2a Fc (SEQ ID NO:333)
hST2-hIL1RAcP-hFc	SEQ ID NO: 327	human ST2 extracellular (SEQ ID NO:328)	human IL-1RAcP extracellular (SEQ ID NO:330)	human IgG1 Fc (SEQ ID NO:332)

Table 3b: 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

Identifier	Sequence
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 TELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTK SFSRTPGK
SEQ ID NO:327 (hST2-hIL1RAcP-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQI QVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPINFRLPEN RISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKL PVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFL IALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHSPNDHVVYE KEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDET RTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPAPRYTVEDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK
SEQ ID NO:328	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE

Identifier	Sequence
(human ST2 extracellular domain)	KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRI ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHS
SEQ ID NO:329 (mouse ST2 extracellular domain)	SKSSWGLENEALIVRCPQRGRSTYPVEWYYSDTNESIPTQKRNRIFVSRDRLKF LPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIPDYLMYSTVRGSDKNF KITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQF THAENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIACSACF GKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHR
SEQ ID NO:330 (human IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKI QNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKN AVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITI DVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQK VPAPRYTVE
SEQ ID NO:331 (mouse IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKYNYSTAHSSGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVVQKDSCFNSAMRFPVHKMYIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTE IVDFHNVLPEGMNLSFFIPLVSNNGNYTCVVTYPENGRLFHLTRTVTVKVVGSPK DALPPQIYSPNDRVVYEKEPGEELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDV TVDITINESVSYSSTEDETRTQILSIKKVTPEDLRRNYVCHARNTKGEAEQAAKVK QKVIPPRYTVE
SEQ ID NO:332 (human IgG1 Fc)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
SEQ ID NO:333 (mouse IgG2a Fc)	EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDD PDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKV NNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIY VEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHE GLHNHHTTKSFSRTPGK
SEQ ID NO:334 (*M. fascicularis* IL-33-6His)	SITGISPITESLASLSTYNDQSITFALEDESYEIYVEDLKKDKKKDKVLLSYYESQH PSSESGDGVDGKMLMVTLSPTKDFWLQANNKEHSVELHKCEKPLPDQAFFVLH NRSFNCVSFECKTDPGVFIGVKDNHLALIKVDYSENLGSENILFKLSEILEHHHHH H

Example 3: IL-4R Antagonistic Antibodies

[0233] Human anti-IL-4R antibodies were generated as described in US Patent No. 7,608,693. The exemplary IL-4R antibody used in the following example is a mouse antibody specific for mouse IL-4R and has the following amino acid sequences: a heavy chain variable region (HCVR) comprising SEQ ID NO: 335 and a light chain variable domain (LCVR) comprising SEQ ID NO: 336. The human anti-IL-4R antibody, referred to as dupilumab, specifically binds to human IL-4Rα and comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 337 and a light chain variable region (LCVR) comprising SEQ ID NO: 338, a heavy chain complementarity determining region 1 (HCDR1) comprising SEQ ID NO: 339, a HCDR2

comprising SEQ ID NO: 340, a HCDR3 comprising SEQ ID NO: 341, a light chain complementarity determining region 1 (LCDR1) comprising SEQ ID NO: 342, a LCDR2 comprising SEQ ID NO: 343 and a LCDR3 comprising SEQ ID NO: 344. The full-length heavy chain of dupilumab is shown as SEQ ID NO: 345 and the full length light chain is shown as SEQ ID NO: 346.

Example 4: A chronic House Dust Mite (HDM)-induced fibrosis and severe lung inflammation model to study the role of IL-33 in lung inflammation- Comparison of efficacy of an anti-IL-33 antibody, an IL-4R antibody, or a combination of both.

[0234] Chronic inflammatory airway diseases are a consequence of recurrent episodes of airway inflammation predominantly due to repeated exposure to allergens or other pathogens. In humans, such chronic insults induce a vast array of pathologies that include pulmonary infiltration by immune cells, increased cytokine production, mucus production and collagen deposition (Hirota, (2013) Chest. Sep;144(3):1026-32.; Postma, (2015), N Engl J Med., Sep 24;373(13):1241-9). This increase in inflammatory cytokines and immune cell infiltrates, accompanied by intense airway remodeling leads to airway narrowing, hyperresponsiveness to inhaled triggers such as allergens or pathogens, airway obstruction and loss of lung function.

[0235] To determine the effect of anti-IL-33 inhibition in a relevant *in vivo* model, a chronic house dust mite extract (HDM)-induced fibrosis and severe lung inflammation and remodeling study was conducted in mice that were homozygous for the expression of human IL-33 in place of mouse IL-33 (IL-33 HumIn mice; See US Patent Publication Nos. 2015/0320021 and 2015/0320022). Chronic HDM extract exposure induces severe lung inflammation, resulting in significant cellular infiltrate, cytokine expression, and remodeling. Efficacy of an anti-IL-33 antibody, an anti-mouse IL-4Rα antibody or a combination of both was compared in this model. The anti-mouse IL-4Rα antibody used in this study is designated M1M1875N and comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 335/336. The anti-IL-33 antibody used in this study is designated H4H9675P and comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0236] 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 IL-33 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. Four groups of HDM challenged mice were injected subcutaneously with 25 mg/kg of either the anti-IL-33 antibody H4H9675P, the anti-mouse IL-4Rα antibody M1M1875N, a combination of both antibodies, or an isotype control antibody starting after 11 weeks of HDM challenge and then twice per week until the end of the HDM challenge (4 weeks of antibody treatment). On day 108 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 4.

Table 4: 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	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)
6	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Anti-IL-4Rα antibody (M1M1875N)
7	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Anti-IL-33 (H4H9675P) antibody + Anti-IL-4Rα (M1M1875N) antibody

Lung harvest for cytokine analysis:

[0237] Elevated lung levels of key mediators such as the prototypic type 2 cytokines IL-4, IL-5, and IL-13, as well as cytokines more characteristic of type 1 immune responses, such as IL-1β or TNFα have been involved in human the development of lung diseases (Gandhi, (2016) Nat Rev Drug Discov Jan;15(1):35-50.; Barnes, (2008), Nat Rev Immunol, Mar;8(3):183-92. Lung levels of these inflammatory cytokines were measured in the present study.

[0238] 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 (Thermo Scientific, #87786). 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 1:7 (w/v) tissue to T-PER ratio. Lung samples were mechanically disrupted using the TissueLyser II (Qiagen #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.

[0239] 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.

[0240] Cytokine concentrations in the lung protein extracts were measured using a Proinflammatory Panel 1 (mouse) multiplex immunoassay kit (MesoScale Discovery, # K15048G-2) and a custom mouse 6plex Multi-Spot® immunoassay kit (MesoScale Discovery, #K152A41-4), according to the manufacturer's instructions. Briefly, 50µL/well of calibrators and samples (diluted in Diluent 41) were added to plates pre-coated with capture antibodies and incubated at room temperature while shaking at 700 rpm for 2 hours. The plates were then washed 3 times with 1X PBS containing 0.05% (w/v) Tween-20, followed by the addition of 25µL of Detection Antibody Solution diluted in Diluent 45. After a 2 hour incubation at room temperature while shaking, the plate was washed 3 times, and 150µL of 2X Read Buffer was added to each well. Electrochemiluminescence was immediately read on a MSD Spector® instrument. Data analysis was performed using GraphPad Prism software.

[0241] 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) as shown in Table 5.

Lung cytokines analysis:

[0242] As shown in table 5, the level of the cytokines and chemokines IL-4, IL-5, IL-6, IL-1β and MCP-1 released in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks, with or without treatment with an isotype control antibody were significantly higher than in IL-33 HumIn mice challenged with 1X PBS alone. Similarly, there was a trend towards an increased release of the cytokines IL-13 and TNFα in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks. In contrast, there was a significant reduction in the levels of IL-6, IL-13 and MCP-1 in the lungs of IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. There was a trend towards reduced IL-4, IL-5, IL-1β and TNFα lung levels in IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. The effects on lung cytokines observed with the

combination anti-IL-33 and anti-mouse IL-4Rα antibodies was greater than treatment with either individual antibodies alone.

Table 5: Cytokine concentration in lung protein extracts

Experimental group	Mean [IL-4] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [IL-5] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [IL-13] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [IL-6] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [IL-1β] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [TNFα] in lung protein extracts (pg/mg lung protein) (±SD)	Mean [MCP-1] in lung protein extracts (pg/mg lung protein) (±SD)
1. 1X PBS challenge (n=5)	0.13 (±0.17)	0.80 (±1.41)	ND	4.75 (±3.39)	1.97 (±1.67)	2.86 (±1.01)	4.12 (±1.12)
2. HDM challenge 11 weeks (n=4)	5.71 (±3.76) *	7.31 (±3.67)	0.20 (±0.03)	293.1 (±139.3) *	181.8 (±131.0) *	17.39 (±8.90)	43.06 (±24.21)
3. HDM challenge 15 weeks (n=4)	2.70 (±1.71)	5.13 (±3.20)	0.19 (±0.03)	308.3 (±390.1)	51.79 (±16.97)	15.38 (±8.11)	105.6 (±106.5) *
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	5.46 (±3.38) **	7.00 (±4.50) *	0.22 (±0.02)	395.0 (±270.1) **	162.3 (±166.5) **	19.57 (±14.81)	141.7 (±126.3) **
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	1.15 (±1.38)	1.93 (±1.90)	0.20 (±0.02)	136.8 (±164.1)	122.9 (±194.1)	17.05 (±4.48) *	16.64 (±6.40)
6. HDM challenge 15 weeks + anti-mouse IL-4Rα antibody (n=5)	2.88 (±2.43)	13.13 (±12.81) **	0.16 (±0.03)	18.24 (±12.43)	26.73 (±20.94)	7.85 (±4.89)	11.63 (±8.69)
7. HDM challenge 15 weeks +	0.47 (±0.13)	0.73 (±0.37)	0.10 (±0.05) ††	7.46 (±2.52) †	3.722 (±1.59)	3.07 (±1.34)	4.62 (±1.27) ††

anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)							

Note: Statistical significance determined by Kruskal-Wallis One-way ANOVA with Dunn's multiple comparison post-hoc test is indicated (*= $p<0.05$, **= $p<0.01$, compared to groups 1: IL33 HumIn mice, Saline challenge; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, compared to group 4: IL33 Humin mice, HDM challenge 15 weeks + Isotype control antibody). ND: Not determined.

Lung harvest for gene expression analysis

[0243] After exsanguination, the accessory lobe of the right lung from each mouse was removed, placed into tubes containing 400 □L of RNA Later (Ambion, #AM7020) 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 (Ambion by Life Technologies, #AM1839) 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 2.5□g) was reverse-transcribed into cDNA using SuperScript® VILO™ Master Mix (Invitrogen by Life Technologies, #11755500). cDNA was diluted to 2ng/□L and 10ng cDNA was amplified with the TaqMan® Gene Expression Master Mix (Applied Biosystems by Life Technologies, #4369542) and the relevant probes (Life Technologies; mouse *B2m*: Mm00437762_m1; mouse *Il4*: Mm00445259_m1; mouse *Il5*: Mm00439646_m1; mouse *Il13*: Mm00434204_m1; mouse *Il9:* Mm00434305_m1; *mouse Il6*: Mm00446190_m1; mouse *Ccl2*: Mm00441242_m1; mouse *Ccl11*: Mm00441238_m1 ; mouse *Ccl24*: Mm00444701_m1; mouse *Tnf*: Mm00443258_m1; mouse *Tgfb1*: Mm01178820_m1; mouse *Il1rl1*: Mm00516117_m1; mouse *Il13ra2*: Mm00515166_m1; mouse *Col15a1*: Mm00456584_m1; mouse *Col24a1*: Mm01323744_m1;) using the ABI 7900HT Sequence Detection System (Applied Biosystems). *B2m* was used as the internal control genes to normalize any cDNA input differences. The reference group used for normalization of all samples was the average of Group 1 samples ('1X PBS Challenge'). Expression of each gene was normalized to *B2m* expression within the same sample and expressed relative to its normalized expression in the reference group (mean ±SD), as shown in table 6.

Lung gene expression analysis

[0244] As shown in table 6, the level of expression of the cytokines, chemokines and collagen genes *Il4*, *Il13*, *Il6*, *Ccl2*, *Tgfb1, Il13ra2* and *Col24a1* in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks, with or without treatment with an isotype control antibody were significantly

increased compared to IL-33 HumIn mice challenged with 1X PBS alone. Similarly, there was a trend towards an increase in expression of the genes *Il5, Il9, Ccl11, Ccl24, Tnf, Il1rl1* and *Col15a1* in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks.

[0245] In contrast, there was a significant reduction in the expression levels of *Il6*, *Ccl2*, *Ccl11* and *Ccl24* in the lungs of IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. There was a trend towards reduced *Il4*, *Il5, Il13, Il9, Tnf, Tgfb1, Il1rl1, Il13ra2, Col15a1* and *Col24a1* expression levels in mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. The effects on gene expression observed with the combination anti-IL-33 and anti-mouse IL-4Rα antibodies was greater than treatment with either individual antibodies alone.

Table 6: Gene expression (TaqMan) in mouse lungs.

Experimental group	Mean Relative *Il4* expression in lung (±SD)	Mean Relative *Il5* expression in lung (±SD)	Mean Relative *Il13* expression in lung (±SD)	Mean Relative *Il9* expression in lung (±SD)	Mean Relative *Il6* expression in lung (±SD)	Mean Relative *Ccl2* expression in lung (±SD)	Mean Relative *Ccl11* expression in lung (±SD)	Mean Relative *Ccl24* expression in lung (±SD)
1. 1X PBS challenge (n=5)	1.03 (±0.28)	1.54 (±1.61)	4.51 (±7.59)	15.91 (±34.81)	1.25 (±1.09)	1.20 (±0.93)	1.24 (±1.07)	1.05 (±0.33)
2. HDM challenge 11 weeks (n=4)	12.78 (±8.45) *	7.13 (±3.49)	114.1 (±68.3) *	38.66 (±30.04)	9.12 (±1.65)	18.86 (±8.40)	13.36 (±5.05)	15.44 (±12.02)
3. HDM challenge 15 weeks (n=4)	6.27 (±3.39)	4.20 (±1.51)	58.05 (±31.61)	30.63 (±20.54)	8.92 (±4.55)	22.61 (±13.37) *	8.65 (±3.20)	4.58 (±1.91)
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	10.98 (±5.46) *	5.50 (±3.16)	92.51 (±75.96) *	19.51 (±10.29)	13.80 (±6.98) **	24.53 (±9.13) **	12.14 (±7.82)	12.41 (±8.73)
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	2.80 (±3.11)	1.74 (±1.11)	12.91 (±12.93)	0.00 (±0.00)	3.87 (±3.00)	5.20 (±2.44)	6.21 (±3.55)	1.45 (±2.09)

6. HDM challenge 15 weeks + anti-mouse IL-4Rα antibody (n=5)	1.87 (±1.03)	7.98 (±6.52)	69.56 (±66.86) *	63.50 (±92.04)	2.77 (±1.39)	2.97 (±1.86)	1.00 (±0.18)	0.44 (±0.34)
7. HDM challenge 15 weeks + anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)	1.37 (±0.35)	1.56 (±0.97)	9.34 (±3.10)	0.57 (±1.27)	1.04 (±0.31) ††	1.08 (±0.24) †† §	0.72 (±0.28) †	0.15 (±0.10) ††

Table 6 Continued: Gene expression (TaqMan) in mouse lungs.

Experimental group	Mean Relative *Tnf* expression in lung (±SD)	Mean Relative *Tgfb1* expression in lung (±SD)	Mean Relative *Il1rl1* expression in lung (±SD)	Mean Relative *Il13ra2* expression in lung (±SD)	Mean Relative *Col15a1* expression in lung (±SD)	Mean Relative *Col24a1* expression in lung (±SD)
1. 1X PBS challenge (n=5)	1.02 (±0.24)	1.00 (±0.11)	1.11 (±0.58)	1.59 (±1.96)	1.00 (±0.10)	1.02 (±0.16)
2. HDM challenge 11 weeks (n=4)	1.45 (±0.41)	1.40 (±0.27)	3.03 (±0.88) *	48.43 (±34.21)	2.75 (±0.96)	24.55 (±7.97) **
3. HDM challenge 15 weeks (n=4)	1.58 (±0.43)	1.32 (±0.33)	2.53 (±0.79) *	32.07 (±13.45)	3.00 (±1.22)	17.25 (±5.29) *
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	1.59 (±0.78)	1.37 (±0.12) *	3.45 (±1.48) *	52.02 (±40.63)	3.80 (±0.96) *	23.58 (±6.18) ***
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	1.38 (±0.27)	1.22 (±0.24)	0.99 (±0.47)	13.54 (±12.25)	1.64 (±0.30)	10.58 (±5.42)

6. HDM challenge 15 weeks + anti-mouse IL-4Rα antibody (n=5)	1.00 (±0.25)	1.13 (±0.20)	3.38 (±1.97)	1.89 (±0.59)	1.24 (±0.28)	7.08 (±4.56)
7. HDM challenge 15 weeks + anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)	0.68 (±0.08) §	1.09 (±0.12)	1.12 (±0.57)	1.89 (±0.27)	0.74 (±0.21) $^{\dagger\dagger\,\S}$	1.76 (±0.15) †

Note: Statistical significance determined by Kruskal-Wallis One-way ANOVA with Dunn's multiple comparison post-hoc test is indicated (*= $p<0.05$, **= $p<0.01$, ***= $p<0.01$ compared to groups 1: IL33 HumIn mice, Saline challenge; $^{\S}p<0.05$, $^{\S\S}p<0.01$, compared to group 3: IL33 Humin mice, HDM challenge 15 weeks; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, compared to group 4: IL33 Humin mice, HDM challenge 15 weeks + Isotype control antibody;).

