DIAGNOSIS AND TREATMENTS RELATING TO TH2 INHIBITION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of provisional U.S. Application No. 61/459,760 filed December 16, 2010, provisional U.S. Application No. 61/465,425 filed March 18, 2011, provisional U.S. Application No. 61/484,650 filed May 10, 2011, provisional U.S. Application No. 61/574,485 filed August 2, 2011, and provisional U.S. Application No. 61/557,295 filed November 8, 2011, all of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 17, 2011, is named P4569R1WO_PCTSequenceListing.txt and is 73,118 bytes in size.

FIELD

[0003] Methods of diagnosing and treating disorders related to TH2 inhibition, including but not limited to asthma, are provided. Also provided are methods of selecting or identifying patients for treatment with certain therapeutic agents that are TH2 pathway inhibitors.

BACKGROUND

[0004] Asthma is a complex disease with increasing worldwide incidence. Among other events, eosinophilic inflammation has been reported in the airways of asthma patients. The pathophysiology of the disease is characterized by variable airflow obstruction, airway inflammation, mucus hypersecretion, and subepithelial fibrosis. Clinically, patients may present with cough, wheezing, and shortness of breath. While many patients are adequately treated with currently available therapies, some patients with asthma have persistent disease despite the use of current therapies.

[0005] A plethora of drugs are on the market or in development for treating asthma. One of the numerous targets for asthma therapy is IL-13. IL-13 is a pleiotropic TH2 cytokine produced by activated T cells, NKT cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models. Despite the

many links between IL-13, IL-4 and asthma in the literature, many IL13 and/or IL4 antagonists therapies have had disappointing results in the clinic. Currently, no IL-13 or IL-4 antagonist therapy has been approved for use in asthma. Furthermore, moderate to severe asthmatic patients continue to lack good, alternative treatment options. Thus, there is a need to identify better therapies for treating asthma and improved methods for understanding how to treat asthma patients.

[0006] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety for any purpose.

SUMMARY

[0007] This application provides therapeutic agents for inhibiting the TH2 pathway and better methods of using the same. This application also provides better methods for diagnosing disease for use in treating the disease optionally with the TH2 pathway inhibitor.

[0008] The methods of treatment and diagnosis as provided herein can be applied to patients suffering from asthma, eosinophilic disorder, respiratory disorders, IL-13 mediated disorder and/or IgE-mediated disorder, or symptoms related to those disorders. Patients suffering from asthma-like symptoms, include patients that have not been diagnosed with asthma may be treated according to the methods provided herein.

[0009] According to one embodiment, a patient treated according to the methods provided herein suffers from asthma, an eosinophilic disorder, a respiratory disorder, an IL-13 mediated disorder and/or an IgE-mediated disorder, or symptoms related to those disorders, and does not have cancer or a neoplasm. According to another embodiment, the patient treated according to the methods provided herein is suffering from asthma, eosinophilic disorder, respiratory disorders, IL-13 mediated disorder and/or IgE-mediated disorder, or symptoms related to those disorders, and is 12 years old or older, 18 years old or older or 19 years old or older, or between 12-17 years old or between 18-75 years old.

[0010] In one embodiment, a patient treated with a TH2 pathway inhibitor according to this invention is also treated with one, two, three or more therapeutic agents. In one embodiment, the patient is an asthma patient. According to one embodiment, the patient is treated with the TH2 pathway inhibitor and one, two, three or more therapeutic agents, wherein at least one therapeutic agent, other than the TH2 inhibitor, is a corticosteroid, a leukotriene antagonist, a LABA, a corticosteroid/LABA combination composition, a theophylline, cromolyn sodium, nedocromil sodium, omalizumab, a LAMA, a MABA, a 5-

Lipoxygenase Activating Protein (FLAP) inhibitor, or an enzyme PDE-4 inhibitor. According to one aspect of the invention, a TH2 pathway inhibitor is administered to an asthma patient diagnosed as EIP status, wherein the diagnosis comprises the use of an EID assay (alone or in combination with other assays) to determine the EIP status. In one further embodiment, the asthma patient is uncontrolled on a corticosteroid prior to the treatment. In another embodiment, the asthma patient is also being treated with a second controller. In one embodiment, the second controller is a corticosteroid, a LABA or a leukotriene antagonist. In a further embodiment, the asthma patient is suffering from moderate to severe asthma. Thus, in one embodiment, the patient to be treated with the TH2 pathway inhibitor is a moderate to severe asthma patient who is uncontrolled on a corticosteroid prior to treatment with the TH2 pathway inhibitor, and then is treated with the TH2 pathway inhibitor and one, two, three or more controllers. In one embodiment, at least one of the controllers is a corticosteroid. In a further embodiment, such patient is treated with a TH2 pathway inhibitor, a corticosteroid and another controller. In another embodiment, the patient is suffering from mild asthma but is not being treated with a corticosteroid. It should be understood that the therapeutic agents may have different treatment cycles as compared with the TH2 inhibitor and, consequently can be administered at different times compared to the TH2 inhibitor as a part of the patient's treatment. Therefore, according to one embodiment, a method of treatment according to this invention comprises the steps of administering to a patient a TH2 pathway inhibitory and optionally, administering at least one, two or three additional therapeutic agents. In one embodiment, the TH2 pathway inhibitor is present in a composition with another therapeutic agent. In another embodiment, the TH2 pathway inhibitor is not present in a composition with another therapeutic agent.

[0011] According to another embodiment, the invention comprises a method for treating asthma comprising administering an anti-IL-13 antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10 or an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 as a flat dose. In one embodiment an anti-IL13 antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10 is administered as a 125-1000 mg flat dose (i.e., not weight dependent), by subcutaneous injection or by intravenous injection, at a frequency of time selected from: every 2 weeks, every 3 weeks, and every 4 weeks. In one embodiment an anti-IL13 antibody comprising

HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 is administered as a 125-1000 mg flat dose (i.e., not weight dependent), by subcutaneous injection or by intravenous injection, at a frequency of time selected from: every 2 weeks, every 3 weeks, and every 4 weeks. In one embodiment, the anti-IL13 antibody is lebrikizumab, which is administered as a 125-1000 mg flat dose (i.e., not weight dependent), by subcutaneous injection or by intravenous injection, at a frequency of time selected from: every 2 weeks, every 3 weeks, and every 4 weeks. In another embodiment, the patient is diagnosed with EIP status using a Total Periostin Assay to determine EIP status.

[0012] According to another embodiment, an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10 is administered to treat asthma in a therapeutically effective amount sufficient to reduce the rate of exacerbations of the patient over time or improve FEV1. In yet another embodiment, the invention comprises a method for treating asthma comprising administering an anti-IL-13 antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10 or an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 as a flat dose (i.e., not weight dependent) of 37.5 mg, or a flat dose of 125 mg, or a flat dose of 250 mg. In certain embodiments, the dose is administered by subcutaneous injection once every 4 weeks for a period of time. In certain embodiments, the period of time is 6 months, one year, two years, five years, ten years, 15 years, 20 years, or the lifetime of the patient. In certain embodiments, the asthma is severe asthma and the patient is inadequately controlled or uncontrolled on inhaled corticosteroids plus a second controller medication. In another embodiment, the patient is diagnosed with EIP status using a Total Periostin Assay to determine EIP status and the patient is selected for treatment with an anti-IL13 antibody as described above. In another embodiment, the method comprises treating an asthma patient with an anti-IL13 antibody as described above where the patient was previously diagnosed with EIP status using a Total Periostin Assay to determine EIP status. In one embodiment, the asthma patient is age 18 or older. In one embodiment, the asthma patient is age 12 to 17 and the anti-IL13 is administered in as a flat dose of 250 mg or a flat dose of 125 mg. In one embodiment, the

asthma patient is age 6 to 11 and the anti-IL13 antibody is administered in as a flat dose of 125 mg or a flat dose of 62.5 mg.

[0013] The present invention provides a periostin assay. In one embodiment, the periostin assay is a Total Periostin Assay. In another embodiment, the Total Periostin Assay comprises the use of one or more of the anti-periostin antibodies of this invention to bind to the Total Periostin in a biological sample obtained from a patient. In yet another embodiment of this invention, the biological sample is serum obtained from whole blood. In one embodiment, the biological sample is obtained from an asthma patient. In a further embodiment, the asthma patient is a moderate to severe asthma patient. In yet a further embodiment the moderate to severe asthma patient is uncontrolled on a corticosteroid and optionally, is being treated with one, two, three or more controllers.

[0014] The anti-periostin assays and antibody assays disclosed herein can be used for other diseases in which periostin is elevated such as idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), and cancer.

[0015] The present invention provides a therapeutic agent that is a TH2 pathway inhibitor for use in treating asthma or a respiratory disorder in a patient, wherein the patient expresses elevated levels of total periostin. In one embodiment, the target for inhibition in the TH2 pathway is selected from: IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33 and IgE; and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). In one embodiment, the patient to be treated according to the methods of the present invention is suffering from mild to severe asthma, optionally moderate to severe asthma, and whose asthma is uncontrolled on a corticosteroid. In a further embodiment, the serum level of Total Periostin in the moderate to severe asthmatic patient who is uncontrolled on a corticosteroid is greater than 20ng/ml, 21 ng/ml, 22 ng/ml, 23 ng/ml, 24ng/ml or 25ng/ml in a E4 Assay. In yet a further embodiment, the patient to be treated additionally has elevated expression levels of any one, combination or all CEA, TARC (CCL17) and MCP-4 (CCL13)mRNAs or proteins. In yet a further embodiment, the patient to be treated in addition to having elevated expression levels of periostin as described herein, has a FE_{NO} level greater than 21 ppb, or greater than 35 ppb.

[0016] The present invention provides the use of a therapeutic agent that binds a TH2 induced asthma pathway target in the preparation of a medicament for the treatment of a

patient having asthma or a respiratory disorder, wherein the patient expresses elevated levels of total periostin, and wherein the target is IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33 and IgE; and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI or FcepsilonRII/CD23 (receptors for IgE). In one embodiment, the patient is EIP. According to one embodiment, the patient was determined to have EIP by using an assay according to this invention. In yet another embodiment, the assay is a Total Periostin Assay. In another embodiment, the assay measures the level of Total Periostin in a biological sample obtained from the patient. In one embodiment, the assay measures the level of Total Periostin protein in the serum sample obtained from the patient.

[0017] The present invention comprises a kit or article for manufacture for diagnosing an asthma subtype in a patient comprising:

[0018] (1) determining the levels of Total Periostin in a serum sample obtained from the patient and optionally the protein expressions levels for one or more proteins selected from TARC and MCP-4; and

[0019] (2) instructions for measuring the expression levels of the Total Periostin and optionally the TARC and/or MCP-4 proteins in the serum sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype.

[0020] In yet another embodiment, methods of identifying an asthma patient or a respiratory disorder patient who is likely to be responsive to treatment with a TH2 Pathway Inhibitor are provided. In certain embodiments, the methods comprise determining whether the patient is Eosinophilic Inflammation Positive (EIP) using an Eosinophilic Inflammation Diagnostic Assay (EIDA), wherein the EIP status indicates that the patient is likely to be responsive to treatment with a TH2 Pathway Inhibitor.

[0021] In another embodiment, methods of identifying an asthma patient or a respiratory disorder patient who is likely to suffer from severe exacerbations are provided. In certain embodiments, the methods comprise determining whether the patient is EIP using an EIDA, wherein the EIP status indicates that the patient is likely to suffer from an increase in severe exacerbations.

[0022] In yet still another embodiment, methods of identifying an asthma patient or a respiratory disorder patient who is less likely to be responsive to treatment with a TH2

Pathway Inhibitor are provided. In certain embodiments, the methods comprise determining whether the patient is Eosinophilic Inflammation Negative (EIN) using an EIDA, wherein the EIN status indicates that the patient is less likely to be responsive to treatment with the TH2 Pathway Inhibitor.

[0023] In another embodiment, methods of monitoring an asthma patient being treated with a TH2 Pathway inhibitor are provided. In certain embodiments, the methods comprise determining whether the patient is EIP or EIN using an EIDA. In one embodiment, the method comprises determining a treatment regimen for the TH2 Pathway Inhibitor. In one embodiment, the determination of EIP indicates continuing therapy with the TH2 Pathway Inhibitor and the determination of EIN indicates discontinuing therapy with the TH2 Pathway Inhibitor.

[0024] In certain embodiments, the EIDA used in methods described above comprises the steps of: (a) determining the amount of Total Periostin in a sample obtained from an asthma patient; (b) comparing the amount of Total Periostin determined in step (a) to a reference amount; and (c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step (b). In certain embodiments, the Total Periostin is serum periostin, which periostin is a measured using an immunoassay. In certain embodiments, the immunoassay is a sandwich immunoassay. In certain embodiments, the sandwich immunoassay is performed by an Elecsys® analyzer (Roche Diagnostics GmbH). In certain embodiments, the sandwich immunoassay is an E4 Assay. In one embodiment, the reference amount for EIP is 23 ng/ml greater when using the E4 Assay in step (a). In one embodiment, the reference amount for EIP is 50 ng/ml or greater when using the Elecsys® analyzer in step (a). In one embodiment, the reference amount for EIN is 21 ng/ml or lower when using the E4 Assay in step (a). In one embodiment, the reference amount for EIN is 48 ng/ml or lower when using the Elecsys® analyzer in step (a).

[0025] In certain embodiments, the patient according to the methods described above is suffering from moderate to severe asthma. In certain embodiments, the asthma or respiratory disorder is uncontrolled on a corticosteroid. In certain embodiments, the corticosteroid is an inhaled corticosteroid. In certain embodiments, the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®. In one embodiment, the patient is also being treated with a second controller. In certain embodiments, the second controller is a long acting bronchial dilator (LABD). In certain embodiments, the LABD is a long-acting beta-2 agonist (LABA), leukotriene receptor

antagonist (LTRA), long-acting muscarinic antagonist (LAMA), theophylline, or oral corticosteroids (OCS). In certain embodiments, the LABD is Symbicort®, Advair®, Brovana®, Foradil®, Perforomist™ or Serevent®.

[0026] In certain embodiments, the TH2 Pathway Inhibitor according to the methods above inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935), or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8). In certain embodiments, the TH2 Pathway Inhibitor is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent. In certain embodiments, the TH2 Pathway Inhibitor is an anti- anti-IL-13 antibody. In certain embodiments, the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab. In certain embodiments, the anti-IL-13 antibody is a bispecific antibody that also binds IL-4. In certain embodiments, the TH2 Pathway Inhibitor is an anti-IgE antibody. In certain embodiments, the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti- M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-

H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25 [SQNTLVPWT].