Lung harvest for pulmonary cell infiltrate analysis

[0246] Pulmonary infiltration by immune cells is observed in multiple airway inflammatory diseases, including asthma and COPD. Neutrophilic lung inflammation has been associated with lower lung function and severe tissue remodeling in asthma patients (Wenzel *et.al.*, (2012), Nat Med 18(5):716-725) and with increased pulmonary damage in COPD patients (Meijer, *et.al.,* (2013), Expert Rev. Clin. Immunol. 9(11):1055-1068). Eosinophilic lung inflammation is a hallmark of type 2 inflammation usually seen in atopic diseases (Jacobsen, *et.al.*, (2014), Clin. Exp., Allergy, 44(9):1119-1136). In humans, high CD4/CD8 ratios are observed in patients with granulomatous lung diseases and other chronic inflammatory conditions (Costabel, *et.al.,* (1997), Eur. Respir. J. 10(12):2699-2700; Guo, *et.al.*, (2011), Ann. Clin. Biochem, 48(Pt4):344-351). Flow cytometry was used in the present study to determine the level of cellular infiltration in the lungs of HDM-exposed mice.

[0247] After exsanguination, the caudal lobe of the right lung from each mouse was removed, chopped into cubes that were approximately 2 to 3 mm in size, and then placed into a tube containing a solution of 20 µg/mL DNAse (Roche, #10104159001) and 0.7 U/mL Liberase TH (Roche, #05401151001) diluted in Hank's Balanced Salt Solution (HBSS) (Gibco, #14025), which was incubated in a 37°C water bath for 20 minutes and vortexed every 5 minutes. The

reaction was stopped by adding ethylenediaminetetraacetic acid (EDTA, Gibco, #15575) at a final concentration of 10mM. Each lung was subsequently dissociated using a gentleMACS dissociator® (Miltenyi Biotec, #130-095-937), then filtered through a 70 µm filter and centrifuged. The resulting lung pellet was resuspended in 1 mL of 1X red blood cell lysing buffer (Sigma, #R7757) to remove red blood cells. After incubation for 3 minutes at room temperature, 3 mL of 1X DMEM was added to deactivate the red blood cell lysing buffer. The cell suspensions were then centrifuged, and the resulting cell pellets were resuspended in 5 mL of MACS buffer (autoMACS Running Buffer; Miltenyi Biotec, #130-091-221). The resuspended samples were filtered through a 70µm filter and $1x10^6$ cells per well were plated in a 96-well V-bottom plate. Cells were then centrifuged and the pellets were washed in 1X PBS. After a second centrifugation, the cell pellets were resuspended in 100µL of LIVE/DEAD® Fixable Blue Dead Cell Stain (Life Technologies, # L23105) diluted at 1:500 in 1X PBS to determine cell viability and incubated for 20 minutes at room temperature while protected from light. After one wash in 1X PBS, cells were incubated in a solution of MACS buffer containing 10µg/mL of purified rat anti-mouse CD16/CD32 Fc Block, (Clone: 2.4G2; BD Biosciences, #553142) for 10 minutes at 4°C. The cells were then incubated in the appropriate 2x antibody mixture (described in Table 7) diluted in MACS buffer 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 (BD Biosciences, #554655) and then incubated for 15 minutes at 4°C while protected from light. The cells were subsequently washed, resuspended in MACS buffer, and then transferred to BD FACS tubes (BD Biosciences, #352235) for analysis of cellular infiltrates by flow cytometry.

[0248] CD4 and CD8 T cells were defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, $CD3^+$, $CD19^-$, $CD4^+$, $CD8^-$ and live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, $CD3^+$, $CD19^-$, $CD4^-$, $CD8^+$ respectively. Activated CD4 T cells were defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, $CD3^+$, $CD19^-$, $CD4^+$, $CD8^-$, and $CD69^+$. Activated CD8 T cells were defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, $CD3^+$, $CD19^-$, $CD4^-$, $CD8^+$, and $CD69^+$. Activated B cells were defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, $CD3^-$, $CD19^+$, and $CD69^+$. ST2+ CD4+ T cells were defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, CD3+, CD19-, ST2+ and $CD4^+$. Eosinophils were defined as live, $CD45^+$, $GR1^-$, $CD11c^{lo}$, $SiglecF^{hi}$. Alveolar macrophages were defined as live, $CD45^+$, $GR1^-$, $CD11c^{Hi}$, $SiglecF^{hi}$. Data for activated cells is expressed as frequency of activated cells ($CD69^+$) within the parent population (CD4, ± SD). Data for ST2+ CD4+ T cells is expressed as frequency of T cells (defined as cells that were live, $CD45^+$, SSC^{Lo}, FSC^{Lo}, CD3+ and CD19-). Data for Eosinophils and Alveolar macrophages is expressed as frequency of live cells. CD4/CD8 T cells ratio is calculated as the ratio of the frequency of CD4 T to the frequency of CD8 T cells within the live population. All data is shown in Table 8.

Table 7: Antibodies Used for Flow Cytometry Analysis

Antibody	Fluorochrome	Manufacturer	Catalogue Number	Final dilution
CD45.2	PerCP-Cy5.5	eBioscience	45-0454	1/800
Siglec-F	BV 421	BD	562681	1/200
F4/80	APC	eBioscience	17-4801-82	1/200
Ly6G	BUV395	BD	563978	1/200
Ly6C	PE-Cy7	BD	560593	1/100
CD11c	PE	eBioscience	12-0114-82	1/200
CD11b	FITC	eBioscience	53-0112-82	1/200
CD19	BV650	BD	562701	1/400
CD3	PE-Cy7	BD	552774	1/200
CD4	BV421	BioLegend	100438	1/200
CD8	BUV 395	BD	563786	1/400
NKp46 (CD335)	FITC	eBioscience	11-3351	1/800
CD69	PE	eBioscience	12-0691	1/200
CD25	BV510	BioLegend	102042	1/200
ST2	APC	BioLegend	145306	1/200

Pulmonary cell infiltrate analysis:

[0249] As shown in table 8, the frequency of eosinophils, activated B cells, activated CD8 cells, ST2+ Cd4+ T cells and CD4/CD8 T cells ratio in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks, with or without treatment with an isotype control antibody were significantly higher than in IL-33 HumIn mice challenged with 1X PBS alone. Similarly, there was a trend towards an increased frequency of activated CD4 T cells in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks. There was a trend towards a decreased frequency of alveolar macrophages detected by flow cytometry in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks, in the absence or presence of an isotype control antibody treatment. The frequency of alveolar macrophages was significantly increased in the lungs of IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. Similarly, there was a trend towards reduced frequency

of eosinophils, activated CD4 and CD8 T cells, activated B cells, ST2+ CD4+ T cells as well as CD4/CD8 T cells ratio in the lungs of mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. The effects on frequency of eosinophils, alveolar macrophages, activated CD8 T cells, ST2+ CD4+ T cells and CD4/CD8 ratio in the lung observed for the combination anti-IL-33 and anti-mouse IL-4Rα antibodies shows a trend towards greater efficacy than treatment with either individual antibodies alone.

Table 8: Frequency of pulmonary cell infiltrate as determined by flow cytometry

Experimental group	Mean Frequency of Eosinophils in the live population (±SD)	Mean Frequency of Alveolar Macrophages in the live population (±SD)	Mean CD4/CD8 T cells ratio (±SD)	Mean Frequency of Activated cells in CD4 T cells population (±SD)	Mean Frequency of Activated cells in CD8 T cells population (±SD)	Mean Frequency of Activated cells in B cells population (±SD)	Mean Frequency of ST2+ CD4+ cells in T cells population (±SD)
1. 1X PBS challenge (n=5)	1.45 (±0.92)	5.05 (±1.64)	3.00 (±1.48)	13.12 (±9.89)	3.26 (±1.64)	0.39 (±1.17)	3.25 (±4.15)
2. HDM challenge 11 weeks (n=4)	17.08 (±3.94) *	2.34 (±0.93)	6.42 (±2.71)	49.95 (±8.76)	9.58 (±7.44)	4.67 (±1.47) **	32.60 (±12.23)
3. HDM challenge 15 weeks (n=4)	15.40 (±3.99) *	4.92 (±1.55)	6.95 (±0.71) **	58.53 (±5.76)	15.68 (±3.03) *	3.70 (±1.44) *	37.33 (±8.98) *
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	15.00 (±3.35) *	2.33 (±1.60)	7.49 (±1.28) *	57.75 (±7.64)	14.59 (±3.82)	3.90 (±1.48) *	37.96 (±16.71) *
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	8.51 (±7.52)	7.44 (±4.18)	4.03 (±1.28)	48.22 (±5.66)	13.86 (±5.21)	1.72 (±0.72)	19.24 (±5.72)
6. HDM challenge 15 weeks +	12.30 (±7.83)	9.93 (±5.18)	5.56 (±2.22)	53.42 (±6.52)	13.11 (±6.26)	2.14 (±1.23)	35.01 (±9.83) *

anti-mouse IL-4Rα antibody (n=5)							
7. HDM challenge 15 weeks + anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)	3.78 (±1.60)	14.64 (±3.86) †	2.96 (±0.93)	42.52 (±9.79)	7.90 (±1.30)	1.74 (±0.91)	11.78 (±3.73)

Note: Statistical significance determined by Kruskal-Wallis One-way ANOVA with Dunn's multiple comparison post-hoc test is indicated (*= $p<0.05$, **= $p<0.01$, compared to groups 1: IL33 HumIn mice, Saline challenge; †$p<0.05$, compared to group 4: IL33 Humin mice, HDM challenge 15 weeks + Isotype control antibody).

Lung harvest for quantification of histopathology:

[0250] The inflammatory pattern observed in this model is accompanied by widespread and severe structural changes in HDM-exposed lungs, with evidence of goblet cell metaplasia, increases in sub-epithelial collagen deposition and significant pulmonary consolidation. These pathologies are known features of human inflammatory respiratory diseases that contribute to decline of lung function and airway hyperreactivity (James, (2007) Eur Respir J., Jul;30(1):134-55; Jeong, (2007) Radiographics May-Jun;27(3):617-37).

[0251] After exsanguination, the left lungs were removed and placed into plates containing a 3 mL solution of 4% (w/v) paraformaldehyde (Boston Bioproducts, # BM-155) in 1X phosphate buffered saline and stored at room temperature for 3 days. Lung samples were then blotted dry and transferred to tubes containing 70% ethanol for histological analysis. The samples were sent to Histoserv, Inc (Germantown, MD) for paraffin embedding, sectioning and periodic acid Schiff (PAS) or Hematoxylin and Eosin (H&E) staining.

Quantification of Goblet cell metaplasia:

[0252] Goblet cell metaplasia and mucus hyper-secretion are hallmarks of many pulmonary diseases including asthma, chronic obstructive pulmonary disease, and cystic fibrosis (Boucherat, (2013) Exp Lung Res. 2013 May-Jun;39(4-5):207-16). Excessive mucus production leads to airway obstruction and affects several important outcomes such as lung function, health-related quality of life, exacerbations, hospitalizations, and mortality in humans (Ramos, FL, *et.al.,* (2014), Int J Chron Obstruct Pulmon Dis, Jan. 24; 9:139-150). □PAS-positive goblet cells and total epithelial cells were counted in a millimeter length of the primary bronchus.

Goblet cell metaplasia is expressed as the frequency of PAS-positive cells in a millimeter of bronchial epithelium (%, ± SD) as shown in Table 9.

Quantification of lung consolidation:

[0253] "Lung consolidation" is defined as the accumulation of solid or liquid material in the alveolar space. Lung consolidation is a compound endpoint likely reflecting the combination of cellular infiltrate, hyperplasia, and mucus production, used here as a measurement of gross pathology. The fraction of lung area occupied by the crystal bodies was quantified on Movat pentachrome stained paraffin-embedded lung sections using ImageJ software (NIH, Bethesda, MD). Using the particle analysis function, total lung area in the section, as well as consolidated area in the section were measured. The fraction of consolidated lung area is given by the ratio of both measurements, as shown in Table 9.

Quantification of sub-epithelial fibrosis

[0254] "Sub-epithelial fibrosis" is defined as an excess of interstitial collagen deposition beneath the pulmonary epithelium (Redington, *et.al.,* (1997), Thorax, April; 52(4):310-312). Increased sub-epithelial fibrosis has been reported to be specifically associated with asthma in humans (Boulet, *et.al.,(*1997) Chest, July; 112(1):45-52; James, AL and Wenzel, S., (2007), Eur Respir J, July, 30(1):134-155). In the present model, sub-epithelial fibrosis was measured on Masson's trichrome stained paraffin-embedded lung sections using HaLo software (Indica Labs, NM). Using the 'Layer thickness' tool, the thickness of the collagen layer beneath the bronchial epithelium was recorded multiple times, with about 30 µm intervals, across a millimeter of the primary bronchus. Sub-epithelial fibrosis is expressed as the mean thickness of the collagen layer beneath the epithelium (µm, ± SD) as shown in Table 9.

Analysis of lung histopathology:

[0255] As shown in table 9, there was a trend towards an increase in goblet cell metaplasia in the lungs of IL-33 HumIn mice receiving HDM for 15 weeks, with or without treatment with an isotype control antibody compared to IL-33 HumIn mice challenged with 1X PBS alone. Similarly, there was a significant increase in lung consolidation, as well as in sub-epithelial collagen thickness, in IL-33 HumIn mice receiving HDM for 15 weeks.

[0256] In contrast, there was trend towards a reduction in goblet cell metaplasia and sub-epithelial collagen thickness, and a significant reduction in lung consolidation in IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody during this time period. The effects on goblet cell metaplasia, lung consolidation and sub-epithelial collagen thickness observed for the combination anti-IL-33 and anti-mouse IL-4Rα antibodies showed a trend towards greater efficacy than treatment with either individual antibodies alone.

Table 9: Quantification of histopathology in mouse lungs

Experimental group	Mean Goblet cell metaplasia (% PAS-positive cells) (±SD)	Mean lung consolidation (% ±SD)	Mean sub-epithelial collagen thickness (μm) (±SD)
1. 1X PBS challenge (n=5)	32.94 (±43.61)	6.97 (±3.72)	25.90 (±4.00)
2. HDM challenge 11 weeks (n=4)	59.98 (±39.01)	70.70 (±12.94)	81.76 (±25.37) *
3. HDM challenge 15 weeks (n=4)	92.15 (±10.16)	83.21 (±3.65) **	82.12 (±23.04) *
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	81.60 (±17.56)	84.16 (±5.85) **	63.11 (±11.87)
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	39.22 (±18.93)	58.82 (±18.26)	70.99 (±23.85)
6. HDM challenge 15 weeks + anti-mouse IL-4Rα antibody (n=5)	79.82 (±25.02)	57.79 (±18.72)	57.62 (±15.34)
7. HDM challenge 15 weeks + anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)	19.69 (±8.80)	35.01 (±20.68)	48.19 (±18.58)

Note: Statistical significance determined by Kruskal-Wallis One-way ANOVA with Dunn's multiple comparison post-hoc test is indicated (**= $p<0.01$, compared to groups 1: IL33 HumIn mice, Saline challenge).

Serum collection for IgE and HDM-specific IgG1 levels measurement:

[0257] To determine the total IgE concentration in the serum samples for each mouse, a sandwich ELISA OPTEIA kit (BD Biosciences, #555248) was used according to the manufacturer's instructions. Serum samples were diluted and incubated with anti-IgE capture antibody coated on 96-well plates. Total IgE was detected by biotinylated anti-mouse IgE secondary antibody. Purified horseradish peroxidase (HRP)-labeled mouse IgE was used as a standard. The chromagen 3,3',5,5'-tetramethylbenzidine (TMB) (BD OPTEIA substrate reagent set, BD, #555214) was used to detect HRP activity. A stop solution of 1 M sulfuric acid was then added, and absorbance at 450nm was measured on a Molecular Devices SpectraMax M5 plate reader. Data analysis was performed using Prism™ software. The mean amounts of circulating IgE levels in serum for each experimental group are expressed as ng/mL (± SD) as shown in Table 10.

[0258] To determine the HDM specific IgG1 levels in the serum samples from each mouse, an ELISA was utilized. HDM (Greer, #XPB70D3A2.5) coated plates were incubated with serially diluted mouse serum samples, followed by incubation with a rat anti-mouse IgG1-HRP

conjugated antibody (BD Biosciences, # 559626). All samples were developed with a TMB solution and analyzed as described above. Relative levels of circulating IgG1 in serum were represented as titer units (titer units were calculated by multiplying the measured OD by a dilution factor required to achieve OD450 that was greater than two times background). The mean circulating HDM-specific IgG1 levels in serum for each experimental group are expressed as titer x 10^6 (±SD) as shown in Table 10.

Analysis of the circulation levels of IgE and HDM-specific IgG1

[0259] As shown in table 10, there was a significant increase in circulating levels of IgE in the serum of IL-33 HumIn mice receiving HDM for 15 weeks, with or without treatment with an isotype control antibody in IL-33 HumIn mice challenged with 1X PBS alone. Similarly, there was a trend towards an increased level of circulating HDM-specific IgG1 in the serum of IL-33 HumIn mice receiving HDM for 15 weeks. In contrast, there was a significant decrease in circulating levels of IgE and a trend towards a decrease in circulating levels of HDM-specific IgG1 in the serum of IL-33 HumIn mice treated with a combination of anti-IL-33 and anti-mouse IL-4Rα antibodies during the last four weeks of the chronic HDM challenge as compared to IL-33 HumIn mice administered HDM with an isotype control antibody.

Table 10: Circulating levels of IgE and HDM-specific IgG1 in mouse serum.