[0027] In another aspect, uses of a kit for detecting Total Periostin in a sample obtained from an asthma patient for stratifying/classifying asthma patients into likely responders and non-responders for therapeutic treatment with a TH2 Pathway Inhibitor. In certain embodiments, the Total Periostin is detected using an EIDA, which EIDA comprises the steps of: (a) determining the amount of Total Periostin in a sample obtained from an asthma patient; (b) comparing the amount of Total Periostin determined in step (a) to a reference amount; and (c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step (b). In certain embodiments, the Total Periostin is serum periostin, which periostin is a measured using an immunoassay. In certain embodiments, the immunoassay is a sandwich immunoassay. In certain embodiments, the sandwich immunoassay is performed by an Elecsys® analyzer (Roche Diagnostics GmbH). In certain embodiments, the sandwich immunoassay is an E4 Assay. In one embodiment, the reference amount for EIP is 23 ng/ml greater when using the E4 Assay in step (a). In one embodiment, the reference amount for EIP is 50 ng/ml or greater when using the Elecsys® analyzer in step (a).

[0028] In certain embodiments, the patient according to the uses described in the paragraph above is suffering from moderate to severe asthma. In certain embodiments, the asthma or respiratory disorder is uncontrolled on a corticosteroid. In certain embodiments, the corticosteroid is an inhaled corticosteroid. In certain embodiments, the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®. In one embodiment, the patient is also being treated with a second controller. In certain embodiments, the second controller is a long acting bronchial dilator (LABD). In certain embodiments, the LABD is a long-acting beta-2 agonist (LABA), leukotriene receptor antagonist (LTRA), long-acting muscarinic antagonist (LAMA), theophylline, or oral corticosteroids (OCS). In certain embodiments, the LABD is Symbicort®, Advair®, Brovana®, Foradil®, Perforomist™ or Serevent®.

[0029] In certain embodiments, the TH2 Pathway Inhibitor according to the uses above inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as,

anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935), or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8). In certain embodiments, the TH2 Pathway Inhibitor is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent. In certain embodiments, the TH2 Pathway Inhibitor is an anti- anti-IL-13 antibody. In certain embodiments, the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab. In certain embodiments, the anti-IL-13 antibody is a bispecific antibody that also binds IL-4. In certain embodiments, the TH2 Pathway Inhibitor is an anti-IgE antibody. In certain embodiments, the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti- M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25 [SQNTLVPWT].

[0030] In yet another aspect, kits for measuring the Total Periostin in a biological sample obtained from an asthma patient or a patient suffering from a respiratory disorder are

provided, wherein the kit comprises a first nucleic acid molecule that hybridizes to a second nucleic acid molecule, wherein the second nucleic acid molecule encodes Total Periostin or a portion thereof, or the kit comprises an antibody that binds to Total Periostin. In certain embodiments, the kit comprises a package insert containing information describing the uses provided above.

[0031] In still yet another aspect, kits for diagnosing an asthma subtype in a patient are provided, the kits comprising: (1) determining the levels of Total Periostin in a serum sample obtained from the patient and optionally the protein expressions levels in the serum sample for one or more proteins selected from TARC and MCP-4; and (2) instructions for measuring the levels of the Total Periostin and optionally TARC and/or MCP-4 in the serum sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype. In certain embodiments, the kit further comprises a package insert for determining whether an asthma patient or respiratory disorder patient is EIP or EIN. In certain embodiments, the kit further comprises a package insert for determining whether an asthma patient is likely to respond to a TH2 Pathway Inhibitor. In certain embodiments, the kit further comprises a package insert containing information describing any of the uses provided above. In certain embodiments, the kit further comprises an empty container to hold a biological sample. In certain embodiments, the kit comprises two anti-periostin antibodies for use in an immunoassay for determining Total Periostin levels.

[0032] In another aspect, methods of treating an asthma or a respiratory disorder comprising administering an anti-IL-13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 to a patient suffering from asthma or a respiratory disorder in a 125-500mg flat dose every 2-8 weeks. In certain embodiments, the patient is suffering from moderate to severe asthma. In certain embodiments, the asthma or respiratory disorder is uncontrolled on a corticosteroid. In certain embodiments, the asthma or respiratory disorder is uncontrolled on an inhaled corticosteroid. In certain embodiments, the asthma or respiratory disorder is uncontrolled on a total daily dose of at least 500 mcg fluticasone propionate (FP). In certain embodiments, the corticosteroid is an inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair®, Azmacort®. In certain embodiments, the patient is being treated with a second controller. In certain embodiments, the patient is continuing to be treated with a corticosteroid, optionally an inhaled

corticosteroid, during the treatment with the anti-IL13 antibody. In certain embodiments, the patient is continuing to be treated with a second controller during the treatment with the anti-IL-13 antibody. In certain embodiments, the second controller is a long acting bronchial dilator. In certain embodiments, the long acting bronchial dilator is a LABA, LTRA, LAMA, theophylline, or OCS. In certain embodiments, the patient has been determined to be EIP. In certain embodiments, the patient has been determined to be EIP using a kit a described above. In certain embodiments, the patient has been determined to be EIP using a method as described above. In certain embodiments, the patient is administered a flat dose of 125 mg or 250mg every four weeks. In certain embodiments, the patient is 18 years or older, or the patient is 12-17 years old or 12 years old and older, or the patient is 6-11 years old or 6 years old and older.

[0033] In certain embodiments, the anti-IL-13 antibody is administered subcutaneously. In certain embodiments, the anti-IL-13 antibody is administered using a prefilled syringe or autoinjector device. In certain embodiments, the asthma patient to be treated according to the methods above is 18 years old or older and has serum periostin at $\geq$ 50 ng/mL and is uncontrolled on an inhaled corticosteroid and a second controller medication. In certain embodiments, the serum periostin is measured using an immunoassay, which immunoassay is selected from Elecsys® periostin assay and E4 Assay. In certain embodiments, the serum periostin is measured using a kit as described above. In certain embodiments, the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and a VL comprising SEQ ID NO:10. In certain embodiments, the anti-IL-13 antibody is lebrikizumab. In certain embodiments, the asthma patient to be treated according to the methods described above is 12 years old and above and uncontrolled on an inhaled corticosteroid and a second controller medication.

[0034] In yet another aspect, methods of treating asthma or a respiratory disease comprising administering a therapeutically effective amount of lebrikizumab to the patient are provided. In certain embodiments, the treatment results in a relative improvement in FEV1 of greater than 5% compared to before treatment with lebrikizumab. In certain embodiments, the relative improvement in FEV1 is greater than 8% compared to before treatment with lebrikizumab. In certain embodiments, the treatment results in a reduction in severe exacerbations.

[0035] In still another aspect, methods of treating of a patient suffering from asthma or a respiratory disease comprising administering a TH2 Pathway Inhibitor to the patient

diagnosed as EIP are provided. In certain embodiments, the methods comprise the step of diagnosing the patient as EIP using a Total Periostin assay. In certain embodiments, the methods further comprise the step of retreating the patient with the TH2 Pathway Inhibitor if the patient is determined to be EIP. In certain embodiments, serum from the patient is used to determine whether the patient is EIP.

[0036] In certain embodiments, the EIP status determined according to the methods above uses an EIDA comprising the steps of: (a) determining the amount of Total Periostin in a sample obtained from the patient; (b) comparing the amount of Total Periostin determined in step (a) to a reference amount; and (c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step (b). In certain embodiments, the Total Periostin is serum periostin, which periostin is a measured using an immunoassay. In certain embodiments, the immunoassay is a sandwich immunoassay. In certain embodiments, the sandwich immunoassay is performed by an Elecsys® analyzer (Roche Diagnostics GmbH). In certain embodiments, the sandwich immunoassay is an E4 Assay. In one embodiment, the reference amount for EIP is 23 ng/ml greater when using the E4 Assay in step (a). In one embodiment, the reference amount for EIP is 50 ng/ml or greater when using the Elecsys® analyzer in step (a).

[0037] In certain embodiments, the patient according to the methods described above is suffering from moderate to severe asthma. In certain embodiments, the asthma or respiratory disorder is uncontrolled on a corticosteroid. In certain embodiments, the corticosteroid is an inhaled corticosteroid. In certain embodiments, the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®. In one embodiment, the patient is also being treated with a second controller. In certain embodiments, the second controller is a long acting bronchial dilator (LABD). In certain embodiments, the LABD is a long-acting beta-2 agonist (LABA), leukotriene receptor antagonist (LTRA), long-acting muscarinic antagonist (LAMA), theophylline, or oral corticosteroids (OCS). In certain embodiments, the LABD is Symbicort®, Advair®, Brovana®, Foradil®, Perforomist™ or Serevent®.

[0038] In certain embodiments, the TH2 Pathway Inhibitor according to the methods above inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized

13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR®, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935), or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8). In certain embodiments, the TH2 Pathway Inhibitor is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent. In certain embodiments, the TH2 Pathway Inhibitor is an anti- anti-IL-13 antibody. In certain embodiments, the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, the respective HVRs having the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab. In certain embodiments, the anti-IL-13 antibody is a bispecific antibody that also binds IL-4.

[0039] In certain embodiments, the TH2 Pathway Inhibitor is an anti-IgE antibody. In certain embodiments, the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25 [SQNTLVPWT].

[0040] In another aspect, methods for evaluating adverse events in a patient associated with treatment of asthma with lebrikizumab are provided. In certain embodiments, the methods comprise the steps of monitoring the number and/or severity of events that are exacerbations, community-acquired pneumonia, anaphylaxis, musculoskeletal pains,

musculoskeletal disorders, connective tissue pains or connective tissue disorders. In certain embodiments, the musculoskeletal or connective tissue disorder is arthralgia, back pain, pain in extremity, myalgia, neck pain, arthritis, bone development abnormalities, bursitis, costochondritis, exostosis, flank pain, musculoskeletal chest pain, musculoskeletal pain, pain in jaw or tendinitis.

[0041] In yet another aspect, anti-periostin antibodies are provided. In certain embodiments, the anti-periostin antibody comprises the HVR sequences of SEQ ID NO:1 and the HVR sequences of SEQ ID NO:2. In certain embodiments, the anti-periostin antibody comprises the sequences of SEQ ID NO:1 and SEQ ID NO:2. In certain embodiments, the anti-periostin antibody comprises the HVR sequences of SEQ ID NO:3 and the HVR sequences of SEQ ID NO:4. In certain embodiments, the anti-periostin antibody comprises the sequences of SEQ ID NO:3 and SEQ ID NO:4. In certain embodiments, Total Periostin Assays comprising the use of the above anti-periostin antibodies are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figure 1 provides a schematic of the allergen challenge trial described in Example 1.

[0043] Figure 2 shows allergen-induced changes in FEV1 following challenges in screening (A) and at week 13 (B). FEV1 was measured every 10 minutes for the first 90minutes and then every hour from 2-8 hours following allergen challenge. Error bars represent standard errors of the mean.

[0044] Figure 3 shows serum levels of IgE, CCL13 (MCP-4), and CCL17 (TARC). (A) Serum levels expressed as % predose levels over time in placebo-treated patients and (B) lebrikizumab-treated patients. Lines represent individual patients; groups medians not indicated. Arrows indicated dosing at weeks 0, 4, 8, and 12 (days 0, 28, 56 and 84). (C) Reductions in IgE, CCL13, and CCL17 at Week 13 in individual patients relative to baseline levels of those markers.

[0045] Figure 4 shows lebrikizumab-induced inhibition of the late asthmatic response (LAR) in biomarker-high and biomarker-low patient subgroups. Data are expressed as placebo-corrected mean reduction in AUC (area under the curve) of the LAR at Week 13 (n=5 to 8 active patients/group).

[0046] Figure 5 provides is a schematic of an asthma trial described in Example 2.

[0047] Figure 6 provides the baseline characteristics of patients participating in the asthma trial of Example 2.

[0048] Figure 7 provides results from the asthma trial of Example 2.

[0049] Figure 8 provides FEV1 results from the asthma trial of Example 2 for all efficacy evaluable subjects (A) and for periostin high subjects only (B).

[0050] Figure 9 provides rates of reduction in exacerbations from the asthma trial of Example 2.

[0051] Figure 10 provides percent change of FE_{NO} from the asthma trial of Example 2.

[0052] Figure 11 provides safety results from the asthma trial of Example 2.

[0053] Figure 12 provides is a schematic of an asthma trial described in Example 3.

[0054] Figure 13 provides a schematic of an asthma observational study as described in Example 5.

[0055] Figure 14 shows intra-subject correlation between serum periostin levels across multiple visits in the BOBCAT cohort as described in Example 5. (A) correlation between visit 1 and vist 2; (B) correlation between visit 1 and visit 3; (C) correlation between visit 2 and visit 3; (D) correlation between visit 1 and the mean serum periostin level across all visits; (E) correlation between visit 2 and the mean serum periostin level across all visits; and (F) correlation between visit 3 and the mean serum periostin level across all visits.

[0056] Figure 15 shows that FE_{NO} differentiates moderate-to-severe uncontrolled asthmatics on high-dose ICS according to airway eosinophilic inflammation (BOBCAT cohort) as described in Example 5. (A) Asthmatics with > 3% sputum eosinophils had significantly elevated FE_{NO} compared to asthmatics with < 3% sputum eosinophils ($p<0.001$ by Wilcoxon rank-sum test). (B) Asthmatics with > 22 eosinophils/mm2 total bronchial tissue had a trend for elevated FE_{NO} compared to asthmatics with < 22 eosinophils/mm2 total bronchial tissue ($p=0.07$ by Wilcoxon rank-sum test). (C) A composite airway eosinophil score where 0 = sputum eosinophils <3% AND tissue ("Bx") eosinophils < 22/mm2; 1 = EITHER sputum eosinophils >3% OR tissue eosinophils > 22/mm2 (exclusive); 2 = BOTH sputum eosinophils >3% AND tissue eosinophils > 22/mm2 demonstrated a strong positive trend for increasing FE_{NO} levels with increasing composite airway eosinophil score ($p=0.001$ by logistic regression). Serum periostin status is indicated as in the legends. (D) Serum periostin and FE_{NO} were both elevated in most subjects with elevated sputum or tissue eosinophils, but subsets of subjects had elevation of only periostin or FE_{NO}. Most subjects lacking elevated sputum AND tissue eosinophils exhibited low serum periostin and low

FE_{NO}. (E) Receiver operating characteristic (ROC) curve analysis of the sensitivity and specificity of serum periostin, FE_{NO}, blood eosinophils, and serum IgE for composite airway eosinophil status. AUC = area under the curve.

[0057] Figure 16 provides the percentage of patients with no treatment failure in the asthma trial described in Example 3.

[0058] Figure 17 shows a comparison between serum periostin levels as measured on assays similar to the Example 4 Assay (E4 Assay) or the Elecsys® periostin assay (Example 7) for samples obtained from the clinical trials described in Example 3 and Example 5.

[0059] Figure 18 shows serum periostin pharmacodynamics in periostin-low patients (A) and in periostin-high patients (B) as described in Example 2.

[0060] Figure 19 shows the percent change in median periostin levels over time in placebo and lebrikizumab-treated patients as described in Example 2.

[0061] Figure 20 shows the correlation between % change in FEV1 at week 12 and body weight for individuals in the Phase II study described in Example 2 (A) and the Phase II study described in Example 3 (B) as described in Example 6.

[0062] Figure 21 shows the predicted proportion of patients with steady-state trough concentrations above various levels as described in Example 6.