Experimental group	Mean circulating IgE levels (µg/mL) (±SD)	Mean circulating HDM-specific IgG1 levels (Titer x 10^6) (±SD)
1. 1X PBS challenge (n=5)	2.16 (±2.02)	ND
2. HDM challenge 11 weeks (n=4)	50.16 (±8.35)	1.18 (±0.15)
3. HDM challenge 15 weeks (n=4)	131.38 (±106.84) *	1.88 (±0.81)
4. HDM challenge 15 weeks+ isotype control antibody (n=4)	193.07 (±78.96) ***	1.62 (±0.62)
5. HDM challenge 15 weeks + anti-IL-33 antibody (n=5)	45.74 (±45.74)	1.76 (±0.98)
6. HDM challenge 15 weeks + anti-mouse IL-4Rα antibody (n=5)	11.12 (±8.65)	0.99 (±0.56)
7. HDM challenge 15 weeks + anti-IL-33 + anti-mouse IL-4Rα antibodies (n=5)	6.45 (±5.79) †	0.75 (±0.30)

Note: Statistical significance determined by Kruskal-Wallis One-way ANOVA with Dunn's multiple comparison post-hoc test is indicated (*= $p<0.05$, **= $p<0.01$, ***= $p<0.001$, compared

to groups 1: IL33 HumIn mice, Saline challenge; †p<0.05, compared to group 4: IL33 Humin mice, HDM challenge 15 weeks + Isotype control antibody). ND: Not determined.

[0260] A combination of H4H9675P and anti-mIL-4Rα treatment initiated in the context of severe, mixed inflammation improves all inflammatory parameters measured, reducing most to baseline levels. Additionally, additive effects are observed on some of the most pernicious endpoints, including composite lung gross pathology, goblet cell metaplasia, lung cellular infiltration, and cytokine levels. Therefore, blocking both pathways simultaneously has the potential to impact multiple inflammatory mediators in the context of severe mixed inflammation and tissue pathology, and normalize multiple parameters to baseline.

Example 5: Epitope Mapping H4H9675P Binding to IL33 by Hydrogen Deuterium Exchange

[0261] In order to determine the epitopes of human IL33 recognized by an anti-IL33 antibody, H4H9675P, hydrogen-deuterium (H/D) exchange studies were performed for the antibody co-complexed with human IL33. For the experiments recombinant human IL33 expressed with a C-terminal hexahistidine tag (SEQ ID NO: 356) was used. A general description of the H/D exchange method has been set forth in Ehring et al. (1999) *Analytical Biochemistry* 267(2):252-259 and Engen and Smith (2001) *Anal. Chem.* 73:256A-265A. H/D exchange experiments were performed on an integrated Waters HDX/MS platform, consisting of a Leaptec HDX PAL system for the deuterium labeling, a Waters Acquity M-Class (Auxiliary solvent manager) for the sample digestion and loading, a Waters Acquity M-Class (μBinary solvent manager) for the analytical column gradient, and Synapt G2-Si mass spectrometer for peptic peptide mass measurement.

[0262] The labeling solution was prepared in 10 mM PBS buffer in D_2O at pD 7.0 (equivalent to pH 6.6). For deuterium labeling, 3.8 μL of hIL33-MMH (96 pmol/μL) or hIL33-MMH premixed with the antibody in a 1:1 molar ratio was incubated with 56.2 μL D_2O labeling solution for various time-points (2 min, 10 min, and undeuterated control = 0 sec). The deuteration was quenched by transferring 50 μL of the sample to 50 μL of pre-chilled 0.2 M TCEP, 6 M guanidine chloride in 100 mM phosphate buffer at pH 2.5 (quench buffer) and the mixed sample was incubated at 1.0 °C for two minutes. The quenched sample was then injected into a Waters HDX Manager for online pepsin/protease XIII digestion. The digested peptides were trapped onto an ACQUITY UPLC BEH C18 1.7-μm, 2.1 × 5 mm VanGuard pre-column at 0°C and eluted to an ACQUITY UPLC BEH C18 1.7-μm, 1.0 × 50 mm column using a 9-minute gradient separation of 5%-40% B (mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile). The mass spectrometer was set at cone voltage of 37 V, scan time of 0.5 s, and mass/charge range of 50-1700 Th.

[0263] For the identification of the peptides from hIL33-MMH, LC- MS^E data from undeuterated

sample were processed and searched against the database including human IL33, pepsin, and their randomized sequences via Waters ProteinLynx Global Server (PLGS) software. The identified peptides were imported to DynamX software and filtered by two criteria: 1) minimum products per amino acid: 0.3, and 2) replication file threshold: 3. DynamX software then automatically determined deuterium uptake of each peptide based on retention time and high mass accuracy (<10ppm) across multiple time points with 3 replicates at each time point.

[0264] Using the online pepsin/protease XIII column coupled with MS^E data acquisition, a total of 68 peptides from hIL33-MMH were identified in the absence or presence of H4H9675P, representing 95% sequence coverage. Eleven peptides had significantly reduced deuteration uptake (centroid delta values > 0.4 daltons with p-values < 0.05) when bound to H4H9675P and are listed in Table 11. The recorded peptide mass corresponds to the average value of the centroid MH^+ mass from three replicates. These peptides, corresponding to amino acids 1-12 and 50-94 of SEQ ID NO: 349), had a slower deuteration rate by the binding of H4H9675P. These identified residues also correspond to residues 112-123 and 161-205 of human IL-33 as defined by Uniprot entry O95760 (IL33_HUMAN; see also SEQ ID NO: 348). These data provide support for amino acid residues 112-123 and 161-205 of SEQ ID NO: 348, or amino acid residues 1-12 and 50-94 of SEQ ID NO: 349 defining at least in part the binding region in IL-33 for antibody H4H9675P.

Table 11. Human IL33 peptides with significant reduced deuteration upon binding to H4H9675P

Residue numbers based on SEQ ID NO: 349	2min Deuteration			10 min Deuteration		
	IL33 Centroid MH^+	IL33+H4H9675P Centroid MH^+	Δ	IL33 Centroid MH^+	IL33+H4H9675P Centroid MH^+	Δ
1-9	893.43±0.06	891.89±0.02	-1.54	893.60±0.03	892.00±0.08	-1.60
1-10	1023.15±0.06	1021.53±0.05	-1.62	1023.31±0.01	1021.69±0.03	-1.63
1-11	1186.18±0.06	1184.39±0.13	-1.80	1186.43±0.02	1184.61±0.03	-1.82
1-12	1300.27±0.07	1297.88±0.04	-2.39	1300.60±0.03	1298.65±0.01	-1.95
50-61	1458.12±0.06	1456.36±0.00	-1.75	1458.27±0.08	1456.34±0.01	-1.93
52-67	1791.16±0.12	1789.89±0.25	-1.27	1791.20±0.02	1790.27±0.02	-0.93
52-72	2353.80±0.12	2351.27±0.05	-2.53	2353.89±0.03	2351.95±0.10	-1.94
53-70	1945.19±0.10	1944.26±0.07	-0.93	1945.25±0.02	1944.84±0.03	-0.41
53-72	2189.95±0.13	2188.03±0.03	-1.92	2190.02±0.02	2188.58±0.04	-1.44
71-81	1253.89±0.07	1253.08±0.18	-0.81	1254.07±0.02	1253.52±0.16	-0.55
71-94	2815.35±0.05	2814.48±0.00	-0.87	2816.06±0.12	2815.10±0.12	-0.96

Example 6: Effect of an IL-33 antibody (REGN3500) and an IL-4R antibody (dupilumab), alone or in combination, in a 19-week model of allergen induced lung inflammation using IL-33-, IL-4-, and IL-4Ralpha-humanized mice

[0265] The effect of an IL-33 antibody alone, an IL-4R antibody alone, or a combination of both antibodies was first tested in Example 4 above, using mice that were homozygous for the expression of human IL-33 in place of mouse IL-33 (IL-33 HumIn mice; See US Patent Publication Nos. 2015/0320021 and 2015/0320022). A fully human anti-IL-33 antibody (REGN3500), and an anti-mouse IL-4Rα antibody or a combination of both was compared in this model and the results described in Example 4.

[0266] Since neither the human anti-IL-33 antibody (REGN3500), nor the human anti-IL-4R antibody (dupilumab) bind their respective murine target proteins, genetically modified mice, in which mouse IL-33, IL-4, and the ectodomain of IL-4Rα were replaced with the corresponding human sequences (*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$), were generated. The *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mouse strain was validated as a tool to study the effect of REGN3500 and dupilumab administration using a 4-week model of HDM exposure-induced lung inflammation. In this model, HDM-exposed *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice demonstrated immune responses similar to *wild-type* mice as assessed by evaluation of eosinophilic lung infiltration.

[0267] The study described below was conducted to determine if simultaneous blockade of the IL-33 and IL-4/IL-13 pathways could have a greater impact on lung inflammation than blocking either pathway alone. In this study, *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice were intranasally (IN) exposed to HDM or saline for 19 weeks. A control group of *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice was sacrificed after 11 weeks of HDM exposure to assess disease severity at the onset of antibody treatment. Nineteen-week HDM-exposed mice either received no antibody treatment, or received twice-weekly subcutaneous (SC) antibody injections from week 12 to week 19 of HDM exposure for a total of 8 weeks and 16 doses. The following antibodies were administered at a final protein dose of 11 mg/kg: (a) 11 mg/kg isotype control antibody, (b) 1 mg/kg REGN3500 + 10 mg/kg isotype control antibody (c) 10 mg/kg dupilumab + 1 mg/kg isotype control antibody, or (d) 1 mg/kg REGN3500 + 10 mg/kg dupilumab. The effect of REGN3500 and dupilumab treatment, alone or in combination, on HDM-exposed mice was assessed for the following pathological markers of airway inflammation:

- Gross pathology (relative lung weight)
- Lung tissue infiltration by type 1 inflammatory cells (neutrophils, quantified by lung protein levels of the neutrophil marker Myeloperoxidase [MPO]) and type 2 inflammatory cells (total and activated [CD11c^{Hi}] eosinophils and ST2^{+} CD4^{+} T cells, quantified by flow cytometry)
- Inflammatory cytokine lung protein levels (human IL-4 and mouse IL-5, IL-6, IL-1β, TNFα, IFNγ, GROα, and MCP-1, quantified by immunoassay)

- Circulating levels of the systemic marker of inflammation, serum amyloid A [SAA] protein (quantified by immunoassay)

Materials and Methods

Test System

IL-33-, IL-4-, and IL-4Rα Ectodomain-Humanized Mice

[0268] Neither REGN3500 nor dupilumab bind mouse IL-33 or mouse IL-4Rα respectively. Therefore, in order to test REGN3500 and dupilumab side by side and in combination, genetically modified mice were generated in which mouse IL-33, IL-4, and the ectodomain of IL-4Rα were replaced with the corresponding human sequences (*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$). This triple-humanized mouse strain was generated using VelociGene® technology at Regeneron Pharmaceuticals (Valenzuela, DM, *et al.* Nat Biotechnol. (2003), Jun;21(6):652–9, Poueymirou, WT, *et al.* Nat Biotechnol. (2007) Jan;25(1):91–9) by crossing the previously characterized IL-4/IL-4Rα ectodomain-humanized mouse strain *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ with the previously characterized IL-33-humanized strain *Il33*$^{hu/hu}$.

Lung Inflammation Mouse Model

[0269] The mouse lung inflammation model employs repeated intranasal (IN) exposure to HDM extract that serves as the source of house dust mite allergen (Johnson, et al.; American Journal of Respiratory and Critical Care Medicine; (2004) Feb 1;169 (3):378-85), a significant cause of indoor allergy in humans (Calderon, et al., (2015), Respiratory allergy caused by house dust mites: What do we really know? J Allergy Clin Immunol. 2015 Jul;136 (1):38–48). Chronic exposure to HDM is reported to induce severe lung inflammation resulting in significant pulmonary cellular infiltrate, cytokine expression, and remodeling. In particular, it has been demonstrated that mice chronically exposed to HDM exhibit lung inflammation of mixed type 1/type 2 phenotypes such as tissue infiltration by type 1 and type 2 inflammatory cells (neutrophils and eosinophils, respectively), increased serum IgE, increased serum HDM-specific IgG1, as well as induction of type 2 inflammatory cytokines such as IL-5 and IL-13 (Johnson, *et al.* (2004), American Journal of Respiratory and Critical Care Medicine; Feb 1;169 (3):378-85; Johnson, *et al*. (2011). PloS ONE. Jan 20;6 (1):e16175; Llop-Guevara, *et al*., (2008), PloS ONE, Jun 11;3(6):e2426.

Experimental Design

Four-Week HDM Exposure-Induced Lung Inflammation Model

[0270] Female mice of the genotypes indicated in Table 12 were randomized into 2 groups each per genotype. Saline (20 µL) or 50 µg HDM diluted in 20 µL of saline solution were administered IN 3 times per week for 4 weeks. All mouse strains were of a mixed C57BL/6NTac

/ 129S6SvEvTac background. Mice were sacrificed 4 days after the last exposure, lungs were harvested, and eosinophilic lung infiltration was determined.

Table 12: Experimental Protocol for 4-week HDM Model

Group	Genotype	N	Exposure Reagent	Duration of Exposure (weeks)
A	*Wild type*	3	20 µL Saline	4
B	*Wild type*	5	50 µg HDM	4
C	*Il33*$^{hu/hu}$	5	20 µL Saline	4
D	*Il33*$^{hu/hu}$	5	50 µg HDM	4
E	*Il4ra*$^{hu/hu}$	5	20 µL Saline	4
F	*Il4ra*$^{hu/hu}$	5	50 µg HDM	4
G	*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$	5	20 µL Saline	4
H	*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$	5	50 µg HDM	4
I	*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$	4	20 µL Saline	4
J	*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$	5	50 µg HDM	4

Wild type = C57BL/6NTac / 129S6SvEvTac

Nineteen-Week HDM Exposure-Induced Lung Inflammation Model

[0271] *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice used in this study were of a mixed background C57BL/6NTac (72%) / 129S6SvEvTac (28%); female mice were randomized into 7 separate groups. The HDM exposure and treatment or control dosing protocol for each group of mice is shown in Table 13. Saline (20 µL) or 50 µg HDM diluted in 20 µL of saline solution were administered IN 3 times per week for 19 weeks. A control group of *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice were sacrificed after 11 weeks of HDM exposure to assess disease severity at the onset of antibody treatment. Nineteen-week HDM-exposed mice either received no antibody treatment, or received twice-weekly subcutaneous (SC) injections from week 12 to week 19 of HDM exposure for a total of 16 antibody doses as indicated in Table 13. In brief, the following antibodies were administered at a final protein dose of 11 mg/kg: 11 mg/kg isotype control antibody (group D), 1 mg/kg REGN3500 + 10 mg/kg isotype control antibody (group E), 10 mg/kg dupilumab + 1 mg/kg isotype control antibody (group F), or 1 mg/kg REGN3500 + 10

mg/kg dupilumab (group G). For the purpose of this document, the dual antibody treatment groups (D-G) will only be identified by the therapeutic antibody (REGN3500 and/or dupilumab). On day 134 of the study, 4 days after the last IN exposure and antibody injection, all mice were sacrificed, blood was collected via cardiac puncture, and lungs were harvested for analysis.

Table 13: Experimental Protocol for 19-week HDM Model

Group	N	Exposure Reagent	Duration of Exposure (weeks)	Antibody Administration	Antibody Dose (mg/kg)
A	5	20 µL Saline	19	None	None
B	9	50 µg HDM	11	None	None
C	9	50 µg HDM	19	None	None
D	9	50 µg HDM	19	$IgG4^P$ Control	11
E	7	50 µg HDM	19	REGN3500 + $IgG4^P$ Control	1 + 10
F	8	50 µg HDM	19	dupilumab + $IgG4^P$ Control	10 + 1
G	8	50 µg HDM	19	REGN3500 + dupilumab	1 + 10

$IgG4^P$ Control = isotype-matched control antibody, REGN1945.

Mouse Husbandry

[0272] For the entire duration of each experiment, animals remained housed in the Regeneron animal facility under standard conditions, and were allowed to acclimate for at least 7 days prior to being placed on study. All animal experiments were performed in accordance with the guidelines for the Institutional Animal Care and Use Committee at Regeneron.

Specific Procedures

Relative Lung Weight Measurement

[0273] A terminal body weight measurement was recorded before sacrifice. After exsanguination, the left lung from each mouse was removed and placed into a tube containing a solution of 4% paraformaldehyde. Wet weight of the left lung for each mouse was recorded on a Mettler Toledo New Classic MS scale. To determine relative lung weights, the ratio of lung wet weight (in mg) to body weight (in g) was calculated by dividing the lung wet weight by the body weight.

Analysis of Cellular Pulmonary Infiltrates

[0274] After exsanguination, the caudal lobe of the right lung from each mouse was removed, placed into a tube containing a solution of 20 µg/mL DNase I and 0.7 U/mL Liberase TH diluted

in Hank's Balanced Salt Solution (HBSS), and cut into pieces that were approximately 2 to 3 mm in size. The tubes containing diced lung lobes 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. Then, 2 mL of autoMACS buffer was added 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 4 mL of 1x Red Blood Cell Lysing Buffer to lyse red blood cells. After incubation for 3 minutes at room temperature, 2.5 times volume 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 1 mL of DPBS. The resuspended samples were each filtered through a 50 µm cup-type filcon and transferred to a 2 mL deep well plate. The plate was centrifuged for 4 min at 400 x g and each sample resuspended in 1mL DPBS. 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 100 µL of LIVE/DEAD Fixable Dead Cell Stain diluted at 1:500 in 1X DPBS to determine cell viability. Cells were incubated with the viability dye for 15 minutes at room temperature while protected from light. After one wash in 1X DPBS, cells were incubated with purified rat anti-mouse CD16/CD32 Fc Block diluted 1:50 in 50 µL of autoMACS buffer for 15 minutes at 4°C. The cells were then incubated in the appropriate 2x antibody mixture diluted in Brilliant Stain Buffer (described in Table 14) for 30 minutes at 4°C while protected from light. After antibody incubation, the cells were washed twice in autoMACS 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 autoMACS buffer. Cell suspensions were then filtered into a new U-Bottom plate through an AcroPrep Advance 96 Filter Plate 30-40 µm. 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).

Gating Strategy for Eosinophils (Total and Activated)

[0275] Eosinophils were defined as intact, single, live cells (low LIVE/DEAD viability dye signal), $CD45^+$, $F4/80^+$, $Ly6G^-$, $SiglecF^+$. Data for eosinophils were expressed as frequency of live cells. Within the eosinophil population, activated eosinophils were defined as intact, single, live, $CD45^+$, $F4/80^+$, $Ly6G^-$, $SiglecF^+$, $CD11c^{Hi}$ and expressed as frequency of total eosinophils.

Gating Strategy for $ST2^+$ $CD4^+$ T cells

[0276] $ST2^+$ $CD4^+$ T cells were defined as intact, single, live, $CD45^+$, $CD3^+$, $CD19^-$, $CD4^+$, $CD8^-$, $ST2^+$. Data for $ST2^+$ $CD4^+$ T cells were expressed as frequency of $CD4^+$ T cells (intact, single, live, $CD45^+$, $CD3^+$, $CD19^-$, $CD4^+$, $CD8^-$).

Table 14: Antibodies Used for Flow Cytometry Analysis

Antibody	Fluorochrome	Manufacturer	Catalog #	Lot #	Final dilution
Mix 1:	**Total and Activated Eosinophils**				
CD45	Alexa Fluor 700	BioLegend	103128	B191240/ B211311	1/200
Siglec-F	BV421	BD	562681	4234913/ 6007723	1/200
F4/80	PE	BD	565410	5168713/ 5257914	1/500
Ly6G	BUV395	BD	563978	5156800/ 7103737	1/200
CD11c	PerCP-Cy5.5	BD	560584	5148566/7074758	1/200
Mix 2:	**$ST2^+$ $CD4^+$ T Cells**				
CD45	Alexa Fluor 700	BioLegend	103128	B211311	1/200
CD19	BUV737	BD	564296	6315651	1/200
ST2	PerCP-eFluor710	eBioscience	H6-9335-82	E17254-105	1/200
CD3	PE-Cy7	BD	552774	7074769	1/200
CD8	BUV395	BD	563786	6245983	1/200
CD4	BV786	BD	563331	7075503	1/200

Determination of Lung Protein Levels

[0277] After exsanguination, the cranial and middle lobes of the right lung from each mouse were removed, weighed, and placed into tubes containing a solution of tissue protein extraction reagent (T-PER) supplemented with a protease inhibitor cocktail. To achieve a final 1:8 (w/v) lung tissue weight to T-PER volume ratio, 8 µL of T-PER solution (containing the protease inhibitor cocktail) were added per mg of tissue. Lung samples were mechanically homogenized using the TissueLyser II. 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. Cytokines and MPO concentrations were expressed as the total amount of protein per lobe examined (ng/lung lobe and µg/lung lobe respectively).

Cytokines Multiplex Immunoassay

[0278] Mouse cytokines (IL-5, IL-13, IL-6, IL-1β, IL-12p70, TNFα, IFNγ, GROα and MCP-1) concentrations in the lung protein extracts were measured using a multiplex immunoassay kit

(Custom mouse 10-Plex, MSD), according to the manufacturer's instructions. Briefly, lung homogenate samples were diluted and incubated on plates pre-coated with capture antibodies. Calibrator proteins provided by the manufacturer were used as standards. Cytokines in the homogenates was detected by tagged detection antibodies incubated with Read Buffer. Electrochemiluminescence was immediately read on a MSD Spector® instrument. Data analysis was performed using GraphPad Prism software. The lowest concentration of standard within the linear range of each assay was defined as the assay's lower limit of quantification (LLOQ) for the respective cytokine. The LLOQ values for the individual cytokines tested were as follows: IFNγ=0.2 pg/mL, IL-1β=1.6 pg/mL, IL-5=0.2 pg/mL, IL-6=1.4 pg/mL, IL-12p70=125.8 pg/mL, IL-13=24.4 pg/mL, GROα: 0.5 pg/mL, MCP-1= 9.8 pg/mL, TNFα=2.4 pg/mL.

Human IL-4 ELISA

[0279] Human IL-4 concentrations in the lung protein extracts were measured using a sandwich ELISA kit according to the manufacturer's instructions (Human IL-4 Quantikine ELISA, R&D Systems). Briefly, lung homogenates were diluted and incubated on 96-well plates pre-coated with anti-human IL-4 capture antibody. Purified human IL-4 was used as a standard. Captured human Il-4 was detected using HRP-conjugated anti-human IL-4 detection antibody. HRP activity was detected using the chromagen 3,3',5,5'-tetramethylbenzidine (TMB). A stop solution was then added, and the optical density at 450nm (OD_{450}) was measured on a Molecular Devices SpectraMax M5 plate reader. Data analysis was performed using GraphPad Prism software. The lowest concentration of standard within the linear range of the assay was defined as the assay's LLOQ=31.25 pg/mL.

MPO ELISA

[0280] MPO concentrations in the lung protein extracts were measured using a sandwich ELISA kit according to the manufacturer's instructions (mouse MPO ELISA kit, Hycult Biotech). Briefly, lung homogenates were diluted and incubated on 96-well plates pre-coated with anti-MPO capture antibody. Purified mouse MPO was used as a standard. Captured MPO was detected using biotinylated anti-mouse MPO detection antibody. Purified HRP-conjugated streptavidin was used to detect biotinylated anti-mouse MPO. HRP activity was detected using the chromagen 3,3',5,5'-tetramethylbenzidine (TMB). A stop solution was then added, and the optical density at 450nm (OD_{450}) was measured on a Molecular Devices SpectraMax M5 plate reader. Data analysis was performed using GraphPad Prism software. The lowest concentration of standard within the linear range of the assay was defined as the assay's LLOQ=156.3 ng/mL.

Serum Collection

[0281] 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. The resulting supernatant, designated serum, was transferred into clean polypropylene plates and used to determine circulating antibody levels as described below.

Determination of SAA Levels in Serum by ELISA

[0282] Total SAA concentrations in the serum samples for each mouse were determined using a commercial immunoassay (Quantikine ELISA, R&D Systems) according to the manufacturer's instructions. Briefly, serum samples were diluted and incubated on 96-well plates previously coated with monoclonal anti-mouse SAA capture antibody. Recombinant mouse SAA was used as a standard. Captured SAA was detected using HRP-conjugated polyclonal anti-mouse SAA detection antibody. HRP activity was detected using the colorimetric HRP substrate TMB. A stop solution of diluted hydrochloric acid was then added, and the OD_{450} was measured on a Molecular Devices SpectraMax M5 plate reader. The concentration of circulating SAA in serum for each sample was determined as ng/mL and graphed as µg/mL. Data analysis was performed using GraphPad Prism software. The lowest concentration of standard within the linear range of the assay was defined as the assay's LLOQ= 31.2 ng/mL.

Determination of Serum IgE Levels by ELISA

[0283] Total IgE concentrations in the serum samples for each mouse were determined using a colorimetric sandwich ELISA OPTEIA kit according to the manufacturer's instructions. Briefly, serum samples were diluted and incubated on 96-well plates previously coated with anti-IgE capture antibody. Purified mouse IgE was used as a standard. Captured IgE was detected using biotinylated anti-mouse IgE detection antibody. Purified HRP-conjugated streptavidin was used to detect biotinylated anti-mouse IgE. HRP activity was detected using the TMB. A stop solution of 2N sulfuric acid was then added, and the OD_{450} was measured on a Molecular Devices SpectraMax M5 plate reader. The concentration of circulating IgE in serum for each sample was expressed as µg/mL. Data analysis was performed using GraphPad Prism software. The lowest concentration of standard within the linear range of the assay was defined as the assay's LLOQ = 78.15 ng/mL.

Determination of Serum HDM-Specific IgG1 Levels by ELISA

[0284] A colorimetric ELISA assay was developed to determine the levels of HDM-specific IgG1 in serum samples. Plates were coated with HDM at a concentration of 4 µg/mL in phosphate

buffered saline (PBS) overnight at 4°C, washed, blocked with a solution of 0.5% BSA in PBS for 1 hour at room temperature, and incubated with serially diluted mouse serum samples. After 1 hour at room temperature, plates were washed and IgG1 antibodies captured onto the plates were detected by incubation with rat anti-mouse IgG1 HRP-conjugated antibody for 1 hour at room temperature. HRP activity was detected using TMB. A stop solution of 2N sulfuric acid was then added, and OD_{450} was measured on a Molecular Devices SpectraMax M5 plate reader. Relative levels of IgG1 in serum were represented as titer units. Titer units were calculated by multiplying the measured OD_{450} by the dilution factor required to achieve an OD_{450} reading that was greater than 2 times background OD_{450}. Data analysis was performed using GraphPad Prism software. The lowest dilution factor used in the assay was defined as the assay's LLOQ = 100.

Determination of Human Target-specific IgG4 Antibody Levels by ELISA

[0285] The concentration of human antibody (REGN3500, dupilumab, or $IgG4^P$ isotype control) in the serum samples for each mouse was determined using a colorimetric sandwich ELISA developed to detect human IgG4 antibodies. Microtiter wells were coated with the antigen specific for the human antibody to be measured, i.e. Human IL-33 (REGN3931) to capture REGN3500, Human IL-4Rα (REGN560) to capture REGN668, Natural Fel d 1 to capture REGN1945, at a concentration of 2 µg/mL in PBS overnight at 4°C. Wells were washed four times with 0.05% Tween 20 in DPBS, blocked with a solution of 5% BSA in DPBS for 3 hours at room temperature and incubated with serially diluted mouse serum samples or serially diluted calibration standards. Purified antibodies (REGN3500, REGN668, and $IgG4^P$ control antibody) were used as standards for calibration and quantitation of the respective antibody concentrations in serum. After 1 hour at room temperature, plates were washed 7 times and human IgG4 captured onto the plates was detected using a biotinylated mouse anti-human IgG4-specific monoclonal antibody followed by incubation with Poly HRP, Streptavidin conjugated. HRP activity was detected using a TMB substrate according to manufacturer's instructions. After 10 minutes, absorbance was measured at 450 nm using a Molecular Devices SpectraMax multimode plate reader. The lowest concentration of standard (REGN3500, REGN668, or $IgG4^P$ control antibody) used for calibration (0.002 µg/mL) was defined as this assay's LLOQ. Data analysis was performed using GraphPad Prism™ (GraphPad Software, CA). The concentration of human antibody in serum for each sample was expressed as µg/mL.

Statistical Analyses

[0286] Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software, CA).

Statistical Analysis of Data from Characterization of IL-33-, IL-4-, and IL-4Rα-Humanized Mice in the 4-week HDM Exposure Model

[0287] Results were interpreted by two-way analysis of variance (ANOVA) followed by the Tukey's post hoc test for multiple comparisons. Differences were considered to be statistically significant when $p \leq 0.05$.

Statistical Analysis of Data from REGN3500/Dupilumab Treatment in 19-week HDM Exposure-induced Lung Inflammation Model

[0288] Normality of the data was evaluated using the Shapiro-Wilk 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 ANOVA followed by Tukey's 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 Dunn's post hoc test for multiple comparisons. Differences were considered to be statistically significant when $p \leq 0.05$.

RESULTS

Characterization of IL-33-, IL-4-, and IL-4Rα Ectodomain-Humanized Mice

[0289] *Wild type*, *Il33*$^{hu/hu}$- and *Il4ra*$^{hu/hu}$-single humanized, *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$-double humanized, and *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$*Il33*$^{hu/hu}$-triple humanized mice were exposed IN to saline or HDM 3 times per week for 4 weeks. Mice were sacrificed 4 days after the last exposure and lungs were harvested to assess the lung infiltration by activated eosinophils identified by high CD11c expression. Individual mouse data and statistical analyses are provided in Figure 2. Triple-humanized *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice showed robust response to HDM similar to *wild-type* mice, as indicated by a significant increase in the frequency of activated eosinophils in lung tissue following 4 weeks of HDM exposure. *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ and *wild-type* mice also showed similar frequencies of activated eosinophils in lung tissue in the absence of HDM exposure (saline-exposed control mice). No statistically significant differences were observed comparing HDM-exposed *wild-type* mice with HDM-exposed mice from any of the tested humanized mouse strains, with the exception of *Il4ra*$^{hu/hu}$ single humanized mice. The lack of statistically significant HDM exposure-induced increases in percentage of activated eosinophilic lung infiltration in *Il4ra*$^{hu/hu}$ mice is likely due to the fact that mouse IL-4 does not signal via the human IL-4Rα

receptor. Human IL-33, on the other hand, has been shown to signal via the murine receptor complex (REGN3500-MX-16069). Additionally, no statistically significant differences were observed comparing saline-exposed *wild-type* mice with saline-exposed mice from any of the tested humanized mouse strains. These findings validate the *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mouse strain for use as a mouse model of HDM-exposure induced lung inflammation.

Effect of REGN3500 and Dupilumab Treatment in 19-week HDM-exposed Mice

[0290] *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice were exposed IN to saline or HDM 3 times per week for 11 or 19 weeks. Four groups of 19-week HDM-exposed mice received twice-weekly SC injections of antibodies from week 12 to week 19; all other groups received no treatment (none, light gray boxes). Antibodies were administered alone or in combination at final protein doses of 11 mg/kg as follows: 11 mg/kg isotype control antibody, 1 mg/kg REGN3500 + 10 mg/kg isotype control antibody, 10 mg/kg dupilumab + 1 mg/kg isotype control antibody, or 1 mg/kg REGN3500 + 10 mg/kg dupilumab. One cohort of mice was sacrificed after 11 weeks of exposure to determine inflammatory profile at the start of antibody treatment (11-week exposure group). The other 4 cohorts were sacrificed on day 134 (19-week exposure groups), four days after the last exposure and antibody injection. Whole blood was collected by cardiac puncture for serum isolation and lungs were harvested for further analysis. All groups comprised mice of the same strain, (*Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$) unless noted otherwise.

Analysis of Gross Lung Pathology

[0291] Relative lung weight was significantly increased in 19-week HDM-exposed mice compared with saline-exposed control mice Figure 3. This is likely due to increased cellular infiltration, collagen deposition, muscle hypertrophy, and mucus production. In HDM-exposed mice, the combined administration of REGN3500 and dupilumab significantly blocked HDM exposure-induced increases in relative lung weight compared with mice administered isotype control antibody (Figure 3). A trend towards reduced relative lung weight was also observed in HDM-exposed mice dosed with REGN3500 alone.

Analysis of Pulmonary Cell Infiltrates

[0292] Four days after the last antibody injection, the mouse lungs were harvested and the caudal lobe of the right lung was dissociated into a single cell suspension for flow cytometric analysis of eosinophils. Eosinophils were defined as intact, single, live, CD45^{+}, F4/80^{+}, Ly6G^{-}, SiglecF^{+} and activated eosinophils were further defined as CD11c^{Hi}. Lung infiltration by activated eosinophils was reported as frequency (%) of total lung eosinophils in (A) and overall lung eosinophilic infiltration was reported as frequency (%) of total lung eosinophils in live (intact, single, live) cells. Compared with saline-exposed control mice, exposure to HDM for 19

weeks significantly increased cellular pulmonary infiltration, as assessed by flow cytometry for detection of total and activated lung eosinophils (Figure 4A and 4B) and lung $ST2^+$ $CD4^+$ T cells ($ST2^+$ $CD4^+$ T cells were defined as intact, single, live, $CD45^+$, $CD3^+$, $CD19^-$, $CD4^+$, $CD8^-$, $ST2^+$ and reported as frequency of $CD4^+$ T cells.) (Figure 5), or by immunoassay for detection of lung MPO protein levels as a marker for neutrophils (MPO protein levels were measured by enzyme-linked immunosorbent assay. Lung MPO protein levels are expressed as MPO protein amount (µg) per lung lobe.) (Figure 6).

[0293] Combined administration of REGN3500 and dupilumab in 19-week HDM-exposed mice, but not of either antibody alone, significantly reduced levels of lung infiltration by activated eosinophils compared with administration of an isotype control antibody. Notably, levels of lung infiltration by activated eosinophils in mice administered REGN3500 and dupilumab in combination were also significantly reduced compared with 11-week HDM exposure-induced levels, which corresponds to the onset of treatment Figure 4A. While administration of either antibody alone did not result in significant effects, a trend towards reduced pulmonary infiltration by activated eosinophils was observed in dupilumab-administered mice. HDM-exposed mice administered REGN3500 and dupilumab in combination also showed a trend towards a reduction in HDM-induced lung infiltration by total eosinophils (Figure 4B).

[0294] In 19-week HDM-exposed mice administered REGN3500 alone or in combination with dupilumab, levels of lung infiltration by $ST2^+$ $CD4^+$ T cells were significantly reduced compared with levels in isotype control-administered mice, and compared with 11-week HDM exposure-induced levels at the onset of treatment (Figure 5). Similar blocking of infiltration (mean frequencies within 1.02-fold) was observed for REGN3500 alone and in combination with dupilumab, indicating that this pathology is mainly driven by IL-33.

[0295] Similar to eosinophilic infiltration, combined administration of REGN3500 and dupilumab showed a stronger effect on blocking neutrophilic lung infiltration than either antibody alone. HDM exposure-induced increases in lung protein levels of myeloperoxidase (MPO), a marker of neutrophilic infiltration, were significantly blocked by combined administration of REGN3500 and dupilumab compared with isotype control (Figure 6). While administration of either antibody alone did not result in significant effects, a trend towards reduced lung MPO protein levels was observed in REGN3500-administered mice.

Lung Tissue Cytokine Level Analysis

[0296] The effect of HDM exposure and antibody treatment on lung protein levels (total protein per lobe) was assessed for mouse cytokines IL-5, IL-13, IL-6, IL-1β, IL-12p70, TNFα, IFNγ, GROα, and MCP-1, and for the human cytokine hIL-4.

[0297] Lungs (cranial and middle lobes of the right lung) were harvested and protein levels of the indicated mouse cytokines were measured by multiplexed immunoassay. Human IL-4 protein levels (hIL-4) were detected using a commercially available ELISA kit. IL-5 (Figure 7A) and IL-6 (Figure 7B) protein levels were measured by multiplexed immunoassay. Lung tissue cytokine protein levels were calculated as protein amount (pg) per lung lobe. A false-colored heat map (not shown here) was generated to denote the relative cytokines ranging from light yellow to dark blue. A scale of relative lung cytokine levels was created by defining the lowest and highest recorded mean lung protein level for each separate cytokine as 0% (light yellow) and 100% (dark blue), respectively. Relative lung cytokine protein levels (%) were indicated by numbers and by color in the heat map. Statistical significance was determined by Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc test.