[0063] Figure 22 shows the effect of lebrikizumab on serum CCL17 (TARC) levels over time for the Example 2 study (A) and for the Example 3 study (B) as described in Example 6.

[0064] Figure 23 shows the simulated population PK profiles at each of the Phase III doses, 250 mg every 4 weeks, 125 mg every 4 weeks, and 37.5 mg every 4 weeks as described in Example 6.

DETAILED DESCRIPTION

[0065] Unless defined otherwise, 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. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

[0066] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice

versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0067] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

[0068] The term "Total Periostin" as used herein refers to at least isoforms 1, 2, 3 and 4 of periostin. Human periostin isoforms 1, 2, 3 and 4 are known in the art as comprising the following amino acid sequences: NP_006466.2 (SEQ ID NO:19); NP_001129406.1 (SEQ ID NO:20), NP_001129407.1 (SEQ ID NO:21), and NP_001129408.1 (SEQ ID NO:22), respectively, according to the NCBI database. In addition, applicants have detected an additional form of periostin. This new isoform is referred to herein as "isoform 5" and has been partially sequenced. Isoform 5 comprises the amino acid sequence of SEQ ID NO:23. In one embodiment, the isoforms of periostin are human periostins. In a further embodiment, the term Total Periostin includes isoform 5 of human periostin in addition to isoforms 1-4. In another embodiment, Total Periostin is Total Serum Periostin or Total Plasma Periostin (i.e., Total Periostin from a serum sample obtained from whole blood or a plasma sample obtained from whole blood, respectively, the whole blood obtained from a patient).

[0069] The term "Total Periostin Assay" refers to an assay that measures the levels of Total Periostin in a biological sample. In one embodiment, the Total Periostin levels are measured using anti-periostin antibodies. In another embodiment, the anti-periostin antibodies are the anti-periostin antibodies described herein. In another example, the Total Periostin Levels are measured using one or more nucleic acid sequences antisense to mRNA encoding periostin isoforms 1-4. In yet another example, the Total Periostin Assay is the assay described in Example 4 ("Example 4 Assay" or "E4 Assay"). In one embodiment, the Total Periostin Assay comprises the use of (1) an antibody comprising the sequences SEQ ID NO:1 and SEQ ID NO:2 (the "25D4" antibody) and/or an antibody comprising the sequences of SEQ ID NO:3 and SEQ ID NO:4 (the "23B9" antibody) to bind periostin in a biological sample, (2) an antibody comprising the variable region sequences SEQ ID NO:1 and SEQ ID NO:2 and/or an antibody comprising the variable region sequences of SEQ ID NO:3 and SEQ ID NO:4 to bind periostin in a biological sample, (3) an antibody comprising the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 and/or an antibody comprising the HVR sequences of SEQ ID NO:3 and SEQ ID NO:4 to bind periostin in a biological sample, (4) an

antibody comprising the HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 and/or an antibody comprising HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:3 and SEQ ID NO:4.

[0070] As used herein, "Eosinophilic Inflammation Diagnostic Assay," abbreviated "EIDA" is an assay that diagnoses a patient having eosinophilic inflammation in the body or TH2 pathway inflammation in the body by measuring levels of an eosinophilic inflammation marker in a biological sample from a patient, wherein the marker is selected from the group consisting of Periostin mRNA levels or Periostin protein levels, iNOS mRNA levels or iNOS protein levels or FE_{NO} levels or CCL26 mRNA or CCL26 protein levels, serpinB2 mRNA levels or serpinB2 protein levels, serpinB4 mRNA levels or serpinB4 protein levels, CST1 mRNA levels or CST1 protein levels, CST2 mRNA levels or CST2 protein levels, CST4 mRNA levels or CST4 protein levels. In one embodiment, Total Periostin serum or plasma levels are measured. Highly effective examples of assays include, but are not limited to, the example described in Example 4 below (also referred to as the E4 Assay) , or other periostin assays that measure serum or plasma levels of Total Periostin in a biological sample. Two or more assays can be conducted to make a diagnosis of eosinophilic inflammation in a patient. In one embodiment, the EID assay comprises a Total Periostin Assay in combination with a FE_{NO} assay. In another embodiment, the EID assay comprises a Total Periostin Assay +/- FE_{NO} Levels assay in combination with an assay measuring the levels of any one or combination of the following markers: CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10.

[0071] The term "periostin antibody" or "anti-periostin antibody" refers to an antibody that binds to an isoform of periostin. In one embodiment, the periostin is human periostin. In one embodiment, the antibody comprises the sequences SEQ ID NO:1 and SEQ ID NO:2 (the "25D4" antibody) or comprises the sequences of SEQ ID NO:3 and SEQ ID NO:4 (the "23B9" antibody). In another embodiment, the antibody comprises the variable region sequences of SEQ ID NO:1 and SEQ ID NO:2 or comprises the variable region sequences of SEQ ID NO:3 and SEQ ID NO:4. In another embodiment, the antibody comprising the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 or the HVR sequences of SEQ ID NO:3 and SEQ ID NO: In another embodiment, the antibody comprises the HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 and/or an

antibody comprising HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:3 and SEQ ID NO:4.

[0072] Eosinophilic Inflammation Positive (EIP) Patient or Status: refers to a patient who, if a serum or plasma sample from that patient had been tested for serum or plasma periostin levels, respectively, using the E4 Assay (Example 4), would have Total Serum Periostin levels of 20ng/ml or higher (Eosinophilic Positive). According to one embodiment, the Total Periostin levels in a patient who is EIP can be selected from the group consisting of 21ng/ml or higher, 22ng/ml or higher, 23ng/ml or higher, 24ng/ml or higher, 25ng/ml or higher, 26ng/ml or higher, 27ng/ml or higher, 28ng/ml or higher, 29ng/ml or higher, 30ng/ml or higher, 31ng/ml or higher, 32ng/ml or higher, 33ng/ml or higher, 34ng/ml or higher, 35ng/ml or higher, 36ng/ml or higher, 37ng/ml or higher, 38ng/ml or higher, 39ng/ml or higher, 40ng/ml or higher, 41ng/ml or higher, 42ng/ml or higher, 43ng/ml or higher, 44ng/ml or higher, 45ng/ml or higher, 46ng/ml or higher, 47ng/ml or higher, 48ng/ml or higher, 49ng/ml or higher, 50ng/ml or higher, 51ng/ml or higher, 52ng/ml or higher, 53ng/ml or higher, 54ng/ml or higher, 55ng/ml or higher, 56ng/ml or higher, 57ng/ml or higher, 58ng/ml or higher, 59ng/ml or higher, 60ng/ml or higher, 61ng/ml or higher, 62ng/ml or higher, 63ng/ml or higher, 64ng/ml or higher, 65ng/ml or higher, 66ng/ml or higher, 67ng/ml or higher, 68ng/ml or higher, 69ng/ml or higher and 70ng/ml or higher in the serum or plasma. It should be understood that the EIP Status represents the state of the patient, and is not dependent on the type of assay used to determine the status. Thus, other Eosinophilic Inflammation Diagnostic Assays, including other periostin assays such as the Elecsys® periostin assay shown in Example 7, can be used or developed to be used to test for Eosinophilic Inflammation Positive status.

[0073] Eosinophilic Inflammation Negative (EIN) Patient or Status refers to a patient who, if a serum or plasma sample from that patient had been tested for serum or plasma periostin levels, respectively, using the E4 Assay, would have Total Serum Periostin levels less than 20ng/ml. It should be understood that the EIN Status represents the state of the patient, and is not dependent on the type of assay used to determine the status. Thus, other Eosinophilic Inflammation Diagnostic Assays, including other periostin assays such as the Elecsys® periostin assay shown in Example 7, can be used or developed to be used to test for Eosinophilic Inflammation Negative status.

[0074] The term "biological sample" as used herein includes, but is not limited to, blood, serum, plasma, sputum, tissue biopsies (e.g., lung samples), and nasal samples including nasal swabs or nasal polyps.

[0075] FE_{NO} assay refers to an assay that measures FE_{NO} (fractional exhaled nitric oxide) levels. Such levels can be evaluated using, e.g., a hand-held portable device, NIOX MINO (Aerocrine, Solna, Sweden), in accordance with guidelines published by the American Thoracic Society (ATS) in 2005. FE_{NO} may be noted in other similar ways, e.g., FeNO or FENO, and it should be understood that all such similar variations have the same meaning.

[0076] Age of Patients to be tested or treated according to the methods provided herein include: all ages. In one embodiment, the ages are 18+ years old. In another embodiment, the ages are 12+ years old. In yet another embodiment, the ages are 2+ years old. In one embodiment, the ages are 18-75 year olds, 12-75 year olds or 2-75 year olds.

[0077] Asthma is a complex disorder characterized by variable and recurring symptoms, reversible airflow obstruction (e.g., by bronchodilator) and bronchial hyperresponsiveness which may or may not be associated with underlying inflammation. Examples of asthma include aspirin sensitive/exacerbated asthma, atopic asthma, severe asthma, mild asthma, moderate to severe asthma, corticosteroid naïve asthma, chronic asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, newly diagnosed and untreated asthma, asthma due to smoking, asthma uncontrolled on corticosteroids and other asthmas as mentioned in J Allergy Clin Immunol (2010) 126(5):926-938.

[0078] Eosinophilic Disorder means: a disorder associated with excess eosinophil numbers in which atypical symptoms may manifest due to the levels or activity of eosinophils locally or systemically in the body. Disorders associated with excess eosinophil numbers or activity include but are not limited to, asthma (including aspirin sensitive asthma), atopic asthma, atopic dermatitis, allergic rhinitis (including seasonal allergic rhinitis), non-allergic rhinitis, asthma, severe asthma, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angiodema, helminth infections, where eosinophils may have a protective role, onchocercal dermatitis and Eosinophil- Associated Gastrointestinal Disorders, including but not limited to, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis and eosinophilic colitis, nasal micropolyposis and polyposis, aspirin intolerance, asthma and obstructive sleep apnoea. Eosinophil-derived secretory products have

also been associated with the promotion of angiogenesis and connective tissue formation in tumors and the fibrotic responses seen in conditions such as chronic asthma, Crohn's disease, scleroderma and endomyocardial fibrosis (Munitz A, Levi-Schaffer F. Allergy 2004; 59: 268-75, Adamko et al. Allergy 2005; 60: 13-22, Oldhoff, et al. Allergy 2005; 60: 693-6). Other examples include cancer (e.g., glioblastoma (such as glioblastoma multiforme), non-Hodgkin's lymphoma (NHL)), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, pulmonary fibrosis (including idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis secondary to sclerosis), COPD, hepatic fibrosis.

[0079] IL-13 mediated disorder means a disorder associated with excess IL-13 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-13 locally and/or systemically in the body. Examples of IL-13 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease (e.g., Crohn's disease), lung inflammatory disorders (e.g., pulmonary fibrosis such as IPF), COPD, hepatic fibrosis.

[0080] IL-4 mediated disorder means: a disorder associated with excess IL4 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL4 locally and/or systemically in the body. Examples of IL4 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease (e.g., Crohn's disease), lung inflammatory disorders (e.g., pulmonary fibrosis such as IPF), COPD, hepatic fibrosis.

[0081] IL-5 mediated disorder means: a disorder associated with excess IL5 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL5 locally and/or systemically in the body. Examples of IL5 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease (e.g., Crohn's disease), lung inflammatory disorders (e.g., pulmonary fibrosis such as IPF), COPD, hepatic fibrosis.

[0082] IL-9 mediated disorder means: a disorder associated with excess IL9 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL9 locally and/or systemically in the body. Examples of IL9 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease (e.g., Crohn's disease), lung inflammatory disorders (e.g., pulmonary fibrosis such as IPF), COPD, hepatic fibrosis.

[0083] TSLP mediated disorder means: a disorder associated with excess TSLP levels or activity in which atypical symptoms may manifest due to the levels or activity of TSLP locally and/or systemically in the body. Examples of TSLP mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease (e.g., Crohn's disease), lung inflammatory disorders (e.g., pulmonary fibrosis such as IPF), COPD, hepatic fibrosis.

[0084] IgE-mediated disorder means: a disorder associated with excess IgE levels or activity in which atypical symptoms may manifest due to levels of IgE locally and/or systemically in the body. Such disorders include, asthma, atopic dermatitis, allergic rhinitis, fibrosis (e.g., pulmonary fibrosis, such as IPF)

[0085] Asthma-Like Symptom includes a symptom selected from the group consisting of shortness of breath, cough (changes in sputum production and/or sputum quality and/or cough frequency), wheezing, chest tightness, bronchioconstriction and nocturnal awakenings ascribed to one of the symptoms above or a combination of these symptoms (Juniper et al (2000) Am. J. Respir. Crit. Care Med., 162(4), 1330−1334.).

[0086] The term "respiratory disorder" include, but is not limited to asthma (e.g., allergic and non-allergic asthma (e.g., due to infection, e.g., with respiratory syncytial virus (RSV), e.g., in younger children)); bronchitis (e.g., chronic bronchitis); chronic obstructive pulmonary disease (COPD) (e.g., emphysema (e.g., cigarette-induced emphysema); conditions involving airway inflammation, eosinophilia, fibrosis and excess mucus production, e.g., cystic fibrosis, pulmonary fibrosis, and allergic rhinitis. Examples of diseases that can be characterized by airway inflammation, excessive airway secretion, and airway obstruction include asthma, chronic bronchitis, bronchiectasis, and cystic fibrosis.

[0087] Exacerbations (commonly referred to as asthma attacks or acute asthma) are episodes of new or progressive increase in shortness of breath, cough (changes in sputum production and/or sputum quality and/or cough frequency), wheezing, chest tightness, nocturnal awakenings ascribed to one of the symptoms above or a combination of these symptoms. Exacerbations are often characterized by decreases in expiratory airflow (PEF or FEV1). However, PEF variability does not usually increase during an exacerbation, although it may do so leading up to or during the recovery from an exacerbation. The severity of exacerbations ranges from mild to life-threatening and can be evaluated based on both symptoms and lung function. Severe asthma exacerbations as described herein include exacerbations that result in any one or combination of the following hospitalization for

asthma treatment, high corticosteroid use (e.g., quadrupling the total daily corticosteroid dose or a total daily dose of greater or equal to 500 micrograms of FP or equivalent for three consecutive days or more), or oral/parenteral corticosteroid use.

[0088] A TH2 pathway inhibitor is an agent that inhibits the TH2 pathway.

[0089] *Examples of a TH2 pathway inhibitor include inhibitors of the activity of any one* of the targets selected from the group consisting of: ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935) and multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8). Examples of inhibitors of the aforementioned targets are disclosed in, for example, WO2008/086395; WO2006/085938; US 7,615,213; US 7,501,121; WO2006/085938; WO 2007/080174; US 7,807,788; WO2005007699; WO2007036745; WO2009/009775; WO2007/082068; WO2010/073119; WO2007/045477; WO2008/134724; US2009/0047277; and WO2008/127,271).

[0090] A therapeutic agent a provided herein includes an agent that can bind to the target identified herein above, such as a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target identified herein (i.e., siRNA).