[0298] The result for IL-12p70 was below the lower limit of quantification for all groups and is therefore not reported here.

[0299] Eight cytokines (hIL-4, IL-5, IL-6, IL-13, IL-1β, TNFα, GROα, and MCP-1) showed significant increases in lung protein levels in response to 19-week HDM exposure compared with saline-exposed control mice. Only IFNγ did not show significant increases in lung protein levels in response to 19-week HDM exposure compared with saline-exposed control mice, nor were IFNγ levels affected by therapeutic antibody administration compared with 19-week HDM-exposed mice administered isotype control antibody. HDM exposure-induced increases in lung protein levels of 5 cytokines (hIl-4, IL-6, TNFα, GROα, and MCP-1) were significantly blocked by combined administration of REGN3500 and dupilumab, but not by administration of either antibody alone, compared with isotype control antibody-administered mice.

[0300] Another 2 HDM exposure-responsive cytokines (IL-5 and IL-1β) showed trends towards blockade by combined REGN3500 and dupilumab treatment, with 83% and 78% reduction of lung protein levels, respectively, compared with isotype control antibody-administered mice (Figure 7A and 7B). Administration of individual antibodies resulted in less pronounced trends in reduction of IL-5 and IL-1β levels.

Analysis of SAA, a Systemic Marker of Inflammation

[0301] Four days after the last exposure and antibody injection, whole blood was collected by cardiac puncture and serum was isolated. Circulating SAA protein levels were measured using a commercially available ELISA kit. Circulating SAA protein levels are expressed as SAA protein amount (μg) per mL of serum. Circulating protein levels of the systemic marker of

inflammation SAA were significantly increased in 19-week HDM-exposed mice compared with saline-exposed control mice (Figure 8).

[0302] HDM exposure-induced increases in circulating SAA levels were significantly reduced in mice administered REGN3500 alone or in combination with dupilumab (Figure 8), while a trend towards reduced circulating SAA levels was observed in mice administered dupilumab alone.

Quantification of Humoral Allergic Reponses Following HDM Exposure

[0303] Four days after the last exposure and antibody injection, whole blood was collected by cardiac puncture and serum was isolated. Circulating IgE protein levels were measured using a commercially available ELISA kit. Circulating IgE protein levels are expressed as IgE protein amount (µg) per mL of serum.

[0304] Humoral allergic responses were elicited by HDM-exposure as assessed by levels of circulating IgE (Figure 9) and HDM-specific IgG1 (Table 15) at the end of the study (day 134).

[0305] Circulating protein levels of IgE were significantly increased in 19-week HDM-exposed mice compared with saline-exposed control mice (Figure 9). Average titers for circulating HDM-specific IgG1 increased from 1.14E+02 in saline-exposed control mice to levels ranging from 1.37E+06 to 2.43E+06 in mice exposed to HDM for 19 weeks (Table 15). No statistically significant effect of REGN3500, dupilumab, or combination treatment was observed for either of these endpoints, but a trend towards reduced serum IgE levels was observed in mice administered a combination of REGN3500 and dupilumab.

Table 15: Summary of Serum Concentrations of HDM-specific IgG1

HDM-specific IgG1 in Serum (Titer)	Saline 19 weeks	HDM 11 weeks	HDM 19 weeks				
			No Antibody	$IgG4^P$	REGN3500 + $IgG4^P$	Dupilumab + $IgG4^P$	REGN3500 + Dupilumab
Mean	1.14E+02	2.19E+06	2.43E+06	2.14E+06	1.47E+06	1.37E+06	1.21E+06
SD	6.15E+01	1.07E+06	9.81E+05	5.60E+05	1.17E+06	5.79E+05	5.29E+05

Quantification of Serum Concentrations of Human Antibodies

[0306] The serum concentration of human $IgG4^P$ antibodies ($IgG4^P$ isotype control, REGN3500 and dupilumab) were determined at the end of the study (day 134), four days after the last antibody administration, by target-specific anti-human IgG4 ELISA. Average concentrations of human IgG4 antibodies are summarized in Table 16.

Table 16: Human Antibody Serum Levels at End of Study

	Serum Antibody Levels, Mean ± SD (µg/mL)						
	19-wk Saline	11-wk HDM	19-wk HDM	19-wk HDM IgG4^P (11 mg/kg)	19-wk HDM REGN3500 (1 mg/kg) + IgG4^P (10 mg/kg)	19-wk HDM Dupilumab (10 mg/kg) + IgG4^P (1 mg/kg)	19-wk HDM REGN3500 (1 mg/kg) + Dupilumab (10 mg/kg)
REGN3500	<LLOQ	<LLOQ	<LLOQ	n/t	11.4±10.1	n/t	12.7±8.8
Dupilumab	<LLOQ	<LLOQ	<LLOQ	n/t	n/t	8.0±13.5	48.9±27.9
IgG4^P	0.0±0.0[a]	<LLOQ	<LLOQ	54.5±63.9	88.6±76.0	0.1±0.1	n/t

[a] One mouse in this group had serum IgG4^P levels >LLOQ, therefore a rounded value is reported here.

SUMMARY

[0307] Compared to control mice exposed to saline only, mice exposed to HDM for 19 weeks demonstrated significant increases in all but one (lung IFNγ levels) of the 14 measured pathological markers of inflammation when left untreated or after administration of an IgG4^P isotype control antibody.

[0308] Combined administration of REGN3500 and dupilumab significantly blocked 10/13 of the tested HDM exposure-responsive endpoints compared with isotype control antibody (relative lung weight, pulmonary infiltration by activated eosinophils, neutrophils [MPO levels], and ST2$^+$ CD4$^+$ T cells, lung protein levels of cytokines hIL-4, IL-6, TNFα, GROα, and MCP-1, and serum levels of SAA). Furthermore, levels of lung infiltration by activated eosinophils and ST2$^+$ CD4$^+$ T cells were significantly reduced to levels below those observed in 11-week HDM-exposed mice, which corresponds to the onset of antibody treatment. Administration of dupilumab alone did not significantly block any of the 13 tested HDM exposure-responsive endpoints in this model, while administration of REGN3500 alone significantly blocked 2 tested endpoints: ST2$^+$ CD4$^+$ T cell lung infiltration and circulating SAA levels. For these 2 endpoints, blockade mediated by REGN3500 alone was similar to blockade mediated by REGN3500 in combination with dupilumab, suggesting that these pathological markers are mainly driven by IL-33.

[0309] Combined administration of REGN3500 and dupilumab showed a trend towards blocking HDM exposure-induced responses for another 3 HDM exposure-responsive endpoints without reaching statistical significance (pulmonary infiltration by eosinophils [total], lung protein levels

of cytokines IL-5 and IL-1β, and serum protein levels of IgE). For these markers, individual antibody treatment with REGN3500 or dupilumab generally resulted in weaker reduction than combined treatment.

[0310] All antibody treatment groups were associated with detectable serum levels for target-specific human IgG4 antibodies at the end of the study. In mice dosed twice-weekly for 8 weeks with either therapeutic antibody alone or in combination, average serum concentrations of REGN3500 were 11.4±10.1 and 12.7±8.8 µg/mL, respectively, and average serum concentrations of dupilumab were 8.0±13.5 and 48.9±27.9 µg/mL, respectively at the end of the study.

[0311] In conclusion, combined treatment with REGN3500 and dupilumab in a 19-week HDM exposure-induced lung inflammation model using *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice resulted in a more pronounced improvement of almost all of tested lung pathologies and markers of inflammation compared with treatment with either antibody alone.

CONCLUSION

[0312] Combined treatment with REGN3500 and dupilumab in a 19-week HDM exposure-induced lung inflammation model using *Il4ra*$^{hu/hu}$ *Il4*$^{hu/hu}$ *Il33*$^{hu/hu}$ mice resulted in a more pronounced improvement of almost all of tested lung pathologies and markers of inflammation compared with treatment with either antibody alone.

Example 7: Evaluation of SAR440340/REGN3500, or Dupilumab, When Used Alone and When Used as Combination Therapy in Moderate-to-Severe COPD Patients

Study Design

[0313] This study is a randomized, double-blind, placebo-controlled, parallel-group, 24 week proof-of-concept study to assess the efficacy, safety and tolerability of an IL-33 monoclonal antibody (SAR440340/REGN3500), an IL-4R monoclonal antibody (dupilumab, also known as DUPIXENT®), when each is used alone, or when used in combination in patients with moderate-to-severe chronic obstructive pulmonary disease (COPD).

[0314] Eight hundred and thirty two total subjects will participate in the study. The study will consist of four arms, one arm being patients administered subcutaneously (SC) the anti-IL-33 monoclonal antibody (SAR440340/REGN3500) alone; the second arm being patients administered subcutaneously the anti-IL-4R monoclonal antibody (dupilumab) alone; the third arm being patients coadministered both SAR440340/REGN3500 and dupilumab subcutaneously; and the fourth arm being placebo.

[0315] The patients in arm 1 will receive 2 SC injections of SAR440340/REGN3500 every 2 weeks for 24 weeks and coadministered the dupilumab placebo as 1 SC injection every 2 weeks for 24 weeks; the patients in arm 2 will receive 1 SC injection of dupilumab every 2

weeks for 24 weeks and coadministered the SAR440340/REGN3500 placebo as 2 SC injections every 2 weeks for 24 weeks; the patients in arm 3 will receive 2 SC injections of SAR440340/REGN3500 every 2 weeks for 24 weeks and coadministered dupilumab as 1 SC injection every 2 weeks for 24 weeks; the patients in arm 4 will receive matching placebos for SAR440340/REGN3500 and dupilumab administered as 2 and 1 SC injections, respectively, every 2 weeks for 24 weeks.

Study Objectives

[0316] The primary objective of this study is to determine and compare the effects of an interleukin-33 antibody (SAR440340/REGN3500), an interleukin-4 receptor monoclonal antibody (dupilumab), and the coadministration of both, compared to placebo, in patients with moderate-to-severe chronic obstructive pulmonary disease (COPD), who have been treated with an inhaled corticosteroid (ICS), and/or a long acting β2 adrenergic agonist (LABA) and/or long acting muscarinic antagonist (LAMA) background therapy (double or triple therapy), on improving respiratory function, as assessed by post bronchodilator forced volume in 1 second (FEV1), over 24 weeks.

[0317] The secondary objectives will be to evaluate the effects of SAR440340/REGN3500, dupilumab and the coadministration of both, each compared with placebo, on the incidence of moderate-to-severe acute exacerbations of COPD (AECOPD) over 24 weeks of treatment.

[0318] Another secondary objective is to evaluate the effects of SAR440340/REGN3500, dupilumab and the coadministration of both, each compared to placebo on: Pre-bronchodilator FEV1 over 24 weeks; Duration from baseline to first moderate or severe AECOPD event over 24 weeks; Evaluation of clinical symptoms of COPD; Safety and Tolerability.

Inclusion Criteria

[0319] Inclusion criteria for the study are as follows: (1) Patients with moderate-to-severe Chronic Obstructive Pulmonary Disease (COPD) (post bronchodilator Forced Expiratory Volume in one second (FEV1) forced vital capacity (FVC) <70% and post bronchodilator FEV1% predicted <80%, but ≥30%); (2) Patients with COPD Assessment Test (CAT) score ≥ 10 at screening visit 1 and visit 2/Randomization; (3) Patients with reported history of signs and symptoms of chronic bronchitis (chronic productive cough for 3 months in the year up to screening in a patient in whom other causes of chronic cough (eg. gastroesophageal reflux, chronic rhinosinusitis, bronchiectasis) have been excluded; (4) Patients with documented history of ≥ 2 moderate exacerbations or ≥1 severe exacerbation within the year prior to screening; (5) Patients with Standard of Care background therapy, for 3 months prior to Visit 2/Randomization and at a stable dose for at least 1 month prior to the screening visit 1, including either: Double therapy: Long acting β agonist (LABA) + Long acting Muscarinic antagonist (LAMA) or Inhaled

Corticosteroid (ICS) + LABA or ICS + LAMA; or Triple therapy: ICS + LABA + LAMA; (6) Signed written informed consent; and (7) Current or former smokers with a smoking history of ≥10 packs/year.

Exclusion Criteria

[0320] Exclusion criteria for the study are as follows: (1) Age of ≤40 years or > 75 years; (2) Patients with body mass index (BMI) <16; (3) Patients with COPD diagnosed within the 6 months prior to randomization; (4) A current diagnosis of asthma according to the Global Initiative for Asthma (GINA) guidelines; (5) Significant pulmonary disease other than COPD (eg, lung fibrosis, sarcoidosis, interstitial lung disease, pulmonary hypertension, bronchiectasis, eosinophilic granulomatosis with polyangiitis, significant sleep apnea on Bilevel Positive Airway Pressure, etc) or another diagnosed pulmonary or systemic disease associated with elevated peripheral eosinophil counts; (6) Diagnosis of α-1 anti-trypsin deficiency; (7) Advanced COPD with need for chronic (>15 hours/day) oxygen support; (8) Patient with a moderate or severe Acute Exacerbation of COPD event within 4 weeks prior to screening; (9) A patient who has experienced an upper or lower respiratory tract infection within 4 weeks prior to Screening/Visit 1 or during the screening period; (10) Prior history of or planned pneumonectomy or lung volume reduction surgery; (11) Patients with a history of a systemic hypersensitivity reaction to a biologic drug.

Example 8. Evaluation of SAR440340/REGN3500, or Dupilumab, When Used Alone and When Used as Combination Therapy in Moderate-to-Severe Asthma Patients

Study Design

[0321] This study ia a randomized, double-blind, placebo-controlled, parallel-group, 12-week proof-of-concept (PoC) study to assess the efficacy, safety, and tolerability of SAR440340/REGN3500, dupilumab (also known as DUPIXENT®), and the coadministration of SAR440340 and dupilumab in patients with moderate-to-severe asthma who are not well controlled on inhaled corticosteroid (ICS) plus long-acting β2 adrenergic agonist (LABA) therapy.

[0322] Eight hundred total subjects will participate in this study. The study will consist of four arms, one arm being patients administered subcutaneously (SC) the anti-IL-33 monoclonal antibody (SAR440340/REGN3500) alone; the second arm being patients administered subcutaneously the anti-IL-4R monoclonal antibody (dupilumab) alone; the third arm being patients coadministered both SAR440340/REGN3500 and dupilumab subcutaneously; and the fourth arm being placebo.

[0323] The patients in arm 1 will receive SAR440340/REGN3500 administered as 2 subcutaneous (SC) injections every 2 weeks for 12 weeks and coadministration of dupilumab placebo as 1 SC injection every 2 weeks for 12 weeks; the patients in arm 2 will receive

dupilumab administered as 1 SC injection every 2 weeks for 12 weeks and coadministration of SAR440340/REGN3500 placebo as 2 SC injections every 2 weeks for 12 weeks; the patients in arm 3 will receive SAR440340/REGN3500 administered as 2 SC injections every 2 weeks for 12 weeks and coadministration of dupilumab administered as 1 SC injection every 2 weeks for 12 weeks; the patients in arm 4 will receive coadministration of matching placebos for SAR440340/REGN3500 and dupilumab administered as 2 and 1 SC injections, respectively, every 2 weeks for 12 weeks.

Study Objectives

[0324] The primary study objective is to evaluate the effects of SAR440340/REGN3500 with or without dupilumab, compared to placebo, on reducing the incidence of "loss of asthma control" (LOAC) events.

[0325] The secondary study objectives will be to evaluate the effects of SAR440340/REGN3500 and coadministration of SAR440340/REGN3500 and dupilumab, compared with placebo, on forced expiratory volume in 1 second (FEV1); to evaluate the effects of coadministration of SAR440340/REGN3500 and dupilumab, compared with SAR440340/REGN3500 and compared with dupilumab, on FEV1; to evaluate the effects of the concurrent administration of SAR440340/REGN3500 and dupilumab compared to SAR440340/REGN3500 alone and dupilumab alone on reducing the LOAC; and to assess safety and tolerability of SAR440340/REGN3500 alone and in coadministration with dupilumab.

Inclusion criteria

[0326] Inclusion criteria for the study are as follows: **(1)** Adult patients (18 years and above) with a physician diagnosis of asthma for at least 12 months based on the Global Initiative for Asthma (GINA) 2016 Guidelines whose asthma is partially controlled or uncontrolled on ICS/LABA combination therapy with the following criteria: Existing treatment with medium to high dose Inhaled Corticosteroids (ICS) (≥250 mcg of fluticasone propionate twice daily (BID) or equipotent ICS daily dosage to a maximum of 2000 mcg/day of fluticasone propionate or clinically comparable) in combination with a Long Acting Beta-Agonist (LABA) as second controller for at least 3 months with a stable dose ≥1 month prior to Visit 1; (2) Pre-bronchodilator Forced Expiratory Volume in One Second (FEV1) ≥50% but ≤85% of predicted normal at Visit 2/Baseline; (3) Reversibility of at least 12% and 200 mL in FEV1 after administration of 2 to 4 puffs (200-400 mcg) of albuterol/salbutamol or levalbuterol/levosalbutamol during screening (up to 3 opportunities during the same visit are allowed with a maximum of 12 puffs of reliever medication if tolerated by the patient); documented history of 20% variability in pre bronchodilator FEV1 when comparing 2 acceptable spirometric assessments within 6 months prior to Visit 1/Screening, or positive airway

hyperresponsiveness to methacholine within 12 months prior to Visit 1/Screening is considered acceptable to meet this inclusion criterion; (4) Must have experienced, within 1 year prior to Visit 1, any of the following events at least once: Treatment with a systemic steroid (oral or parenteral) for worsening asthma, or hospitalization or emergency medical care visit for worsening asthma; (5) Signed written informed consent.

Exclusion Criteria

[0327] Exclusion criteria for the study are as follows: (1) Patients <18 years or >70 years of age (ie, have reached the age of 71 at the screening visit); (2) Patients with body mass index (BMI) <16; (3) Chronic lung disease (for example, chronic obstructive pulmonary disease [COPD], or idiopathic pulmonary fibrosis [IPF]) which may impair lung function; (4) History of life threatening asthma (ie, severe exacerbation that requires intubation); (5) Co-morbid disease that might interfere with the evaluation of IMP; (6) Patients with any of the following events within the 4 weeks prior to their Screening Visit 1: Treatment with 1 or more systemic (oral and/or parenteral) steroid bursts for worsening asthma, or hospitalization or emergency medical care visit for worsening asthma; (7) Asthma Control Questionnaire 5-question version (ACQ-5) score <1.25 or >3.0 at V2/randomization. During the screening period an ACQ-5 of up to ≤4 is acceptable; (8) Anti-immunoglobulin E (IgE) therapy (eg, omalizumab [Xolair®]) within 130 days prior to Visit 1 or any other biologic therapy (including anti-IL5 mAb) or systemic immunosuppressant (eg, methotrexate) to treat inflammatory disease or autoimmune disease (eg, rheumatoid arthritis, inflammatory bowel disease, primary biliary cirrhosis, systemic lupus erythematosus, multiple sclerosis, etc.) and other diseases, within 2 months or 5 half-lives prior to Visit 1, whichever is longer; (9) Patients with a history of a systemic hypersensitivity reaction to a biologic drug; (10) Patients on or initiation of bronchial thermoplasty within 2 years prior to Visit 1 or plan to begin therapy during the screening period or the randomized treatment period; (11) Current smoker or cessation of smoking within the 6 months prior to Visit 1; (12) Previous smoker with a smoking history >10 pack-years.

We claim:

1. A method for treating an inflammatory disease or disorder, or at least one symptom associated with the inflammatory disease or disorder, the method comprising administering to a subject in need thereof one or more doses of a therapeutically effective amount of an interleukin-33 (IL-33) antagonist in combination with one or more doses of a therapeutically effective amount of an interleukin-4 receptor (IL-4R) antagonist, wherein the administration of the combination results in enhanced therapeutic efficacy as compared to that observed with administration of the IL-33 antagonist alone or the IL-4R antagonist alone.

2. The method of claim 1, wherein the inflammatory disease or disorder is alleviated, or reduced in severity, duration or frequency of occurrence, or at least one symptom associated with the inflammatory disease or disorder is alleviated, or reduced in severity, duration, or frequency of occurrence.

3. The method of either claim 1 or 2, wherein the inflammatory disease or disorder is selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), asthma and COPD overlap syndrome (ACOS), atopic dermatitis, an allergic response, chronic bronchitis, emphysema, chronic rhinosinusitis with or without nasal polyps, inflammatory bowel disease, Crohn's disease, ulcerative colitis, hypersensitivity pneumonitis, multiple sclerosis, arthritis (osteoarthritis, rheumatoid arthritis and psoriatic arthritis), allergic rhinitis, fibrosis, eosinophilic esophagitis, vasculitis, urticaria, Churg Strauss syndrome, inflammatory pain and psoriasis.

4. The method of claim 3, wherein the chronic obstructive pulmonary disease is exacerbated by one or more of the following: asthma, a viral disease, a bacterial infection, an exposure to an allergen, an exposure to a chemical or chemical fumes, or an exposure to an environmental irritant or air pollution.

5. The method of claim 3, wherein the asthma is exacerbated by one or more of the following: a viral disease, a bacterial infection, an exposure to an allergen, an exposure to a chemical or chemical fumes, or an exposure to an environmental irritant or air pollution.

6. The method of claim 3 or 5, wherein the asthma is eosinophilic asthma, non-eosinophilic asthma, steroid resistant asthma or steroid sensitive asthma.

7. The method of claim 3 or 4, wherein the chronic obstructive pulmonary disease results from, or is exacerbated in part by cigarette smoke.

8. The method of any one of claims 1-7, further comprising administering an effective amount of one or more additional therapeutic agents useful for alleviating the inflammatory disease or disorder, or at least one symptom of the inflammatory disease or disorder.

9. The method of claim 8, wherein the one or more additional therapeutic agents 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-1 antagonist, an IL-8 antagonist, an IL-13 antagonist, a different IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-33/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, a TNF inhibitor, an IgE inhibitor, a leukotriene inhibitor, an oral PDE4 inhibitor, a methylxanthine, nedocromil sodium, cromolyn sodium, a long-acting beta 2 agonist (LABA), a long acting muscarinic antagonist (LAMA), an inhaled corticosteroid (ICS) and another IL-33 antagonist.

10. The method of claim 9, wherein another IL-33 antagonist is selected from the group consisting of a different antibody to IL-33, a different IL-33 receptor based trap, an ST2 antibody, a soluble ST2 receptor, an antagonist to an IL-33 receptor other than ST2, an IL-1RAcP antagonist, and an antibody that interacts with an IL-33/ST2 complex.

11. A method for treating a fibrotic disease or disorder, or at least one symptom associated with the fibrotic disease or disorder, the method comprising administering to a subject in need thereof one or more doses of a therapeutically effective amount of an interleukin-33 (IL-33) antagonist in combination with one or more doses of a therapeutically effective amount of an interleukin-4 receptor (IL-4R) antagonist, wherein the administration of the combination results in enhanced therapeutic efficacy as compared to that observed with administration of the IL-33 antagonist alone or the IL-4R antagonist alone.

12. The method of claim 11, wherein the fibrotic disease or disorder is alleviated, or reduced in severity, duration or frequency of occurrence, or at least one symptom associated with the fibrotic disease or disorder is alleviated, or reduced in severity, duration, or frequency of occurrence.

13. The method of claim 11 or 12, wherein the fibrotic disease or disorder is a pulmonary fibrotic disease or disorder.

14. The method of claim 13, wherein the pulmonary fibrotic disease or disorder is selected from the group consisting of idiopathic pulmonary fibrosis, fibrosis associated with acute lung injury or acute respiratory distress, silicosis, radiation-induced fibrosis, bleomycin-induced pulmonary fibrosis, asbestos-induced pulmonary fibrosis, and bronchiolitis obliterans syndrome.

15. A method for preventing or reducing the severity of an allergic response in a subject in need thereof, the method comprising administering one or more doses of a therapeutically effective amount of an IL-33 antagonist in combination with one or more doses of a therapeutically effective amount of an IL-4R antagonist, wherein the administration of the combination results in enhanced therapeutic efficacy for preventing or reducing the severity of an allergic response as compared to that observed with administration of the IL-33 antagonist alone or the IL-4R antagonist alone.

16. The method of any one of claims 1, 11, or 15, wherein the enhanced therapeutic efficacy is measured by any one or more of the following parameters:

a) a reduction in the frequency of one or more of the following: eosinophils, activated B cells, activated CD8 T cells, or CD4/CD8 T cell ratio in a tissue sample;

b) a reduction in one or more of the following: interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-13 (IL-13), monocyte chemoattractant protein-1 (MCP-1), or tumor necrosis factor alpha (TNFα) levels in a tissue sample; or

c) a reduction in the gene expression level of one or more of the following: *Il4, Il5, Il6*, *Il9, Il13*, *Il1rl1*, *Il13ra2*, *tnf, Tgfb1, Ccl2, Ccl11, Ccl24*, *Col15a1* and/or *Col24a1* in a tissue sample.

17. The method of claim 16, wherein the enhanced therapeutic efficacy is further measured by any one or more of the following parameters:

d) a reduction in serum IgE levels;

e) a reduction in goblet cell metaplasia in the lung;

f) an improvement in lung consolidation; or

g) a decrease in sub-epithelial fibrosis in the lung.

18. The method of claim 16, wherein the tissue sample is obtained from the lung, liver, kidney, heart, or whole blood.

19. The method of any one of claims 1-18, wherein the administration of the IL-33 antagonist in combination with the IL-4R antagonist results in an increase in a type 1 immune response, and/or a reduction in a type 2 immune response elicited by the disease, or by the causative agent of the disease or allergy.

20. The method of any one of claims 1-19, wherein the IL-4R antagonist is an antibody or antigen-binding fragment thereof that binds IL-4Rα and prevents the interaction of IL-4 and/or IL-13 with a type 1 or type 2 IL-4α receptor.

21. The method of claim 20, wherein the antibody or antigen-binding fragment thereof that binds IL-4Rα prevents the interaction of IL-4 and/or IL-13 with both type 1 and type 2 IL-4α receptors.

22. The method of either claim 20 or 21, wherein the IL-4Rα antibody or antigen-binding fragment thereof comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:335 or 337 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:336 or 338.

23. The method of any one of claims 20-22, wherein the IL-4Rα antibody or antigen-binding fragment thereof comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 339, the HCDR2 comprises the amino acid sequence of SEQ ID NO:340; the HCDR3 comprises the amino acid sequence of SEQ ID NO:341; the LCDR1 comprises the amino acid sequence of SEQ ID NO:342; the LCDR2 comprises the amino acid sequence of SEQ ID NO:343; and the LCDR3 comprises the amino acid sequence of SEQ ID NO:344.

24. The method of any one of claims 20-23, wherein the IL-4Rα antibody or antigen-binding fragment thereof comprises an HCVR comprising the amino acid sequence of SEQ ID NO: 335 or SEQ ID NO: 337 and an LCVR comprising the amino acid sequence of SEQ ID NO: 336 or SEQ ID NO: 338.

25. The method of any one of claims 20-24, wherein the IL-4Rα antibody or antigen-binding fragment thereof comprises an HCVR/LCVR amino acid sequence pair of either SEQ ID NOs: 335/336 or SEQ ID NOs: 337/338.

26. The method of any one of claims 1-25, wherein the IL-4Rα antagonist is dupilumab or a bioequivalent thereof.

27. The method of any one of claims 1-19, wherein the IL-33 antagonist is an antibody or antigen-binding fragment thereof that binds specifically to IL-33 and blocks the interaction of IL-33 and ST2.

28. The method of any one of claims 1-19, or 27, wherein the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and comprises three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) 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.

29. The method of any one of claims 1-19, or 27-28, wherein the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290 and 308.

30. The method of any one of claims 1-19, or 27-29, wherein the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises 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.

31. The method of any one of claims 1-19, or 27-30, wherein the antibody or antigen-binding fragment thereof that binds specifically to IL-33, comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292 and 310;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294 and 312;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296 and 314;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284 and 318;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286 and 320;

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288 and 322.

32. The method of any one of claims 1-19, or 27-31, wherein the antibody or antigen-binding fragment thereof that binds specifically to IL-33 comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 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 and 308/316.

33. The method of any one of claims 1-19, or 27-32, wherein the IL-33 antibody or antigen-binding fragment thereof interacts with an amino acid sequence ranging from about position 1 to about position 12 of SEQ ID NO: 349, and/or with an amino acid sequence ranging from about position 50 to about position 94 of SEQ ID NO: 349 as determined by hydrogen/deuterium exchange.

34. The method of any one of claims 1-19, or 27-33, wherein the IL-33 antibody or antigen-binding fragment thereof interacts with an amino acid sequence ranging from about position 112 to about position 123 of SEQ ID NO: 348, and/or with an amino acid sequence ranging from about position 161 to about position 205 of SEQ ID NO: 348 as determined by hydrogen/deuterium exchange.

35. The method of any one of claims 1-19, or 27-34, wherein the IL-33 antibody or antigen-binding fragment thereof interacts with either the amino acid sequence of SEQ ID NO: 350, or with the amino acid sequence of SEQ ID NO: 351, or interacts with both SEQ ID NOs: 350 and 351 as determined by hydrogen/deuterium exchange.

36. The method of any one of claims 1-19, or 27-35, wherein the IL-33 antibody or antigen-binding fragment comprises the heavy chain complementarity determining regions (HCDRs) and light chain complementarity determining regions (LCDRs) of a heavy chain variable region/light chain variable region (HCVR/LCVR) amino acid sequence pair of SEQ ID NOs: 274/282.

37. The method of any one of claims 1-19, or 27-36, wherein the IL-33 antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, of SEQ ID NOs: 276-278-280-284-286-288.

38. The method of any one of claims 1-19, or 27-37, wherein the antibody that specifically binds human interleukin-33 (IL-33), or an antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 274; and (b) a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:282.

39. The method of any one of claims 1-19, or 27-38, wherein the IL-33 antibody or antigen-binding fragment thereof competes for binding to IL-33 with a reference antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

40. The method of any one of claims 1-19, or 27-39, wherein the IL-33 antibody or antigen-binding fragment thereof binds to the same epitope on IL-33 as a reference antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

41. The method of any one of claims 1-19, wherein the IL-33 antagonist is 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.

42. The method of claim 41, wherein the IL-33 antagonist is an IL-33 trap further comprising a second IL-33 binding domain (D2) attached to D1 and/or M, wherein D2 comprises an extracellular portion of an IL-1RAcP protein.

43. The method of claim 41, wherein D1 is attached to the N-terminus of M.

44. The method of claim 41, wherein D1 is attached to the C-terminus of M.

45. The method of claim 42, wherein D2 is attached to the N-terminus of M.

46. The method of claim 42, wherein D2 is attached to the C-terminus of M.

47. The method of claim 42, wherein D1 is attached to the N-terminus of D2, and wherein D2 is attached to the N-terminus of M.

48. The method of claim 41, wherein D1 comprises the amino acid sequence of SEQ ID NO: 328 or 329, or an amino acid sequence having at least 90% identity thereto.

49. The method of claim 42, wherein D2 comprises the amino acid sequence of SEQ ID NO: 330 or 331, or an amino acid sequence having at least 90% identity thereto.

50. The method of any one of claims 1-19, wherein the IL-33 antagonist is an IL-33 trap comprising a first IL-33 binding domain (D1) attached to a first multimerizing domain (M1), and a second IL-33 binding domain (D2) attached to a second multimerizing domain (M2), wherein the D1 and/or D2 domains comprise an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

51. The method of claim 50, wherein the IL-33 antagonist comprises a third IL-33 binding domain (D3) that is attached to either D1 or M1, and wherein D3 comprises an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

52. The method of claim 51, wherein the IL-33 antagonist comprises a fourth IL-33 binding domain (D4) that is attached to either D2 or M2, and wherein D4 comprises an IL-33-binding portion of a receptor selected from the group consisting of ST2 and IL-1RAcP.

53. The method of claim 50, wherein D1 is attached to the N-terminus of M1, and D2 is attached to the N-terminus of M2.

54. The method of claim 51, wherein D3 is attached to the N-terminus of D1.

55. The method of claim 51, wherein D3 is attached to the C-terminus of M1.

56. The method of claim 51, wherein D4 is attached to the N-terminus of D2.

57. The method of claim 52, wherein D4 is attached to the C-terminus of M2.

58. The method of claim 52, wherein D3 is attached to the N-terminus of D1, and D1 is attached to the N-terminus of M1; and wherein D4 is attached to the N-terminus of D2, and D2 is attached to the N-terminus of M2.

59. The method of claim 58, wherein D3 is identical or substantially identical to D4 and wherein D1 is identical or substantially identical to D2.

60. The method of claim 59, wherein D3 and D4 each comprise an IL-33-binding portion of an ST2 protein; and wherein D1 and D2 each comprise an extracellular portion of an IL-1RAcP protein.

61. The method of any one of claims 1-19, wherein the IL-33 antagonist is an IL-33 trap comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 327.

62. The method of any one of claims 1-19, or 61, wherein the IL-33 antagonist is an IL-33 trap comprising the amino acid sequence of SEQ ID NO: 327.

63. The method of any one of claims 1-62, wherein the IL-33 antagonist and the IL-4R antagonist are administered in separate formulations to a patient in need thereof.

64. The method of any one of claims 1-62, wherein the IL-33 antagonist and the IL-4R antagonist are co-formulated prior to administration to a patient in need thereof.

65. The method of any one of claims 1-64, wherein the IL-33 antagonist and the IL-4R antagonist are administered to the subject subcutaneously, intravenously, intramuscularly, or intranasally.

66. The method of any one of claims 1-65, wherein the IL-33 antagonist is a monoclonal antibody comprising three heavy chain complementarity determining regions (HCDRs) contained within a heavy chain variable region sequence of SEQ ID NO: 274 and three light chain complementarity determining regions (LCDRs) contained within a light chain variable region (LCVR) sequence of SEQ ID NO: 282 and wherein the IL-4R antagonist is a monoclonal antibody comprising three heavy chain complementarity determining regions (HCDRs) contained within a heavy chain variable region sequence of SEQ ID NO: 337 and three light chain complementarity determining regions (LCDRs) contained within a light chain variable region (LCVR) sequence of SEQ ID NO: 338.

67. The method of any one of claims 1-66, wherein the IL-33 antagonist is a monoclonal antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282 and wherein the IL-4R antagonist is a monoclonal antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 337/338.

68. The method of any one of claims 1-67, wherein the IL-33 antagonist is a monoclonal antibody comprising three HCDRs (HCDR1-HCDR2-HCDR3) having the amino acid sequences of SEQ ID NOs: 276-278-280, respectively and three LCDRs (LCDR1-LCDR2-LCDR3) having the amino acid sequences of SEQ ID NOs: 284-286-288, respectively and wherein the IL-4R antagonist is a monoclonal antibody comprising three HCDRs (HCDR1-HCDR2-HCDR3) having the amino acid sequences of SEQ ID NOs: 339-340-341, respectively and three LCDRs (LCDR1-LCDR2-LCDR3) having the amino acid sequences of SEQ ID NOs: 342-343-344, respectively.

69. The method of any one of claims 1-68, wherein the IL-33 antagonist is REGN3500 and wherein the IL-4R antagonist is dupilumab.