[0091] "An anti-IL13/IL4 pathway inhibitor" refers to a therapeutic agent that inhibits IL-13 and/or IL-4 signaling. Examples of an anti-IL13/IL4 pathway inhibitors includes inhibitors of the interaction of IL13 and/or IL4 with its receptor(s), such inhibitors include, but are not limited to, anti-IL13 binding agents, anti-IL4 binding agents, anti-IL3/IL4 bispecific binding agents, anti-IL4receptoralpha binding agents, anti-IL13receptoralpha1

binding agents and anti-IL13 receptoralpha2 binding agents. Single domain antibodies that can bind IL13, IL4, (including bispecific antibody with a single domain binding IL13 and a single domain binding IL4), IL-13Ralpha1, IL-13Ralpha2 or IL-4Ralpha are specifically included as inhibitors. It should be understood that molecules that can bind more than one target are included.

[0092] "Anti-IL4 binding agents" refers to agent that binds to human IL-4. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-4 sequence with an affinity between 1 uM – 1 pM. Specific examples of anti-IL4 binding agents can include soluble IL4Receptor alpha (e.g., extracellular domain of IL4Receptor fused to a human Fc region), anti-IL4 antibody, and soluble IL13receptoralpha1 (e.g., extracellular domain of IL13receptoralpha1 fused to a human Fc region).

[0093] "Anti-IL4receptoralpha binding agents" refers to an agent that binds to human IL4 receptoralpha. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-4 receptor alpha sequence with an affinity between 1 uM – 1 pM. Specific examples of anti-IL4 receptoralpha binding agents can include anti-IL4 receptor alpha antibodies.

[0094] "Anti-IL13 binding agent" refers to agent that binds to human IL13. Such binding agents can include a small molecule, aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 sequence with an affinity between 1 uM – 1 pM. Specific examples of anti-IL13 binding agents can include anti-IL13 antibodies, soluble IL13receptoralpha2 fused to a human Fc, soluble IL4receptoralpha fused to a human Fc, soluble IL13 receptoralpha fused to a human Fc. According to one embodiment, the anti-IL13 antibody comprises (1) a HVRH1 comprising the amino acid sequence SEQ ID NO 11, (2) HVRH2 comprising the amino acid sequence SEQ ID NO:12, (3) HVRH3 comprising the amino acid sequence SEQ ID NO:13, (4) HVRL1 comprising the amino acid sequence SEQ ID NO:14, (5) HVRL2 comprising the amino acid sequence SEQ ID NO:15, and (6) HVRL3

comprising the amino acid sequence SEQ ID NO:16. In another embodiment, the anti-IL-13 antibody comprises a VH domain comprising the amino acid sequence SEQ ID NO:9 and a VL domain comprising the amino acid sequence SEQ ID NO:10. According to one embodiment, the antibody is an IgG1 antibody. According to another embodiment, the antibody is an IgG4 antibody. According to one embodiment, the IgG4 antibody comprises a S228P mutation in its constant domain.

[0095] Anti-IL13receptoralpha1 binding agents" refers to an agent that specifically binds to human IL13 receptoralpha1. Such binding agents can include a small molecule, aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 receptor alpha1 sequence with an affinity between 1 uM – 1 pM. Specific examples of anti-IL13 receptoralpha1 binding agents can include anti-IL13 receptor alpha1 antibodies.

[0096] "Anti-IL13receptoralpha2 binding agents" refers to an agent that specifically binds to human IL13 receptoralpha2. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 receptor alpha2 sequence with an affinity between 1 uM – 1 pM. Specific examples of anti-IL13 receptoralpha2 binding agents can include anti-IL13 receptor alpha2 antibodies.

[0097] "Anti IgE binding agents" refers to an agent that specifically binds to human IgE. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the anti-IgE antibody comprises a VL sequence comprising the amino acid sequence of SEQ ID NO:17 and a VH sequence comprising the amino acid sequence SEQ ID NO:18.

[0098] "Anti-M1'binding agents" refers to an agent that specifically binds to the membrane proximal M1' region of surface expressed IgE on B cells. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the anti-IgE antibody comprises an antibody described in WO2008/116149 or a variant thereof. According to

another embodiment, the anti-M1' antibody comprises a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25. According to another embodiment, An anti-IgE/M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25. [SQNTLVPWT].

[0099] The term "small molecule" refers to an organic molecule having a molecular weight between 50 Daltons to 2500 Daltons.

[0100] The term "antibody" is used in the broadest sense and specifically covers, for example, monoclonal antibodies, polyclonal antibodies, antibodies with polyepitopic specificity, single chain antibodies, multi-specific antibodies and fragments of antibodies. Such antibodies can be chimeric, humanized, human and synthetic. Such antibodies and methods of generating them are described in more detail below.

[0101] The term "uncontrolled" or "uncontrollable" refers to the inadequacy of a treatment regimen to minimize a symptom of a disease. As used herein, the term "uncontrolled" and "inadequately controlled" can be used interchangeably and are meant to refer to the same state. The control status of a patient can be determined by the attending physician based on a number of factors including the patient's clinical history, responsiveness to treatment and level of current treatment prescribed. For example, a physician may consider factors such as FEV1 <75% predicted or personal best, frequency of need for a SABA in the past 2-4 weeks (e.g., greater than or equal two doses/week), nocturnal awakenings/symptoms in the past 2-4 weeks (e.g., less than or equal to 2 nights/week), limitations on activity in the past 2-4 weeks, daytime symptoms in the past 2-4 weeks

[0102] The term "therapeutic agent" refers to any agent that is used to treat a disease.

[0103] The term "controller" or "preventor" refers to any therapeutic agent that is used to control asthma inflammation. Examples of controllers include corticosteroids, leukotriene receptor antagonists (e.g., inhibit the synthesis or activity of leukotrienes such as montelukast, zileuton, pranlukast, zafirlukast), LABAs, corticosteroid/LABA combination compositions ,

theophylline (including aminophylline), cromolyn sodium, nedocromil sodium, omalizumab, LAMAs, MABA (e.g, bifunctional muscarinic antagonist-beta2 Agonist), 5-Lipoxygenase Activating Protein (FLAP) inhibitors, and enzyme PDE-4 inhibitor (e.g., roflumilast). A "second controller" typically refers to a controller that is not the same as the first controller.

[0104] The term "corticosteroid sparing" or "CS" means the decrease in frequency and/or amount, or the elimination of, corticosteroid used to treat a disease in a patient taking corticosteroids for the treatment of the disease due to the administration of another therapeutic agent. A "CS agent" refers to a therapeutic agent that can cause CS in a patient taking a corticosteroid.

[0105] The term "corticosteroid" includes, but is not limited to fluticasone (including fluticasone propionate (FP)), beclometasone, budesonide, ciclesonide, mometasone, flunisolide, betamethasone and triamcinolone. "Inhalable corticosteroid" means a corticosteroid that is suitable for delivery by inhalation. Exemplary inhalable corticosteroids are fluticasone, beclomethasone dipropionate, budenoside, mometasone furoate, ciclesonide, flunisolide, triamcinolone acetonide and any other corticosteroid currently available or becoming available in the future. Examples of corticosteroids that can be inhaled and are combined with a long-acting beta2-agonist include, but are not limited to: budesonide/formoterol and fluticasone/salmeterol.

[0106] Examples of corticosteroid/LABA combination drugs include fluticasone furoate/vilanterol trifenatate and indacaterol/mometasone.

[0107] The term "LABA" means long-acting beta-2 agonist, which agonist includes, for example, salmeterol, formoterol, bambuterol, albuterol, indacaterol, arformoterol and clenbuterol.

[0108] The term "LAMA" means long-acting muscarinic antagonist, which agonists include: tiotropium.

[0109] Examples of LABA/LAMA combinations include, but are not limited to: olodaterol tiotropium (Boehringer Ingelheim's) and indacaterol glycopyrronium (Novartis)

[0110] The term "SABA" means short-acting beta-2 agonists, which agonists include, but are not limited to, salbutamol, levosalbutamol, fenoterol, terbutaline, pirbuterol, procaterol, bitolterol, rimiterol, carbuterol, tulobuterol and reproterol

[0111] Leukotriene receptor antagonists (sometimes referred to as a leukast) (LTRA) are drugs that inhibit leukotrienes. Examples of leukotriene inhibitors include montelukast, zileuton, pranlukast, and zafirlukast.

[0112] The term "FEV1" refers to the volume of air exhaled in the first second of a forced expiration. It is a measure of airway obstruction. Provocative concentration of methacholine required to induce a 20% decline in FEV1 (PC20) is a measure of airway hyper-responsiveness. FEV1 may be noted in other similar ways, e.g., FEV_1, and it should be understood that all such similar variations have the same meaning.

[0113] The term "relative change in FEV1" = (FEV1 at week 12 of treatment – FEV1 prior to start of treatment) divided by FEV1

[0114] The term "mild asthma" refers to a patient generally experiencing symptoms or exacerbations less than two times a week, nocturnal symptoms less than two times a month, and is asymptomatic between exacerbations. Mild, intermittent asthma is often treated as needed with the following: inhaled bronchodilators (short-acting inhaled beta2- agonists); avoidance of known triggers; annual influenza vaccination; pneumococcal vaccination every 6 to 10 years, and in some cases, an inhaled beta2-agonist, cromolyn, or nedocromil prior to exposure to identified triggers. If the patient has an increasing need for short-acting beta2-agonist (e.g., uses short-acting beta2-agonist more than three to four times in 1 day for an acute exacerbation or uses more than one canister a month for symptoms), the patient may require a stepup in therapy.

[0115] The term "moderate asthma" generally refers to asthma in which the patient experiences exacerbations more than two times a week and the exacerbations affect sleep and activity; the patient has nighttime awakenings due to asthma more than two times a month; the patient has chronic asthma symptoms that require short-acting inhaled beta2-agonist daily or every other day; and the patient's pretreatment baseline PEF or FEV1 is 60 to 80 percent predicted and PEF variability is 20 to 30 percent.

[0116] The term "severe asthma" generally refers to asthma in which the patient has almost continuous symptoms, frequent exacerbations, frequent nighttime awakenings due to the asthma, limited activities, PEF or FEV1 baseline less than 60 percent predicted, and PEF variability of 20 to 30 percent.

[0117] Examples of rescue medications include albuterol, ventolin and others.

[0118] "Resistant" refers to a disease that demonstrates little or no clinically significant improvement after treatment with a therapeutic agent. For example, asthma which requires treatment with high dose ICS (e.g., quadrupling the total daily corticosteroid dose or a total daily dose of greater or equal to 500 micrograms of FP (or equivalent) for at least three consecutive days or more, or systemic corticosteroid for a two week trial to establish if

asthma remains uncontrolled or FEV1 does not improve is often considered severe refractory asthma.

[0119] A therapeutic agent as provided herein can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In one embodiment, the therapeutic agent is inhaled. According to another embodiment, the dosing is given by injections, e.g., intravenous or subcutaneous injections. In yet another embodiment, the therapeutic agent is administered using a syringe (e.g., prefilled or not) or an autoinjector.

[0120] For the prevention or treatment of disease, the appropriate dosage of a therapeutic agent may depend on the type of disease to be treated, the severity and course of the disease, whether the therapeutic agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. The therapeutic agent is suitably administered to the patient at one time or over a series of treatments. The therapeutic agent composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

[0121] Dosing for lebrikizumab, for eosinophilic diseases (including asthma) and for treating other diseases using TH2 therapies: lebrikizumab can be administered 0.1 mg/kg to 100 mg/kg of the patient's body weight. In one embodiment, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight. In another embodiment, the dose is 1 mg/kg to 10 mg/kg of the patient's body weight.

[0122] In an alternative embodiment, lebrikizumab can be administered as a flat dose. In one embodiment lebrikizumab is administered in as a 125-1000mg flat dose (i.e., not weight dependent), by subcutaneous injection or by intravenous injection, at a frequency of time selected from the group consisting of: every 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 1month, 2 months, 3month or 4 months. In another embodiment, if the patient is overweight, lebrikizumab can be administered, e.g., 125-250mg at a frequency of 3 times per month. In one embodiment, the lebrikizumab is administered as a flat dose of 125mg, 250mg

or 500mg every 4 weeks. In another embodiment, the lebrikizumab is administered in a patient >40 kg as a flat dose of 37.5mg, 125mg, 250mg or 500mg every 4 weeks.

[0123] In one embodiment, the patient is 18 years of age or older. In one embodiment, the asthma patient is age 12 to 17 and lebrikizumab is administered in as a flat dose of 250 mg or a flat dose of 125 mg. In one embodiment, the asthma patient is age 6 to 11 and lebrikizumab is administered in as a flat dose of 125 mg..

[0124] "Patient response" or "response" (and grammatical variations thereof) can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression, including slowing down and complete arrest; (2) reduction in the number of disease episodes and/or symptoms; (3) reduction in lesional size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (i.e. reduction, slowing down or complete stopping) of disease spread; (6) decrease of auto-immune response, which may, but does not have to, result in the regression or ablation of the disease lesion; (7) relief, to some extent, of one or more symptoms associated with the disorder; (8) increase in the length of disease-free presentation following treatment; and/or (9) decreased mortality at a given point of time following treatment.

[0125] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen binding arm). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0126] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0127] The terms "anti-target antibody" and "an antibody that binds to target" refer to an antibody that is capable of binding the target with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the target. In one embodiment, the extent of binding of an anti-target antibody to an unrelated, non-target protein is less than

about 10% of the binding of the antibody to target as measured, e.g., by a radioimmunoassay (RIA) or biacore assay. In certain embodiments, an antibody that binds to a target has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-target antibody binds to an epitope of a target that is conserved among different species.

[0128] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0129] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

[0130] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

[0131] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0132] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0133] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0134] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0135] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0136] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0137] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0138] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0139] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0140] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0141] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0142] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

[0143] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs

correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0144] The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter typically being of highest sequence variability and/or involved in antigen recognition. An HVR region as used herein comprise any number of residues located within positions 24-36 (for HVRL1), 46-56 (for HVRL2), 89-97 (for HVRL3), 26-35B (for HVRH1), 47-65 (for HVRH2), and 93-102 (for HVRH3).

[0145] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0146] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0147] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).

[0148] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0149] "Isolated nucleic acid encoding an anti-target antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof),

including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0150] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used according to the methods provided herein may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0151] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0152] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0153] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications,

usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products. The term "package insert" is also used to refer to instructions customarily included in commercial packages of diagnostic products that contain information about the intended use, test principle, preparation and handling of reagents, specimen collection and preparation, calibration of the assay and the assay procedure, performance and precision data such as sensitivity and specificity of the assay.

[0154] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0155] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

[0156] 100 times the fraction X/Y where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be

appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0157] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0158] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0159] The term "target," as used herein, refers to any native molecule from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed target as well as any form of target that results from processing in the cell. The term also encompasses naturally occurring variants of targets, e.g., splice variants or allelic variants.

[0160] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

[0161] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain

may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991).

[0162] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

COMPOSITIONS AND METHODS

[0163] In one aspect, the invention is based, in part, on new diagnostic assays and better methods of treatment. In certain embodiments, antibodies that bind to periostin are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of asthma and other diseases.