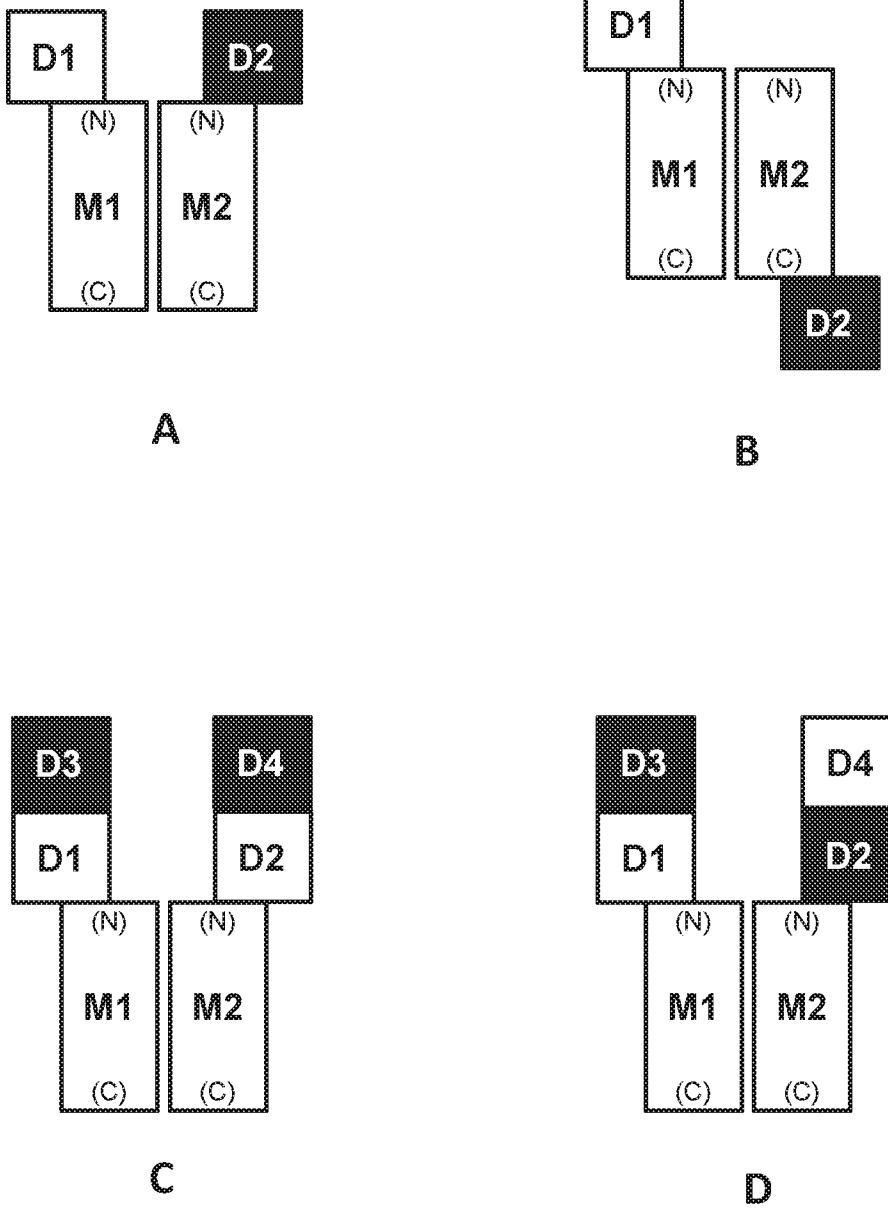
D1
D2
(N)
(N)
M1
M2
(C)
(C)
A
D1
(N)
(N)
M1
M2
(C)
(C)
D2
B
D3
D4
D1
D2
(N)
(N)
M1
M2
(C)
(C)
C
D3
D4
D1
D2
(N)
(N)
M1
M2
(C)
(C)
D

Figure 1

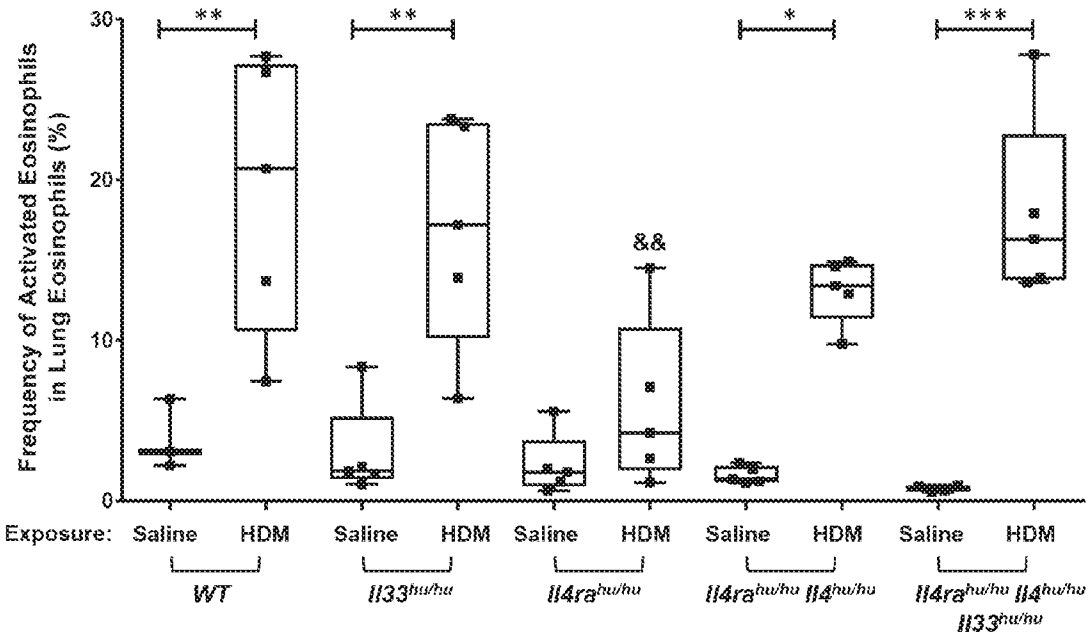
Frequency of Activated Eosinophils in Lung Eosinophils (%)
30
20
10
0
**
**
*
***
&&
Exposure:
Saline
HDM
Saline
HDM
Saline
HDM
Saline
HDM
Saline
HDM
WT
Il33hu/hu
Il4rahu/hu
Il4rahu/hu Il4hu/hu
Il4rahu/hu Il4hu/hu Il33hu/hu

Figure 2

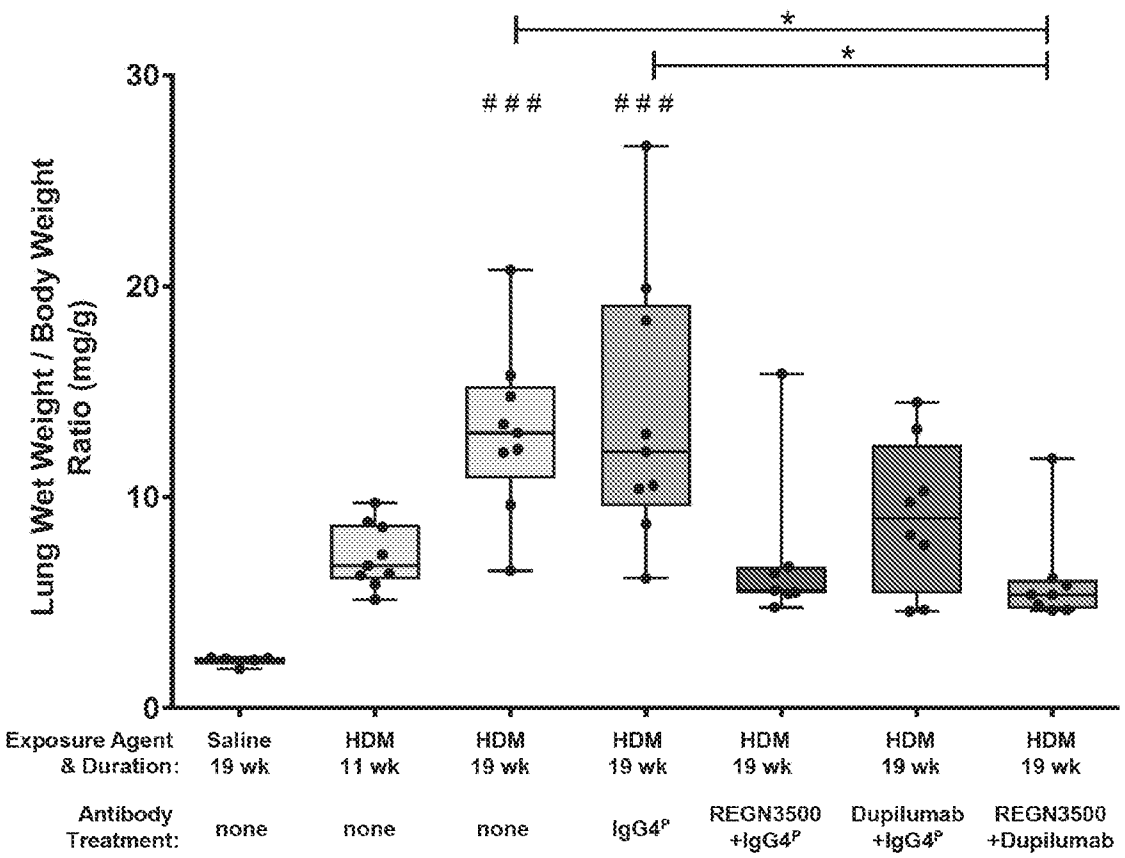
Lung Wet Weight / Body Weight Ratio (mg/g)
30
20
10
0
*
*
###
###
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 3

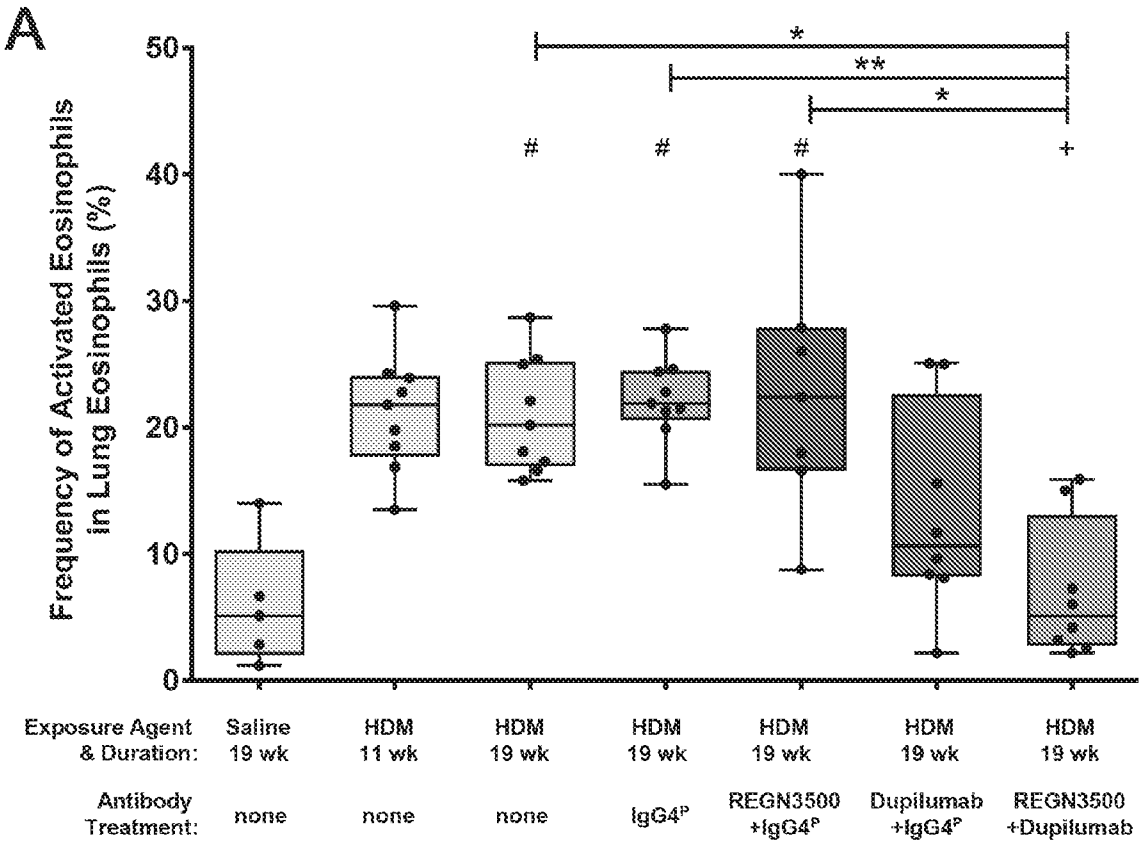
A
Frequency of Activated Eosinophils in Lung Eosinophils (%)
50
40
30
20
10
0
*
**
*
#
#
#
+
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 4A

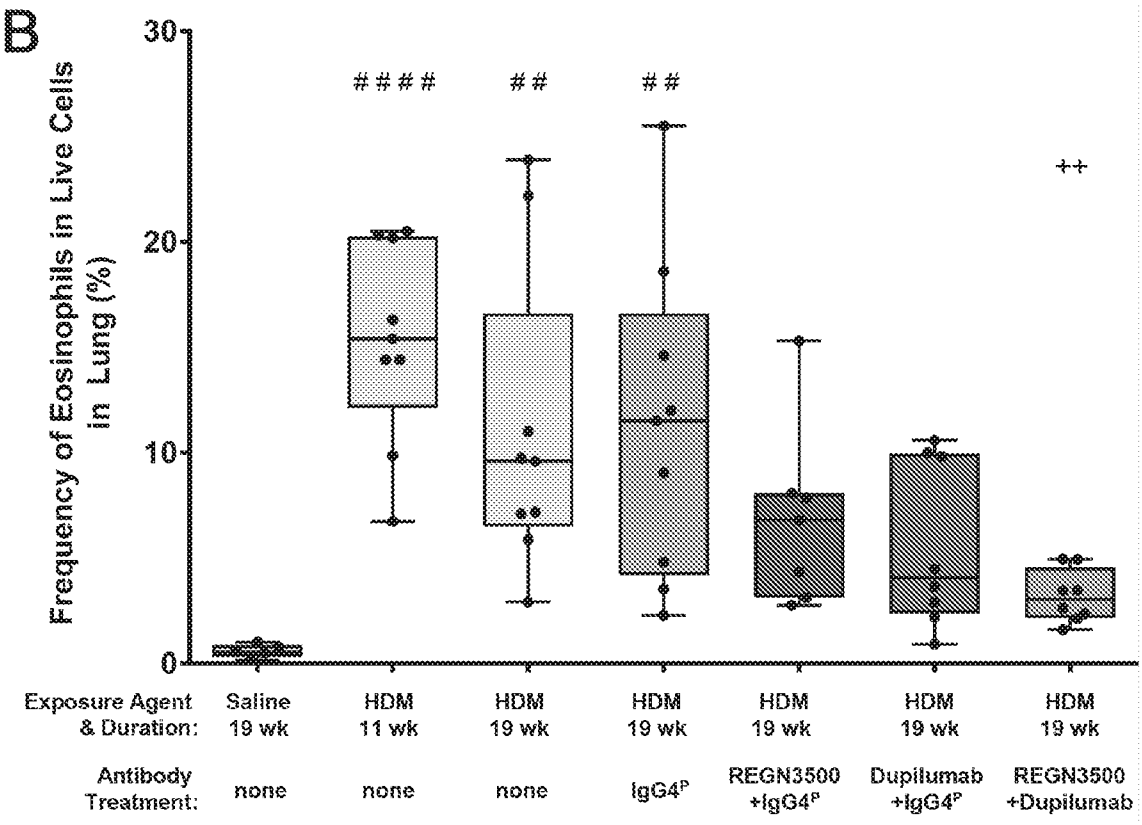
B
Frequency of Eosinophils in Live Cells in Lung (%)
30
20
10
0
####
##
##
++
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 4B

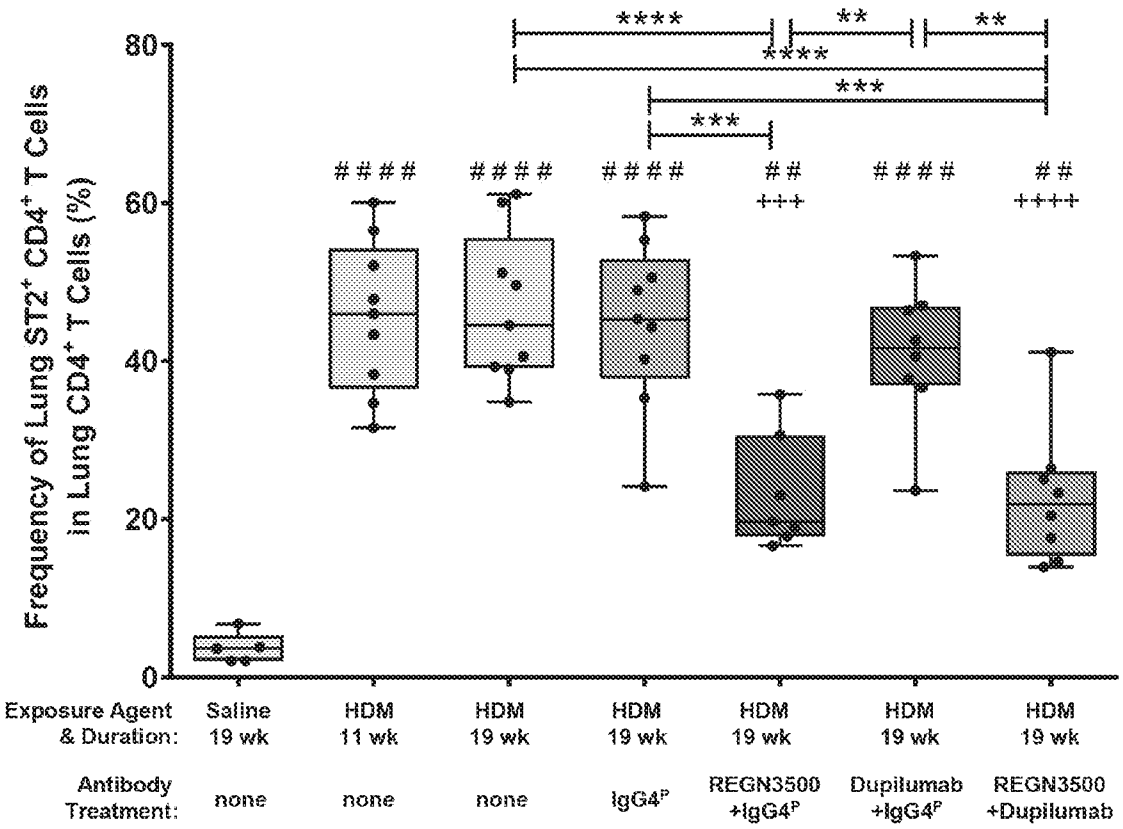
Frequency of Lung ST2+ CD4+ T Cells in Lung CD4+ T Cells (%)
80
60
40
20
0
****
**
**
****
***
***
####
####
####
##
+++
####
##
++++
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 5