Exemplary Antibodies

Anti-periostin Antibodies

[0164] In one aspect, the invention provides isolated antibodies that bind to periostin. In certain embodiments, an anti-periostin antibody that can bind to isoforms 1-4 of human periostin with good affinity.

[0165] . In one embodiment, the antibody comprises the sequences SEQ ID NO:1 and SEQ ID NO:2 (the "25D4" antibody) or comprises the sequences of SEQ ID NO:3 and SEQ ID NO:4 (the "23B9" antibody). In another embodiment, the antibody comprises the variable region sequences SEQ ID NO:1 and SEQ ID NO:2 or comprises the variable region sequences of SEQ ID NO:3 and SEQ ID NO:4. In another embodiment, the antibody comprising the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 or the HVR sequences of SEQ ID NO:3 and SEQ ID NO:4: In another embodiment, the antibody comprises the HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:1 and SEQ ID NO:2 and/or an antibody comprising HVR sequences that are 95% or more identical to the HVR sequences of SEQ ID NO:3 and SEQ ID NO:4.

[0166] In any of the above embodiments, an anti-periostin antibody can be humanized. In one embodiment, an anti-periostin antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

[0167] In another aspect, an anti-periostin antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:1. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-periostin antibody comprising that sequence retains the ability to bind to periostin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:1. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-periostin antibody comprises the VH sequence in SEQ ID NO:1, including post-translational modifications of that sequence.

[0168] In another aspect, an anti-periostin antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:2. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-periostin antibody comprising that sequence retains the ability to bind to periostin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:2. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-periostin antibody comprises the VL sequence in SEQ ID NO:2, including post-translational modifications of that sequence.

[0169] In another aspect, an anti-periostin antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:3. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-periostin antibody comprising that

sequence retains the ability to bind to periostin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:3. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-periostin antibody comprises the VH sequence in SEQ ID NO:3, including post-translational modifications of that sequence.

[0170] In another aspect, an anti-periostin antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:2. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-periostin antibody comprising that sequence retains the ability to bind to periostin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:4. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-periostin antibody comprises the VL sequence in SEQ ID NO:4, including post-translational modifications of that sequence.

[0171] In another aspect, an anti-periostin antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

[0172] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-periostin antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-periostin antibody comprising a VH sequence of SEQ ID NO:1 and a VL sequence of SEQ ID NO:2. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-periostin antibody comprising a VH sequence of SEQ ID NO:3 and a VL sequence of SEQ ID NO:4.

[0173] In a further aspect of the invention, an anti-periostin antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-periostin antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein. In another embodiment, the antibody is a bispecific antibody.

[0174] In a further aspect, an anti-periostin antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

Anti—IL13 Antibodies

[0175] In one aspect, the invention provides isolated antibodies that bind to human IL-13.

[0176] In one embodiment, the anti-IL13 antibody comprises a HVR-L1 comprising amino acid sequence SEQ ID NO:14; an HVR-L2 comprising amino acid sequence SEQ ID NO:15; an HVR-L3 comprising amino acid sequence SEQ ID NO: 16; an HVR-H1 comprising amino acid sequence SEQ ID NO:11; an HVR-H2 comprising amino acid sequence SEQ ID NO: 12; and an HVR-H3 comprising amino acid sequence SEQ ID NO: 13.

[0177] In another embodiment, the antibody comprises the variable region sequences SEQ ID NO:9 and SEQ ID NO:10.

[0178] In any of the above embodiments, an anti-IL-13 antibody can be humanized. In one embodiment, an anti-IL-13 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

[0179] In another aspect, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to human IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, altered inserted and/or deleted in SEQ ID NO:9. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL13 antibody comprises the VH sequence in SEQ ID NO:9, including post-translational modifications of that sequence.

[0180] In another aspect, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:10. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13

antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:10. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-13 antibody comprises the VL sequence in SEQ ID NO:10, including post-translational modifications of that sequence.

[0181] In yet another embodiment, the anti-IL-13 antibody comprises a VL region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:10 and a VH region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9. In yet a further embodiment, the anti-IL-13 antibody comprises a HVR-L1 comprising amino acid sequence SEQ ID NO:14; an HVR-L2 comprising amino acid sequence SEQ ID NO:15; an HVR-L3 comprising amino acid sequence SEQ ID NO: 16; an HVR-H1 comprising amino acid sequence SEQ ID NO:11; an HVR-H2 comprising amino acid sequence SEQ ID NO: 12; and an HVR-H3 comprising amino acid sequence SEQ ID NO: 13.

[0182] In another aspect, an anti-IL-13 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

[0183] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-IL-13 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as or can by competitively inhibited by an anti-IL-13 antibody comprising a VH sequence of SEQ ID NO:9 and a VL sequence of SEQ ID NO:10.

[0184] In a further aspect of the invention, an anti-IL-13 antibody according to any of the above embodiment can be a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-IL13 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein. According to another embodiment, the antibody is a bispecific antibody. In one embodiment, the bispecific antibody comprises the HVRs or comprises the VH and VL regions described above.

[0185] In a further aspect, an anti-IL-13 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

[0186] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M).

[0187] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (125I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [125I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 TM; Packard) is added, and the plates are counted on a TOPCOUNT TM gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0188] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-

hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20TM) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIACORE ® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO TM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0189] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')2, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')2 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0190] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

[0191] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

[0192] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0193] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0194] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0195] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., Methods 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., Methods 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36:61-68 (2005) and Klimka

et al., Br. J. Cancer, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0196] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. J. Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta et al. J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., J. Biol. Chem. 272:10678-10684 (1997) and Rosok et al., J. Biol. Chem. 271:22611-22618 (1996)).

4. Human Antibodies

[0197] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0198] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSETM technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0199] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human

monoclonal antibodies have been described. (See, e.g., Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, Histology and Histopathology, 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Experimental and Clinical Pharmacology, 27(3):185-91 (2005).

[0200] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0201] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., Nature 348:552-554; Clackson et al., Nature 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Marks and Bradbury, in Methods in Molecular Biology 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132(2004).

[0202] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing

hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0203] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0204] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for IL-13 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of IL-13. Bispecific antibodies may also be used to localize cytotoxic agents to cells. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0205] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. J. Immunol. 147: 60 (1991).

[0206] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0207] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to IL-13 as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

[0208] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

Substitution, Insertion, and Deletion Variants

[0209] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Conservative Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn

Original Residue	Exemplary Substitutions	Conservative Substitutions
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0210] Amino acids may be grouped according to common side-chain properties:

[0211] (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

[0212] (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

[0213] (3) acidic: Asp, Glu;

[0214] (4) basic: His, Lys, Arg;

[0215] (5) residues that influence chain orientation: Gly, Pro;

[0216] (6) aromatic: Trp, Tyr, Phe.

[0217] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0218] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant

antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0219] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0220] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0221] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring

residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0222] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Glycosylation variants

[0223] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0224] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0225] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US

Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).

[0226] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

Fc region variants

[0227] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0228] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or

ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcRIII only, whereas monocytes express FcRI, FcRII and FcRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M.S. et al., Blood 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., Int'l. Immunol. 18(12):1759-1769 (2006)).

[0229] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0230] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).)

[0231] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0232] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[0233] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0234] See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Cysteine engineered antibody variants

[0235] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

Antibody Derivatives

[0236] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water

soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0237] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

Recombinant Methods and Compositions

[0238] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-periostin antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one

embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-periostin antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0239] For recombinant production of an anti-periostin antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0240] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in E. coli.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0241] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li et al., Nat. Biotech. 24:210-215 (2006).

[0242] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

[0243] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

[0244] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host

cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

ASSAYS

[0245] Anti-periostin antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Binding assays and other assays

[0246] In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

[0247] In another aspect, competition assays may be used to identify an antibody that competes with IL-13 or periostin for binding to IL13 or periostin, respectively. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by lebrikizumab or another anti-IL13 antibody specified herein or anti-periostin antibody specified herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).

[0248] In an exemplary competition assay, immobilized periostin is incubated in a solution comprising a first labeled antibody that binds to periostin (e.g., 25D4) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to periostin. The second antibody may be present in a hybridoma supernatant. As a control, immobilized periostin is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first

antibody to periostin, excess unbound antibody is removed, and the amount of label associated with immobilized periostin is measured. If the amount of label associated with immobilized periostin is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to periostin. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Activity assays

[0249] In one aspect, assays are provided for identifying anti-IL-13 antibodies thereof having biological activity. Biological activity may include, e.g., activity in asthma. Antibodies having such biological activity in vivo and/or in vitro are also provided.

[0250] In certain embodiments, an antibody of the invention is tested for such biological activity.

Immunoconjugates

[0251] The invention also provides immunoconjugates comprising an anti-periostin antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0252] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., Cancer Res. 53:3336-3342 (1993); and Lode et al., Cancer Res. 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., Current Med. Chem. 13:477-523 (2006); Jeffrey et al., Bioorganic & Med. Chem. Letters 16:358-362 (2006); Torgov et al., Bioconj. Chem. 16:717-721 (2005); Nagy et al., Proc. Natl. Acad. Sci. USA 97:829-834 (2000); Dubowchik et al., Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et al., J. Med. Chem. 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0253] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited

to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0254] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0255] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0256] The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-

SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

Methods and Compositions for Diagnostics and Detection

[0257] In certain embodiments, any of the anti-periostin antibodies provided herein is useful for detecting the presence of periostin in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, plasma, nasal swabs and sputum.

[0258] In one embodiment, an anti-periostin antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of periostin in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-periostin antibody as described herein under conditions permissive for binding of the anti-periostin antibody to periostin, and detecting whether a complex is formed between the anti-periostin antibody and periostin. Such method may be an in vitro or in vivo method. In one embodiment, an anti-periostin antibody is used to select subjects eligible for therapy with an anti-I3 antibody, or any other TH2 pathway inhibitor, e.g. where periostin is a biomarker for selection of patients.

[0259] Exemplary disorders that may be diagnosed using an antibody of the invention are provided herein.

[0260] In certain embodiments, labeled anti-periostin antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes 32P, 14C, 125I, 3H, and 131I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Pharmaceutical Formulations

[0261] Pharmaceutical formulations of an anti-IL-13 antibody or other TH2 pathway inhibitors as described herein are prepared by mixing such antibody or molecule having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0262] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0263] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a controller with the TH2 pathway inhibitor. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0264] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0265] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0266] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

Therapeutic Methods and Compositions

[0267] Eosinophilic inflammation is associated with a variety of illnesses, both allergic and non-allergic (Gonlugur (2006) Immunol. Invest. 35(1):29-45). Inflammation is a restorative response of living tissues to injury. A characteristic of inflammatory reactions is the accumulation of leukocytes in injured tissue due to certain chemicals produced in the tissue itself. Eosinophil leukocytes accumulate in a wide variety of conditions such as allergic disorders, helminthic infections, and neoplastic diseases (Kudlacz et al., (2002) Inflammation 26: 111–119). Eosinophil leukocytes, a component of the immune system, are defensive elements of mucosal surfaces. They respond not only to antigens but to parasites, chemicals, and trauma.

[0268] Tissue eosinophilia occurs in skin diseases such as eczema, pemphigus, acute urticaria, and toxic epidermal necrolysis as well as in atopic dermatitis ([Rzany et al., 1996]). Eosinophils accumulate in the tissue and empty granule proteins in IgE-mediated allergic skin reactions ([Nielsen et al., 2001]). Eosinophils combined with mast cells are likely to cause joint inflammation (Miossec et al., 1997). Eosinophilic inflammation sometimes accompanies joint trauma. Synovial fluid eosinophilia can be associated with diseases such as rheumatoid arthritis, parasitic disease, hypereosinophilic syndrome, Lyme disease, and allergic processes, as well as hemarthrosis and arthrography ([Atanes et al., 1996]). Eosinophilic inflammation can affect bones as well ([Yetiser et al., 2002]). Examples of eosinophilic muscle disease include eosinophilic perimyositis, eosinophilic polymyositis, and focal eosinophilic myositis ([Lakhanpal et al., 1988]). Eosinophilic inflammations affecting skeletal muscles may be associated with parasite infections or drugs or features of some systemic disorders of

hypereosinophilia (e.g., idiopathic hypereosinophilic syndrome and eosinophilia-myalgia syndrome. Eosinophils participate in the inflammatory response to epitopes recognized by autoimmune antibodies ([Engineer et al., 2001]). Connective tissue diseases may lead to neutrophilic, eosinophilic, or lymphocytic vascular inflammations ([Chen et al., 1996]). Tissue and peripheral blood eosinophilia can occur in active rheumatismal diseases. Elevation of serum ECP levels in ankylosing spondylitis, a kind of connective tissue disease, suggests that eosinophils are also involved in the underlying process (Feltelius et al., 1987). Wegener's granulomatosis can rarely present with pulmonary nodules, pleural effusion, and peripheral blood eosinophilia ([Krupsky et al., 1993]).

[0269] Peripheral blood eosinophilia of at least 400/mm3 can occur in 7% of cases of systemic sclerosis, 31% of cases of localized scleroderma, and 61% of cases of eosinophilic fasciitis ([Falanga and Medsger, 1987]). Scleroderma yields an inflammatory process closely resembling Meissner's and Auerbach's plexuses and consists of mast cells and eosinophil leukocytes in the gastrointestinal system. Eosinophil-derived neurotoxins can contribute to gastrointestinal motor dysfunction, as occurs in scleroderma ([de Schryver Kecskemeti and Clouse, 1989]).

[0270] Eosinophils can accompany localized ([Varga and Kahari, 1997]) or systemic ([Bouros et al., 2002]) connective tissue proliferation. They can incite fibrosis by inhibiting proteoglycan degradation in fibroblasts ([Hernnas et al., 1992]), and fibroblasts mediate eosinophil survival by secreting GM-CSF ([Vancheri et al., 1989]). Eosinophils can be found in nasal ([Bacheret et al., 2001]), bronchial ([Arguelles and Blanco, 1983]), and gastrointestinal polyp tissues ([Assarian and Sundareson, 1985]). Likewise, eosinophils can be localized in inflammatory pseudotumors (myofibroblastic tumor). Eosinophils often accompany inflammatory pseudotumors in the orbital region, in which case the condition can mimic angioedema or allergic rhinoconjunctivitis ([Li et al., 1992]).

[0271] Eosinophilic inflammation can be found in tissue trauma (e.g., as a result of surgery or injury). Eosinophilic inflammation can also be associated with cardiovascular illnesses (e.g., eosinophilic myocarditis, eosinophilic coronary arteritis, ischemic heart disease, acute myocardial infarction, cardiac rupture). Necrotic inflammatory processes can also involve eosinophililic inflammation (polymyositis, coronary artery dissection, necrotizing lesions of neuro-Behcet's disease, dementia, cerebral infarction).

[0272] Provided herein are methods of Identifying Eosinophilic Inflammation Positive (EIP) patients predictive for a response to treatment with a TH2 Pathway Inhibitor (or that will be responsive to) by measuring total serum periostin levels in the patient.

[0273] Also provided herein are methods of treating asthma, an Eosinophilic Disorder, an IL-13 mediated Disorder, an IL4 mediated Disorder, an IL9 mediated Disorder, an IL5 mediated Disorder, an IL33 mediated Disorder, an IL25 mediated Disorder, an TSLP mediated Disorder, an IgE-mediated Disorder or Asthma-Like Symptoms comprising administering a TH2 pathway inhibitor to an Eosinophilic Inflammation Positive Patient, wherein the patient was diagnosed as being EIP by measuring total serum periostin levels in the patient.

[0274] In certain embodiments, methods of treating asthma, an Eosinophilic Disorder, an IL-13 mediated Disorder, IL-4 mediated Disorder or an IgE-mediated Disorder comprising administering lebrikizumab to a Eosinophilic Inflammation Positive Patient are provided.

[0275] In certain embodiments, methods of treating asthma, an Eosinophilic Disorder, an IL-13 mediated Disorder, IL-4 mediated Disorder or an IgE-mediated Disorder comprising administering a 125-500mg flat dose of lebrikizumab every 4 weeks to the patient suffering from the disorder are provided.

[0276] Also provided are methods of treating asthma (or Respiratory Disease) comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in a relative change in FEV1 of greater than 5%. In another embodiment, the FEV1 is greater than 6%, 7%, 8%, 9% or10% FEV1. In another embodiment, the patient has been diagnosed as EIP using a Total Periostin assay. In another embodiment, the asthma patient has been diagnosed with a total serum periostin assay.

[0277] In certain embodiments, methods of treating asthma (or Respiratory Disease) comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in a reduction in exacerbation rate of greater than 35%. (other embodiments greater than 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, up to 85%; another embodiment, wherein the patient has been diagnosed as EIP) are provided.

[0278] In certain embodiments, methods of treating asthma (or Respiratory Disease) comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in a reduction in nocturnal awakenings are provided. In one embodiment, the patient is diagnosed by measuring total serum periostin levels in the

patient. In another embodiment, the asthma of the patient is uncontrolled on a corticosteroid. I another embodiment, the patient is diagnosed with EIP.

[0279] Also provided are methods of treating asthma (or Respiratory Disease) comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in an improvement in asthma control. In one embodiment, the patient is diagnosed by measuring total serum periostin levels in the patient. In another embodiment, the asthma is uncontrolled on a corticosteroid treatment. In another embodiment, the patient is diagnosed with EIP

[0280] Methods of treating Asthma (or Respiratory Disease) comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in a reduction of inflammation in the lungs are provided. In one embodiment, the patient is diagnosed by measuring total serum periostin levels in the patient. In another embodiment, the asthma is uncontrollable on a corticosteroid treatment. In another embodiment, the patient is diagnosed with EIP

[0281] In certain embodiments, methods of treating an Eosinophilic Disorder in a patient suffering from the Eosinophilic Disorder and being treated with a corticosteroid comprising administering a therapeutically effective amount of Lebrikizumab to the asthma patient, wherein the treatment results in a reduction or elimination of corticosteroid treatment (amount or frequency) used to treat the disease are provided. In one embodiment, the patient is diagnosed by measuring total serum periostin levels in the patient. In another embodiment, the patient's asthma is uncontrollable on a corticosteroid. In another embodiment, the patient is diagnosed with EIP prior to the treatment.

[0282] Also provided are methods of treating of a patient suffering from asthma (or Respiratory Disease) comprising diagnosing the patient as EIP using a Total Periostin Assay, administering a therapeutically effective amount of TH2 Pathway Inhibitor to the asthma patient, diagnosing the patients EIP status, and retreating the patient with the TH2 Pathway Inhibitor if the status is EIP. The diagnosis being made using Total Periostin Assay alone or in combination with FE_{NO} levels and optionally in combination other biomarkers selected from: CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10. In yet a further embodiment, the patient to be treated in addition to having elevated expression levels of periostin as described herein, has a FE_{NO} level greater than 21 ppb. In still another embodiment, the patient to be treated in addition to having

elevated expression levels of periostin as described herein, has a FE_{NO} level greater than 35 ppb.

[0283] In certain embodiments, methods of Identifying Patients that are Eosinophilic Inflammation Negative (EIN), comprising the step of measuring Total Periostin levels in a patient and determing that the patient is EIN are provided.

[0284] Any of the TH2 pathway inhibitors provided herein may be used in therapeutic methods described herein, especially asthma. In one embodiment, the asthma patient is being treated with a corticosteroid, and has been diagnosed as responsive a TH2 pathway inhibitor using a periostin assay described herein. In a further embodiment, the asthma patient is suffering from moderate to severe asthma. In another embodiment, the patient is suffering from mild asthma but is not being treated with a corticosteroid.

[0285] An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0286] Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0287] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic

agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0288] In certain embodiments, an antibody of the invention is administered as a flat dose (i.e., not weight dependent) of 37.5 mg, or a flat dose of 125 mg, or a flat dose of 250 mg. In certain embodiments, the dose is administered by subcutaneous injection once every 4 weeks for a period of time. In certain embodiments, the period of time is 6 months, one year, two years, five years, ten years, 15 years, 20 years, or the lifetime of the patient. In certain embodiments, the asthma is severe asthma and the patient is inadequately controlled or uncontrolled on inhaled corticosteroids plus a second controller medication. In another embodiment, the patient is diagnosed with EIP status using a Total Periostin Assay to determine EIP status and the patient is selected for treatment with an anti-IL13 antibody as described above. In another embodiment, the method comprises treating an asthma patient with an anti-IL13 antibody as described above where the patient was previously diagnosed with EIP status using a Total Periostin Assay to determine EIP status. In one embodiment, the asthma patient is age 18 or older. In one embodiment, the asthma patient is age 12 to 17 and the anti-IL13 is administered in as a flat dose of 250 mg or a flat dose of 125 mg. In one embodiment, the asthma patient is age 6 to 11 and the anti-IL13 antibody is administered in as a flat dose of 125 mg.

[0289] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-target antibody or anti-periostin antibody.

Articles of Manufacture

[0290] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0291] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-target antibody or anti-periostin antibody

EXAMPLES

EXAMPLE 1 – Allergen Challenge Clinical Study

[0292] A Phase II randomized, double-blind, placebo-controlled study; 5 mg/kg lebrikizumab:placebo (1:1 ratio) subcutaneously q4 weeks for 12 weeks. A high level summary of the trial design is in Table 2 and Figure 1. The primary outcome measure was

allergen-induced late asthmatic response (LAR) at Week 13. Patients received an allergen challenge followed by a methacoline challenge 18-24 hours later during the screening period and at week 13. Serum biomarkers were also assessed to demonstrate IL13 pathway inhibition and to identify patients with an increased benefit from lebrikizumab.

Table 2

Design	Randomized, double-blind, placebo controlled, multiple dose study to evaluate effect of MILR1444A vs placebo on airway hyperresponsiveness to allergen challenge
Population	18-55 year old mild asthma patients
Sample Size	24 (12 per cohort)
Study Duration	12 weeks (and 4 months follow-up)
Schedule, Dose	subcutaneous formulation (SQ), Q4w, (1 active dose level 5mg/kg)
1° endpoint	LAR AUC (Allergen Challenge)
2° endpoint	PC20 (allergen challenge), FEV1, PC20 (Methacholine challenge)

[0293] The patients included in the study were mild asthmatics,18-55 years old, with: (a) Positive skin test (> or = 3mm over negative control) at screening to house dust mite, cat dander, or ragweed; (b) Forced expiry volume in 1 second (FEV1) > or = 70% of predicted; and (c) Early asthmatic response of > or = 20% reduction in FEV1 in 5-30 minutes following allergen challenge, and a late asthmatic resonse (LAR) of > or = 15% reduction in FEV1 in 2-8 hours post challenge.

[0294] Twenty-eight patients were included in analysis of the primary endpoint (n=16 placebo, 12 lebrikizumab). At Week 13, lebrikizumab inhibited the LAR. The mean AUC (area under the curve) of FEV1 2-8 hours after allergen challenge (LAR) in lebrikizumab-treated patients was reduced by 48% vs. placebo (26.3% vs. 50.5%; 95% CI: -19 to 90%), with no effect on the early phase response (EAR) at week 13. The mean AUC of FEV1 and the maximum reduction in FEV1 0-2 hours post-allergen challenge were similar in the lebrikizumab and placebo groups (27.5% vs. 26.4% for both parameters; Table 3). See also Figure 2. No significant difference between the lebrikizumab and placebo groups was observed on airway hyperresponsiveness to methacholine. The arithmetic mean of the methacholine doubling dose in the lebrikizumab group was 0.33 doubling doses higher than

that in the placebo group (1.58 vs. 1.25, 95% CI -0.64 to 1.3), which was not considered a clinically meaningful inhibition.

Table 3

	Screening			Week 13		
	PB N=16	LB N=12	(LB-PB)/PB	PB N=16	LB N=12	(LB-PB)/PB
Early Asthmatic Response						
Max % reduction in FEV1	30.3	38.0	25.4	25.5	29.6	16.1
AUC FEV1 (0-2h postchallenge), %FEV1 x h	34.1	39.9	17.0	26.4	27.5	4.2
Late Asthmatic Response						
Max % reduction in FEV1	24.8	30.8	24.2	16.4	13.8	-15.9
AUC FEV1 (2-8h postchallenge, % FEV1 x h)	73.8	77.0	4.3	50.5	26.3	-47.9

[0295] Lebrikizumab treatment clearly exerted systemic effects on markers of Th2 inflammation, with mean placebo-adjusted reductions of 24%, 25% and 26%, in serum IgE, MCP-4/CCL13, and TARC/CCL17, respectively ($P<0.01$). See Figures 3A and 3B. Serum periostin levels were slightly reduced (5-10%) after treatment. Serum IL-13 levels were mostly below level of detection (<150pg/ml) after treatment. Figure 3C shows reductions in IgE, CCL13 and CCL17 at week 13 in individual patients relative to baseline levels of those markers. In general, periostin,YKL-40, CEA and blood eosinophils levels did not change significantly after lebrikizumab treatment (data not shown).

[0296] Subjects with baseline levels above the median of peripheral blood eosinophils, serum IgE, or serum periostin exhibited a greater placebo-adjusted lebrikizumab dependent reduction in LAR than subjects with baseline levels of these biomarkers below the median. See Figure 4.

[0297] Patients were categorized as "biomarker-high" or "biomarker-low" based on having higher than or lower than the median levels of inflammatory biomarkers at baseline:

serum IgE, CLL13, CCL17, CEA, periostin, YKL-40 and peripheral blood eosiniophils. As shown in Fig. 4, the results for serum IgE, eosinophils, and periostin indicated that patients with elevated (compared to the median) baseline serum IgE, periostin, or increased peripheral blood eosinophils were more likely to respond to IL13 blockade (e.g., by lebrikizumab).

[0298] In conclusion, the study met its primary endpoint: 48% reduction (90% CI [-19%, 90%)]) in mean late phase AUC. Lebrikiuzumab significantly reduced LAR compared to placebo. No safety signal was seen in the safety data. Therapeutic blockade of IL-13 may be an effective treatment for allergic asthma, particularly in patients with elevated markers of airway TH2 inflammation.

EXAMPLE 2 – Asthma Patient Clinical Study I

[0299] A randomized, double-blind, placebo-controlled study was conducted to evaluate the effects of lebrikizumab in patients with asthma who remain inadequately controlled or uncontrolled while on chronic therapy with ICS. Patients continued their standard-of-care therapy which included inhaled corticosteroids (ICS) and could also include a LABA. In this two-arm study, patients were randomly allocated to receive either lebrikizumab or placebo for 6 months. During a 14−20 day run-in period (Visit 1 to Visit 3), patients had to demonstrate compliance with ICS and their ability to use the equipment necessary for daily monitoring throughout the study. Patients were then assessed for study eligibility and randomly allocated (1:1) to study drug (lebrikizumab or placebo) with stratification based on IL-13 signature surrogate status (described further below), LABA use, and study site. The first SC dose occured within 24 hours of random allocation (Study Day 1, i.e., the day of random allocation, regardless of first study drug administration date). Administration of study drug was repeated once every 4 weeks for the next 20 weeks (for a total of six study drug doses providing a 24-week treatment period). Measures of the efficacy of lebrikizumab were assessed during the treatment period.

[0300] Study drug was administered to selected patients by subcutaneous (SC) injection on the following timepoints: Day 1 and at Weeks 4, 8, 12, 16, and 20. Each dose of lebrikizumab was 250 mg. Each placebo dose was 2 mL of the same fluid without lebrikizumab. SC injections were administered in the arm, thigh, or abdomen. A schematic of this trial design can be seen in Figure 5.

[0301] The primary analysis was conducted after all (approximately 200) patients were treated and followed for 24 weeks after random allocation (Day 1). Safety was assessed throughout the study. After the final dose (Week 20) of study drug, patients were monitored

for an additional 12 weeks; the first 4 weeks after the final dose were considered part of the (24-week) treatment period, and the final 8 weeks constituted the follow-up period. The 8-week follow-up period, together with the last 4 weeks of the treatment period, allowed for monitoring of patients for 3–4 half-lives following the last dose. Therefore, patients generally participated in the study for a total of approximately 34 weeks. See Figure 5. Efficacy evaluable patients (n= 180) were defined for decision making purposes to exclude subjects for two reasons: (1) not part of target population in key characteristics and (2) unreliable baseline FEV1 from which to measure treatment effect. See Figure 6 for baseline characteristics of patients participating in this trial.

Table 4

Design	1:1 randomized, double-blind, placebo-controlled study to evaluate efficacy and safety of lebrikizumab vs placebo
Population	18-65 year old patients with asthma who are inadequately controlled on inhaled corticosteroids (ICS)
Sample Size	Approximately 218
Study Duration	2 week screening period, 24 week treatment period, 8 week safety follow-up period
Dose	250 mg, every 28 days, for a total of 6 doses
1° endpoint	Relative change in FEV1 from baseline to Week 12
2° endpoints	Relative change in FEV1 from baseline to Week 24. Relative change in FEV1 from baseline to Week 12 or patients with IL-13 signature surrogate positive status. Rate of asthma exacerbations during the 24-week period.

[0302] Key Inclusion Criteria included the following: Ability to perform spirometry at Visits 1-3 as per study specific Pulmonary Function Test (PFT) Manual; Chest radiograph within 12 months of Visit 1 with no evidence of a clinically significant abnormality; Ability to complete study materials as measured by compliance with diary completion and PEF measurements between Visits 1- 3; Uncontrolled asthma selected on all the following criteria:

- Diagnosis of asthma > 12 months
- Bronchodilator response at Visit 1 *or* 2
- Prebronchodilator FEV1 ≥ 40% and ≤ 80% predicted at Visits 1 and 3
- Use of ICS ≥ 200 μg and ≤ 1000 μg total daily dose of fluticasone propionate (FP) or equivalent for at least consecutive 6 months prior to Visit 1
- Visit 3 Asthma Control Questionnaire (ACQ) s score ≥ 1.5 despite ICS compliance.