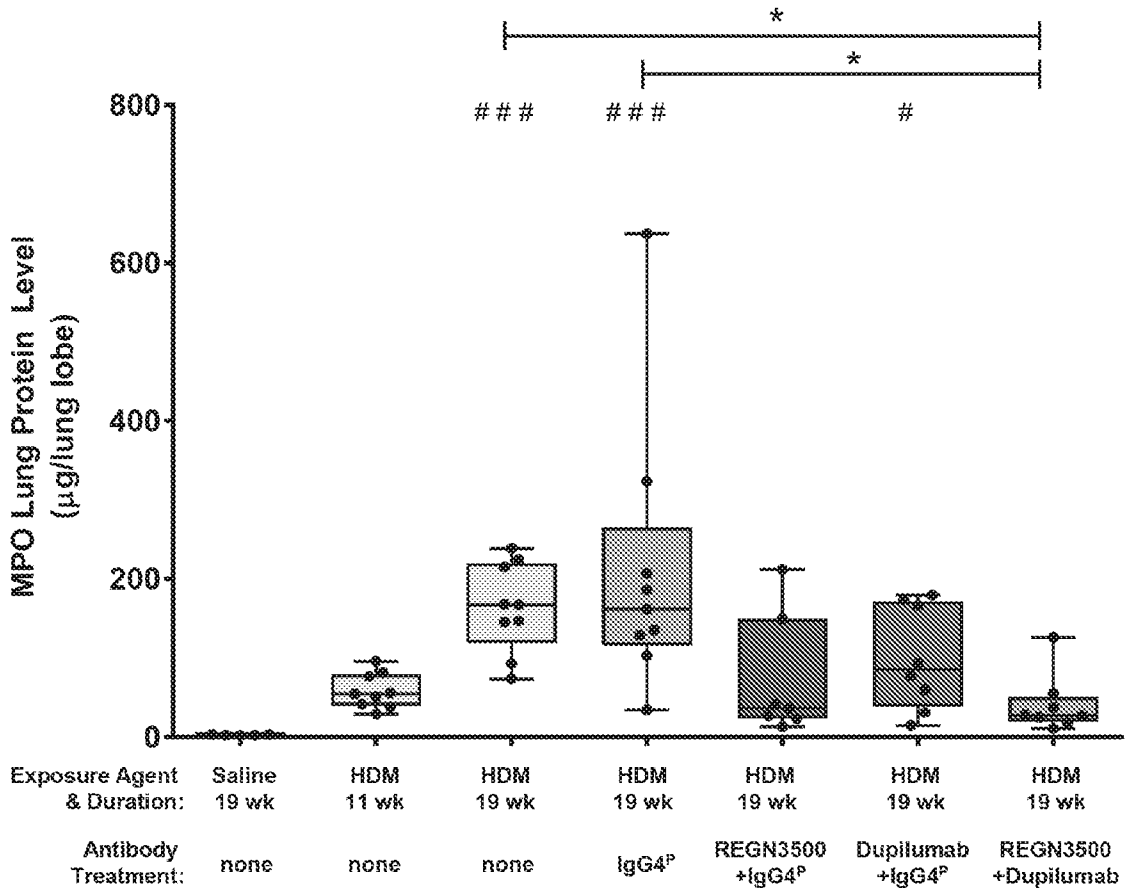
*
*
###
###
#
800
600
400
200
0
MPO Lung Protein Level
(µg/lung lobe)
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 6

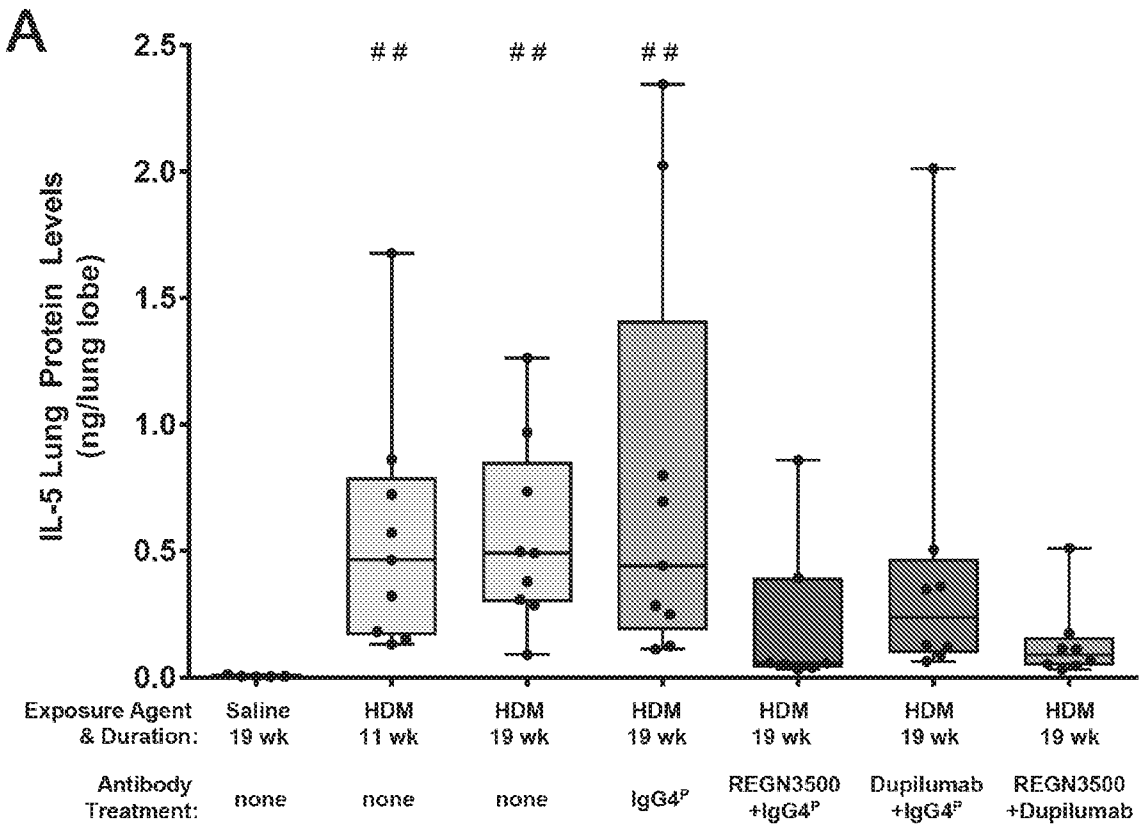
A
IL-5 Lung Protein Levels (ng/lung lobe)
2.5
2.0
1.5
1.0
0.5
0.0
# #
# #
# #
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 7A

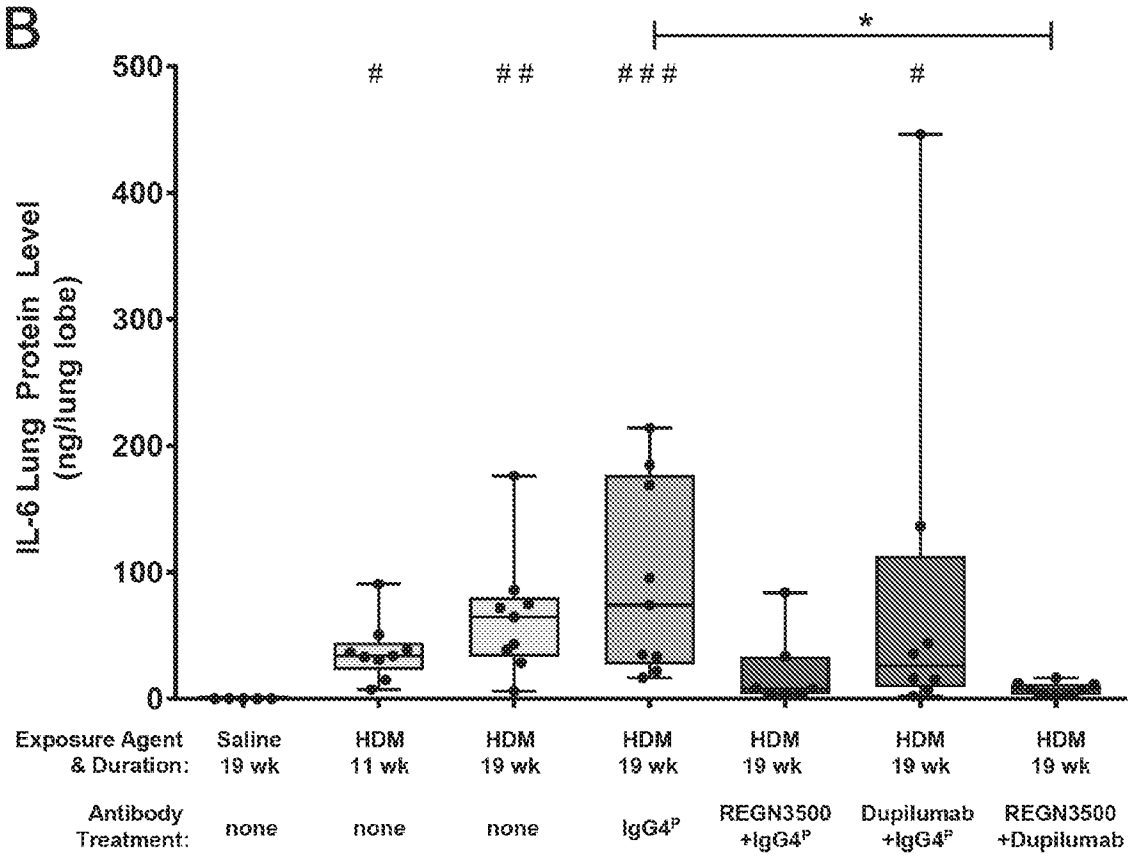
B
IL-6 Lung Protein Level (ng/lung lobe)
500
400
300
200
100
0
#
# #
# # #
#
*
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 7B

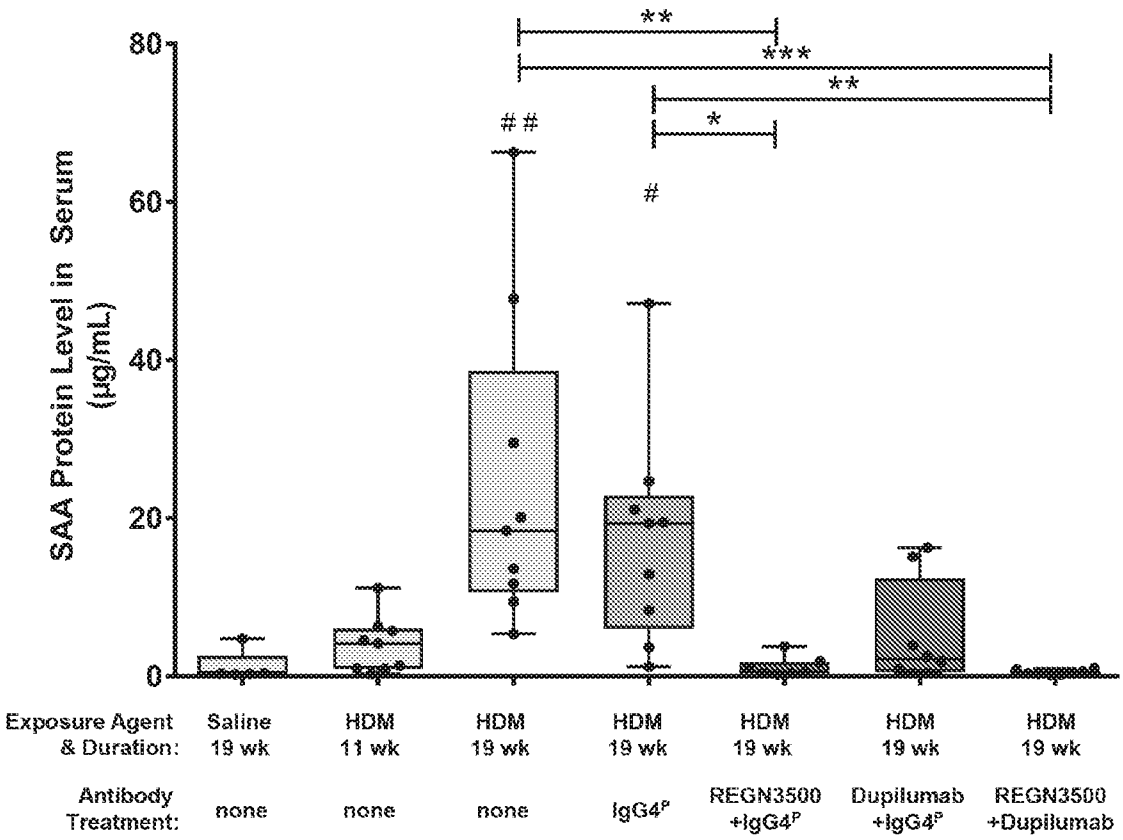
SAA Protein Level in Serum (µg/mL)
80
60
40
20
0
**
***
**
*
# #
#
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 8

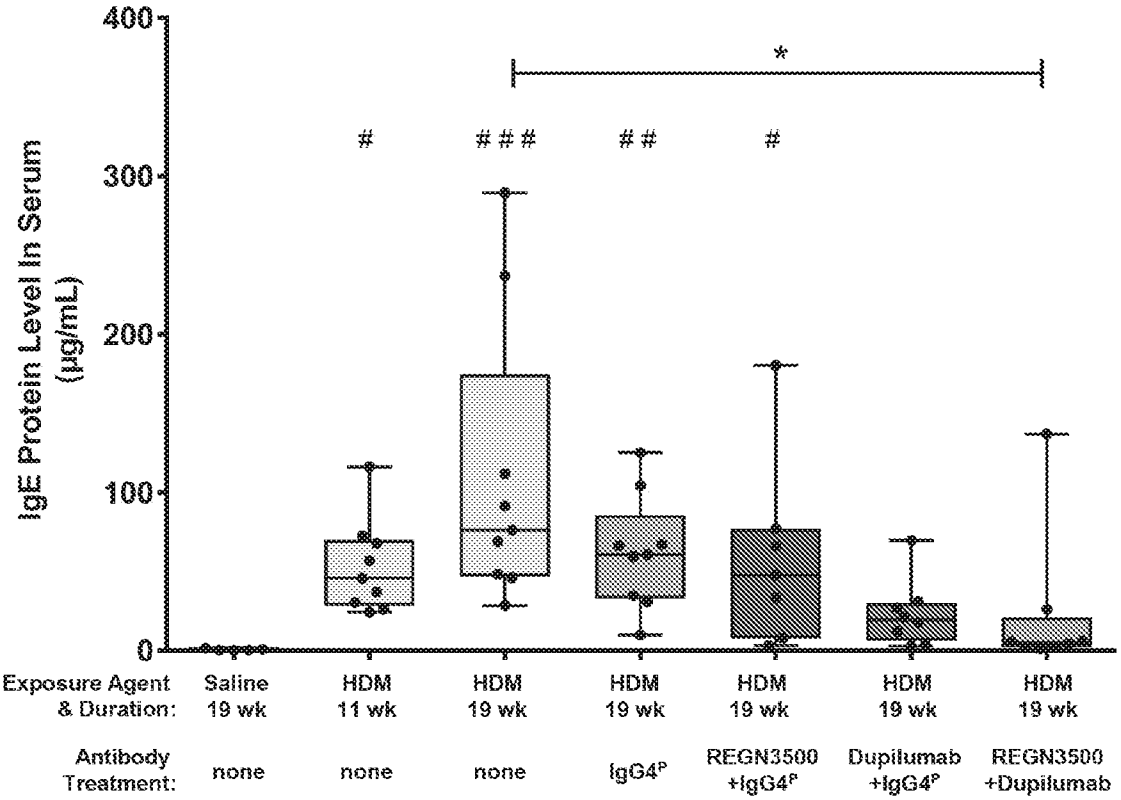
IgE Protein Level In Serum (µg/mL)
400
300
200
100
0
*
#
###
##
#
Exposure Agent & Duration:
Saline 19 wk
HDM 11 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
HDM 19 wk
Antibody Treatment:
none
none
none
IgG4P
REGN3500 +IgG4P
Dupilumab +IgG4P
REGN3500 +Dupilumab

Figure 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/064041

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 C07K16/28 A61K39/00 C07K14/715
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 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, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 027 015 A1 (REGENERON PHARMA [US]) 8 June 2016 (2016-06-08) paragraph [0157] - paragraph [0163] -----	1-69
X	US 2014/271642 A1 (MURPHY ANDREW J [US] ET AL) 18 September 2014 (2014-09-18) cited in the application paragraph [0102] claims 1-7, 25-48 -----	1-19, 41-62
X	US 2014/271658 A1 (MURPHY ANDREW J [US] ET AL) 18 September 2014 (2014-09-18) cited in the application paragraph [0133] - paragraph [0134] -----	1-19, 27-40, 66-68
X	US 2009/074793 A1 (MARTIN JOEL H [US] ET AL) 19 March 2009 (2009-03-19) paragraph [0063] - paragraph [0064] -----	1-26
	-/--	

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

[X] See patent family annex.

* Special categories of cited documents :

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"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
23 January 2018	09/02/2018

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	Rojo Romeo, Elena

2

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Anonymous: "Evaluation of SAR440340 and as Combination Therapy With Dupilumab in Moderate-to-Severe Asthma Patients - Full Text View - ClinicalTrials.gov", , 1 January 2018 (2018-01-01), XP055443191, Retrieved from the Internet: URL:https://clinicaltrials.gov/ct2/show/NCT03387852?sfpd_d=14 [retrieved on 2018-01-22] the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/064041

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		EP 2511300 A2	17-10-2012

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/064041

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