[0303] Key Exclusion Criteria included the following:

[0304] Medical conditions:

- Asthma exacerbation, significant airflow obstruction, or respiratory infection from Visits 1 – 3
- Known malignancy or current evaluation for a potential malignancy
- Known immunodeficiency, including but not limited to HIV infection
- Pre-existing lung disease other than asthma, including active infections
- Uncontrolled clinically significant medical disease

[0305] Exposures:

- Current smoker or former smoker with a lifetime smoking history of >10 pack-years
- Prohibited concomitant medications (Steroids other than ICS, short-acting bronchodilators other then SABA, immunomodulatory agents)
- Pregnancy or not willing to use highly effective contraception

[0306] Given the practical difficulties of measuring IL-13 in the lung itself, eosinophil and IgE levels in peripheral blood were used as a surrogate measure, denoted as "IL-13 signature surrogate." IL-13 signature surrogate–positive patients were defined as patients with total IgE >100 IU/mL and blood eosinophils ≥0.14 x 10xe9 cells/L, whereas the IL-13 signature surrogate–negative patients had a total IgE ≤100 IU/mL or blood eosinophils < 0.14 x 10xe9 cells/L. The criteria for the IgE and eosinophil levels that determined patients' status for IL-13 signature surrogate were established from patients with asthma in whom IL-13 induced gene expression in the bronchial epithelium (IL-13 signature) was correlated with peripheral blood IgE and eosinophils (Corren et al., N Engl J Med 365:1088-1098 [2011]).

[0307] The efficacy of lebrikizumab was assessed using multiple measures of asthma activity, including pulmonary function (i.e., FEV1, peak flow including variability in peak flow, response to methacholine at selected centers, fractional exhaled nitric oxide [FE_{NO}]) and measures of disease activity or control (i.e., patient-reported outcomes [PROs], use of rescue medication, rate of exacerbations). Change in FEV1 was the primary outcome. The following markers after treatment with lebrikizumab: TARC (CCL17), MCP-4 (CCL13) and IgE for pharmacodynamic analyses.

Primary Efficacy Outcome Measure

[0308] The primary efficacy outcome measure was the relative change in pre-bronchodilator FEV1 (volume) from baseline to Week 12.

Secondary Efficacy Outcome Measures

[0309] The secondary efficacy outcome measures were the following: Relative change in pre-bronchodilator FEV1 (volume) from baseline to Week 24; Relative change in pre-bronchodilator FEV1 (volume) from baseline to Week 12 for patients with IL-13 signature surrogate positive status; Change in Asthma Control Questionnaire (ACQ) score from baseline to Week 12; Change in Asthma Symptom Score as measured by the Asthma Control Daily Diary (ACDD) from baseline to Week 12; Change in morning pre-bronchodilator peak flow value from baseline to Week 12; Rate of asthma exacerbations during the 24-week treatment period; Rate of severe asthma exacerbations during the 24-week treatment period; Change in rescue medication use (measured by number of puffs per day of rescue medication or nebulized rescue medication) from baseline to Week 12.

Exploratory Measures:

[0310] The following exploratory outcome measures were assessed: Change in the number of days per week with well controlled asthma, as measured by the ACDD from baseline to Week 12; Change in weekly frequency of nocturnal awakening due to asthma from baseline to Week 12; Change in FE_{NO} levels from baseline to Week 12.

Initial Observations

[0311] In this study of patients with poorly controlled asthma, lebrikizumab treatment was associated with a statistically significant improvement in pre-bronchodilator FEV1, the primary outcome variable. The improvement in FEV1 occurred soon after the initiation of treatment, indicating that IL-13 inhibition impacted measures of airflow relatively quickly. Although lebrikizumab treatment did not lead to statistically significant reductions in protocol-defined and severe exacerbations using the Elecsys® periostin assay (described below, due to unavailable values to contribute to the analysis), a trend towards a decrease in rates of severe exacerbations, especially for periostin high patients, was observed. However, using the E4 Assay as described below, lebrikizumab treatment did lead to statistically significant reductions in protocol-defined and severe exacerbations (84% in the periostin high subgroup (95% CI 14%, 97%, p=0.03). See also below. Furthermore, the FE_{NO} subgroup analysis did achieve a statistically significant reduction in severe exacerbations (p=0.04). Lebrikizumab treatment did not improve asthma symptoms as measured by the symptom-only version of the ACQ5 (which excluded rescue SABA use and FEV1) or the daily diary measures.

[0312] The reduction in serum Th2 chemokines, CCL13 and CCL17, and IgE supports a lebrikizumab-mediated biologic effect that underlies the clinical impact measured in the airway. The slight increase in blood eosinophil count is consistent with an overall reduced trafficking of eosinophils from the blood to the lung compartment following inhibition of eosinophil-attracting chemokines. The finding that lebrikizumab decreased FE_{NO} is consistent with this suggestion. However, lebrikizumab may have decreased FE_{NO} by indirectly inhibiting nitric oxide synthase expression via IL-13 blockade, rather than by modifying eosinophilic inflammation (which is also thought to impact FE_{NO}).

[0313] The patient eligibility criteria for this study required reversibility of at least 12% to 400 μg of albuterol (with no SABA use for at least 4 hours and no LABA use for at least 12 hours before visits). This was to ensure that patients had "room to move" when looking for an overall relative change of at least 10% in lung function. This simple clinical test may limit the ability to generalize these data to unselected, more general asthma patient populations. Indeed, the most common reason for patient ineligibility was failure of this test (in 92 of 263 patients who failed screening).

[0314] In this study, we first hypothesized that the combination of high serum IgE and high blood eosinophil count was a surrogate for identifying patients with increased expression of IL-13 related genes in the lung (IL-13 signature surrogate, or Th2 high). While the data were still masked to treatment assignment, we wrote a statistical analysis plan to use differentiation based on serum periostin levels. As described in more detail below, this subgroup analysis showed that the effectiveness of lebrikizumab treatment was enhanced in periostin-high relative to periostin-low patients, as observed with both a more robust increase in FEV1 and a greater decline in FE_{NO}, as well as a significant interaction test. These findings suggest that the prespecified marker, serum periostin, could potentially be used to select asthma patients who may be more responsive to lebrikizumab treatment.

[0315] The baseline characteristics of the patients participating in the study are shown in Figure 6. The numbers refer to the mean (SD) unless otherwise noted. Periostin high refers to patients with baseline periostin above 23ng/ml according to the E4 Assay described below. Periostin low refers to patients with baseline periostin below 23ng/ml according to the E4 Assay described below.

[0316] The relative changes in FEV1 (liters) at 12 weeks and at 24 weeks (95% confidence intervals) for patients treated are shown in Figure 7. As compared to the IL-13

signature positive patients, the periostin positive patients experienced a higher relative change in FEV1.

[0317] Figure 7 shows that the placebo corrected relative change in FEV1 from baseline to 12 weeks for all-comers was 5.9% (95% CI 0.9%, 10.9%, p=0.2) and 10% for the periostin high subgroup (95% CI 2.5%, 17.5%, p=0.01)). The placebo corrected relative change in FEV1 from baseline to 24 weeks for all-comers was 4.8% (95% CI 0.3%, 9.3%, p=0.04) and 6.1% for the periostin high subgroup (95% CI -1.4%, 13.6%. p=0.11). Figure 8 shows the relative change in FEV1 throughout the treatment period (32 weeks) for all efficacy evaluable subjects (A) and for periostin high subjects only (B). The efficacy of lebrikizumab at 32 weeks of treatment did not wane suggesting that it may be possible to decrease the frequency in which lebrikizumab is administered.

[0318] We also examined the effect of lebrikizumab treatment on post-bronchodilator FEV1 as an exploratory outcome and an indirect surrogate for airway remodeling. Change in post-bronchodilator FEV1 at 20 weeks was measured after four inhalations of 100mcg albuterol. At baseline before study drug administration, the mean post-bronchodilator FEV1 was 2.50 liters (0.71) and 2.54 liters (0.73) in the placebo and lebrikizumab groups, respectively. This corresponded to 77.9% predicted in both groups. The overall placebo-corrected change in post-bronchodilator FEV1 was 4.9% (95% CI 0.2% to 9.6%; p=0.04). In the periostin high group, lebrikizumab treatment increased the post-bronchodilator FEV1 at 20 weeks (8.5%; 95% CI 1.1% to 16%; p=0.03). There was no evidence of improvement in post-bronchodilator FEV1 in the periostin low group (1.8%; 95% CI –4.1 to 7.7; p=0.55). In addition, lebrikizumab increased the absolute post-bronchodilator FEV1 in the periostin high group (170 mls; 95% CI 10 to 320 ml). We conclude that lebrikizumab improved post-bronchodilator FEV1 in uncontrolled asthma patients who had evidence of increased Th2 airway inflammation (periostin high). Based upon this data, lebrikizumab may have the potential to reduce airway remodeling in asthma.

[0319] Figure 9 shows the exacerbation rate and severe exacerbation rate observed at 24 weeks of treatment. The exacerbation rate was reduced by 37% in lebrikizumab treated all-comers compared to placebo (95% CI -22%, 67%, p=0.17) and by 61% in the periostin high subgroup (95% CI -6%, 86%, p=0.07). The severe exacerbation rate was reduced by 51% (95% CI -33%, 82%, p=0.16) for all comers and by 84% in the periostin high subgroup (95% CI 14%, 97%, p=0.03). We also examined the rates of severe exacerbations at 32 weeks, the end of the follow-up period, 12 weeks after the final dose of lebrikizumab in the ITT

population. As shown in Table 10, severe exacerbation rates across all patients at 32 weeks were significantly reduced by 50% in lebrikizumab treated patients compared with placebo (p=0.03). The rate of severe exacerbations was lowest in periostin-high patients treated with lebrikizumab (0.13); however, the rate reduction from placebo did not reach statistical significance in the subanalyses. We conclude that Lebrikizumab had a significant benefit in reducing severe exacerbations in patients inadequately controlled by ICS. The greatest reduction in severe exacerbations was seen in patients with high periostin levels prior to treatment. The duration of observation to capture events is important in powering studies for this end-point and 32-weeks observation compared to 24 weeks may be needed to detect at least a 50% reduction in approximately 200 subjects.

Table 10. Severe exacerbation rate at 32 weeks, 12 weeks post-final dose.

Group*	Lebrikizumab, severe exacerbation rate	Placebo, severe exacerbation rate	Rate reduction (95% CI) p-value
All patients (n=218)	0.17	0.34	50% (9, 72) p=0.03
Periostin-high (n=110)	0.13	0.32	61% (–1, 85) p=0.06
Periostin-low (n=101)	0.23	0.40	43% (–30, 75) p=0.18

[0320] Lebrikizumab met its primary endpoint and was very effective in reducing severe exacerbations. The data suggests that the periostin status of a patient can be prognostic of exacerbations events and to a lesser extent FEV1. The data supports the predictive value of periostin levels for determining response to lebrikizumab treatment. For example, using the E4 assay, those patients with serum or plasma periostin levels below 20ng/ml are less likely to benefit from lebrikizumab whereas those patients with serum or plasma periostin levels above 20ng/ml are more likely to benefit from lebrikizumab. Furthermore, those patients with serum or plasma periostin levels above 23ng/ml (as determined by the E4 assay) have even a greater likihood of benefiting from lebrikizumab treatment.

[0321] Patient Reported Outcomes: The ACQ and mini-AQLQ did not demonstrate consistent differences between treatment groups. After several visits, the ACDD data demonstrated some differences in the mean scores between treatment groups in well

controlled days, asthma symptoms, nocturnal awakening, and rescue medication use, all favoring lebrikizumab treatment. For all endpoints in the ACDD data (well controlled days, asthma symptioms, nocturnal awakening, and rescue medication use), there was a greater placebo corrected treatment effect in periostin high subjects compared to all subjects; however, none of the differences between treatment groups at 12 weeks were statistically significant.

[0322] Lung function: Improvement in lung function (PEF) from baseline was observed for most time points in all lebrikizumab treated subjects and at all time points for periostin high lebrikizumab treated subjects. Placebo treated subjects declined from baseline at all time points.

[0323] Lung Inflammation: FE_{NO} declined (improved) throughout the study for both lebrikizumab treated subjects and those in the periostin high subset, while placebo treated subjects had increases in FE_{NO} (worsening). The percent change in FE_{NO} at 12 weeks was -20.1% for lebrikizumab treated subjects (n=83) versus 15% for placebo-treated subjects (n=86) [$p<0.01$]. The percent change in FE_{NO} at 12 weeks was -24.2% for lebrikizumab treated subjects versus 17.5% for placebo-treated subjects (n=44) [$p<0.01$]. Differences between treatment groups at 12 weeks were statistically significant. See Figure 10. In a post hoc analysis, high baseline FE_{NO} was also associated with efficacy in improving FEV1 and a lower severe exacerbation rate compared to placebo. However we noted that baseline FE_{NO} showed greater intra-patient variability during the run-in than did periostin (19.8% versus 5.0%).

[0324] We also examined the data to determine whether patients who were both periostin-low and FE_{NO} -high benefited from lebrikizumab treatment to the same degree as periostin-high patients. At baseline, we observed a moderate correlation between FE_{NO} and serum periostin ($rs = 0.31$, $P<0.001$; N=210 with matching data). Using median cutoffs, periostin and FE_{NO} status generally but incompletely overlapped (Table 11). For the data shown in Table 11, the periostin cut-off was 25 ng/mL using the E4 Assay described below and the FE_{NO} cut-off was 21 ppb as measured using standard methodology known in the art. Evaluation of FEV1 based on composite periostin and FE_{NO} status revealed that the placebo-adjusted effect of lebrikizumab at 12 weeks was greatest in the group with high levels of both periostin and FE_{NO} (Table 12) . In contrast, the observed treatment benefit in the periostin-low/ FE_{NO} -high group was marginal (2.3%, P=0.73). Thus, FE_{NO} does not appear to identify

a subset of periostin-low patients who benefit from lebrikizumab. The relative predictive value of periostin and FE_{NO} for response to lebrikizumab treatment merits further study.

Table 11. Distribution of patients according to baseline periostin and FE_{NO} status.

		FE_{NO} status	
		Low	High
Periostin status	Low	62	39
	High	42	67

p=0.0004 by Fisher's exact test (1-sided)

Table 12. Mean Relative Change From Baseline FEV_1 at 12 Weeks in ITT Patients. The median baseline levels of FE_{NO} or periostin for all patients who met protocol-defined entry criteria were used to define the subsets described in the table (high = median value or higher; low = less than median value).

	All Subjects (n=218)	**Periostin-low FE_{NO}-low (n=62)**	**Periostin-low FE_{NO}-high (n=39)**	**Periostin-high FE_{NO}-low (n=42)**	**Periostin-high FE_{NO}-high (n=67)**
Lebrikizumab	9.8%	2.6%	9.1%	8.6%	16.7%
Placebo	4.3%	1.5%	6.8%	6.3%	5.4%
Difference	5.5%	1.1%	2.3%	2.3%	11.2%
(95% CI)	(0.8%, 10.2%)	(-4.5%, 6.6%)	(-10.7%, 15.2%)	(-9.3%, 13.9%)	(1.9%, 20.5%)

[0325] The pharmacokinetic characteristics of lebrikizumab were similar to those which had been seen in previous studies. Body weight of the subjects had an impact on the pharmacokinetics of lebrikizumab.

[0326] Several markers were evaluated for their ability to provide predictive value in terms of treatment benefit above, in addition to or as an alternative to serum periostin levels. Those markers included CEA, IgE, TARC (CCL17) and MCP-4 (CCL13).

[0327] In addition, we hypothesized that the excess serum periostin in periostin-high patients with asthma is due to the effects of IL-13. Thus, we sought to assess the relative contribution of IL-13 to total systemic periostin levels in both placebo and lebrikizumab-treated patients with uncontrolled asthma. For these experiments, we measured serum periostin using the Elecsys® periostin assay described in Example 7. As shown in Figures 18 and 19, we found that the majority (>90%) patients who were periostin-low at baseline

remained low in both arms of the study (Fig. 18A), whereas 72% of periostin-high patients treated with placebo and 40% treated with lebrikizumab remained periostin-high at Week 12 (Fig. 18B). We conclude that in adults with uncontrolled asthma despite ICS, periostin-high patients exhibited a significant reduction in serum periostin levels upon lebrikizumab treatment as compared with placebo, but periostin-low patients exhibited no significant reduction in response to lebrikizumab. These findings suggest that in uncontrolled asthma patients, excess serum periostin is due to the activity of IL-13, and that inhibition of IL-13 with lebrikizumab decreases serum periostin levels.

[0328] Four lebrikizumab-treated patients experienced serious adverse events (SAEs) (asthma exacerbation [n=2], community-acquired pneumonia, and traumatic pneumothorax related to a car accident). Six placebo patients experienced seven SAEs (asthma exacerbation [n=2], headache, cerebrospinal fluid leak after epidural, shingles, herpes zoster, acute purulent meningitis, and pain medication addiction). See Figure 11 for safety results from this trial.

[0329] The overall frequency of AEs was similar in both treatment arms (lebrikizumab, 74.5%; placebo, 78.6%), as were the frequencies of serious AEs (lebrikizumab, 3.8%; placebo, 5.4%) (Table 9). Musculoskeletal events were more common with lebrikizumab (lebrikizumab, 13.2%; placebo, 5.4%; P=0.045) (Table 9). Twenty-five patients (11.5%) discontinued the study early, including 12 placebo- and 13 lebrikizumab-treated patients.

Table 9

Type of Musculoskeletal or Connective Tissue Disorder	Number of Participants Reporting at Least One Event (%)		
	Placebo (n=112)	Lebrikizumab (n=106)	All (n=218)
Arthralgia	2 (1.8)	3 (2.8)	5 (2.3)
Back pain	2 (1.8)	1 (0.9)	3 (1.4)
Pain in extremity	1 (0.9)	2 (1.9)	3 (1.4)
Myalgia	0	2 (1.9)	2 (0.9)
Neck pain	2 (1.8)	0	2 (0.9)
Arthritis	0	1 (0.9)	1 (0.5)
Bone development abnormal	1 (0.9)	0	1 (0.5)

Bursitis	0	1 (0.9)	1 (0.5)
Costochondritis	0	1 (0.9)	1 (0.5)
Exostosis	0	1 (0.9)	1 (0.5)
Flank pain	0	1 (0.9)	1 (0.5)
Musculoskeletal chest pain	0	1 (0.9)	1 (0.5)
Musculoskeletal pain	0	1 (0.9)	1 (0.5)
Pain in jaw	1 (0.9)	0	1 (0.5)
Tendinitis	0	1 (0.9)	1 (0.5)
Musculoskeletal and connective tissue disorders (Total)	6 (5.4)	14 (13.2)	20 (9.2)

EXAMPLE 3 – Asthma Patient Study II (Dose Ranging Study)

[0330] A randomized, double-blind, placebo controlled, four-arm, dose-ranging study was conducted to further evaluate the relationship between the dose of lebrikizumab and the response in terms of the efficacy, safety and tolerability in patients with asthma who were not on inhaled corticosteroids. See Figure 12 for a schematic design of this trial. Selected patients had a bronchodilator response of at least 15% and a pre-bronchodilator FEV1 $\geq$60% and $\leq$85% predicted with disease stability demonstrated during the run-in period. Selected patients for the study previously managed with ICS could not have received ICS for a minimum of 30 days prior to the first study visit (Visit 1). The study had no withdrawal period; patients were not to be taken off corticosteroids for the sole purpose of becoming eligible for the study.

[0331] The first study-related procedure at Visit 1 began the approximately 2-week run-in period. During the run-in period (Visits 1–3), asthma control (as measured by the Asthma Control Questionnaire [ACQ] score) and pulmonary function were assessed. Patients' asthma was further characterized with skin prick testing and relevant biomarkers including IgE and peripheral blood eosoinophils. The IgE and eosinophil levels were used to classify patients on the basis of their IL-13 signature surrogate status. At the end of the run-in period, eligible patients were randomly allocated (1:1:1:1) to receive one of three doses (500 mg, 250 mg or 125 mg) of lebrikizumab or placebo via SC administration. Study drug was administered four times during the 12-week treatment period. After the final dose of study drug, patients were

monitored for an additional 8-week follow-up period. Therefore, most patients participated in the study for a total of approximately 22 weeks after Visit 1, during which time intermittent PK samples were obtained. Intensive PK sampling was performed with additional PK samples obtained during the study and follow-up period as well as an additional PK sample 16 weeks after the last study drug administration, until 70 subjects completed the intensive PK sampling. Therefore, study participation for the patients in the intensive PK sampling group last approximately 26 weeks after Visit 1. Safety was assessed throughout the study and pre-specified thresholds for treatment failure were defined so that patients could be taken off study drug to resume standard therapy if they have a clinically significant deterioration.

Initial Observations

[0332] Efficacy: The placebo corrected relative change in FEV1 from baseline to 12 weeks for all lebrikizumab treated subjects was 4.1% (95% CI-0.4, 8.6%; p = 0.075) improvement. Throughout the 12 week treatment period, the estimated risk of treatment failure in all lebrikizumab treated patients was 75% lower than placebo treated patients (HR 0.25, 95% CI: 0.11, 0.78; p = 0.001). These results were considered clinically and statistically significant. There was no evidence of a dose response (see Fig. 16).

[0333] Safety: There were no clinically significant imbalances between the lebrikizumab treated and placebo treated patients except for injection site reactions (23% versus 6%, lebrikizumab to placebo respectively).

EXAMPLE 4 – PERIOSTIN ASSAY (E4 Assay)

[0334] A periostin capture ELISA assay that is very sensitive (sensitivity 1.88ng/ml is described below. The antibodies recognize periostin isoforms 1-4 at nM affinity (SEQ ID NOs:5-8)).

[0335] Dilute 80 uL of purified monoclonal antibody, 25D4 (Coat Antibody, SEQ ID NOs: 1 and 2 expressed from a hybridoma or a CHO cell line) with phosphate buffered saline to a final concentration of 2 ug/mL. Coat microtiter plates overnight, covered, at 2-8°C with Coat Antibody 100 µL per well. Wash plate three times with 400 µL wash buffer (PBS/0.05% Tween (polysorbate 20) per well per cycle of wash buffer at room temperature. Add 200 µL per well of blocking buffer to plate. Incubate covered plate at room temp with shaking for 1.5 hours.

[0336] Prepare rhuPeriostin standard curve (Standard Stock of rhuPeriostin = rhuPeriostin isoform 1, R&D systems #3548-F2, 5.25ng/ml, in Assay Diluent (PBS/0.5%bovine serum

albumin (BSA)/0.05% polysorbate 20/0.05% ProClin300, pH7.4). Standard curve diluent = PBS/0.5%BSA/0.05% polysorbate 20, 0.05% ProClin300, pH 7.4. For example:

Std conc (pg/mL)	Procedure
600	80 µL rhuPeriostin, 5.25ng/ml in Assay Diluent + 620 µL standard curve diluent
300	300 µL 600 pg/mL rhuPeriostin + 300 µL standard curve diluent
150	300 µL 300 pg/mL rhuPeriostin + 300 µL standard curve diluent
75	300 µL 150 pg/mL rhuPeriostin + 300 µL standard curve diluent
37.5	300 µL 75 pg/mL rhuPeriostin + 300 µL standard curve diluent
18.75	300 µL 37.5 pg/mL rhuPeriostin + 300 µL standard curve diluent
9.38	300 µL 18.75 pg/mL rhuPeriostin + 300 µL standard curve diluent
0	standard curve diluent

[0337] Prepare Controls and samples. Three controls: Spike Source Control (rhuPeriostin full length, isoform 1, R&D Systems #3548-F2), Normal Matrix Control (normal human serum pool, Bioreclamation, Inc.), High Matrix Control (normal human serum pool, plus 100ng/ml rhuPeriostin spike).

[0338] For example:

[0339] 10 µL Control (or sample) serum + 1.99 mL sample/control diluent = 1:200

300 µL 1:200 dilution + 300 µL sample/control diluent = 1:400

300 µL 1:400 dilution + 300 µL sample/control diluent = 1:800

300 µL 1:800 dilution + 300 µL sample/control diluent = 1:1600

Each dilution is run in singlicate

[0340] Construct Matrix Controls using a normal human serum pool. Use unspiked pooled human serum as the Normal Control. Generate the High Control by spiking 100 ng/mL rhuPOSTN into the pooled serum as described above. Compute mean, standard deviation (SD), and % coefficient of variance (CV, expressed in percent) for the four dilutions for each control on every plate. CV is Quantifies magnitude of variance in replicate measurements with respect to mean of replicates. %CV=100*(SD/mean). Evaluate these mean concentrations across all plates to determine inter-plate precision. This control table is then used to define the Normal and High Control pass/fail criteria, setting allowable variance to ± 20% of the mean concentration for each control.

[0341] Wash plate three times with 400 µL per well per cycle of wash buffer (PBS/0.05% polysorbate 20). Add diluted standards (duplicate wells), controls (all four dilutions), and samples (all four dilutions) to plate, 100 µL per well. Incubate plate covered, at room temperature with shaking for 2 hours at room temp. Dilute 80 uL detection MAb stock I (biotinylated murine anti-human periostin, MAb 23B9, 7.5ug/ml in Assay Diluent) to 12 mL with Assay Diluent = 50 ng/mL. Wash plate four times with 400 µL per well per cycle of wash buffer. Add diluted detection MAb to plate, 100 µL per well. Incubate covered plate at room temp for one hour with shaking. Dilute 80 uL streptavidin-HRP stock I (AMDEX streptavidin-HRP, GE Healthcare #RPN4401 ,approximately 1mg/ml) diluted 1:80 in Assay Diluent to 12 mL with Assay Diluent = 1:12k. Wash plate four times with 400 µL per well per cycle of wash buffer. Add diluted streptavidin-HRP to plate, 100 µL per well. Incubate covered plate at room temp for 45 min. with shaking. Bring Kirkegaard and Perry (KPL) two-step TMB reagents to room temp; do not combine. Wash plate four times with 400 µL per well per cycle of wash buffer. Mix equal volumes of KPL TMB substrate components and add to plate, 100 µL per well. Incubate plate for 20 minutes at room temperature with shaking. Add 1 M phosphoric acid to plate, 100 µL per well. Read plate using 450 nm read wavelength and 650 nm reference wavelength. This assay or an assay similar to the above assay was used in the clinical trial described in Example 3.

[0342] A periostin assay using antibodies against isoform 1 (not Total Periostin) was tested on different asthma patient samples using a similar antibody capture format. Preliminary results indicate that periostin isoform 1 is not as robust as a marker for TH2 inflammation as total periostin.

EXAMPLE 5 – Asthma Patient Observational Study (BOBCAT)

[0343] We previously reported certain biomarker findings based on our studies of biological samples stored in the Airway Tissue Bank at the University of California, San Francisco (UCSF) that had been collected during bronchoscopy performed for research purposes in healthy and asthmatic volunteers. See, e.g., Intn'l Pub. No. WO2009/124090. To verify these findings in a large cohort of moderate-to-severe asthma and generalize them across multiple clinical sites, we conducted a multi-center 3-visit observational study ("BOBCAT") of uncontrolled moderate-to-severe asthmatics (ACQ > 1.50 and FEV1 between 40-80%) on high doses of ICS (> 1000 µg/day fluticasone DPI or equivalent) with collection of induced sputum, endobronchial biopsies, and peripheral blood. We obtained

matched blood and airway data from 59 subjects (see study overview in Fig. 13). Details of the study are provided below.

[0344] BOBCAT (Bronchoscopic exploratory research study Of Biomarkers in Corticosteroid-refractory AsThma) was a multi-center study conducted in the United States, Canada, and United Kingdom to collect matched airway and blood samples in approximately 60 moderate to severe asthmatics. Inclusion criteria required a diagnosis of moderate to severe asthma (confirmed by an FEV1 between 40-80% of predicted as well as evidence within the past 5 years of > 12% reversibility of airway obstruction with a short-acting bronchodilator, or methacholine sensitivity (PC20) < 8 mg/ml) that was uncontrolled (as defined by at least 2 exacerbations in the prior year, or a score of > 1.50 on the Asthma Control Questionnaire (ACQ) (Juniper, E.F., et al., Respir Med 100, 616-621 (2006)) while on a stable dose regimen (> 6 weeks) of high dose ICS (> 1000 μg fluticasone or equivalent per day)) with or without a long-acting beta agonist. Permitted concomitant medications also included leukotriene receptor antagonists and oral corticosteroids. BOBCAT study scheme is depicted in Fig. 13.

[0345] FE_{NO}, bronchoscopy, BAL, induced sputum, and immunohistochemistry for eosinophil counts were performed as previously described (Woodruff, P.G., et al., Am J Respir Crit Care Med 180, 388-395 (2009); Boushey, H.A., et al., N Engl J Med 352, 1519-1528 (2005); Brightling, C.E., et al., Thorax 58, 528-532 (2003); Lemiere, et al., J Allergy Clin Immunol 118, 1033-1039 (2006)). All research protocols were approved by relevant institutional review boards and informed consent was obtained from all subjects prior to enrollment. Patient demographics and lung function data are summarized in Table 5 below.

Table 5. BOBCAT demographic and clinical data.

	UCSF Healthy control	**UCSF cohort 1 Mild-moderate asthma, no ICS**	**UCSF cohort 2 Moderate asthma, on ICS**	**UCSF cohort 3 Moderate asthma, on ICS**	**Leicester Healthy control**	**Leicester Mod-severe asthma, on ICS**	**BOBCAT Mod-severe asthma, on ICS**
N subjects	13	15	24	23	10	27	67
Age	35 ± 11	35 ± 14	36 ± 11	43 ± 14	33 ± 18	36 ± 11	46 ± 12
Sex (M:F)	7:6	8:7	7:17	10:13	4:6	18:9	32:35
FEV1 (% predicted)	99 ± 15	85 ± 12	84 ± 14	75 ± 19	99 ± 12	79 ± 18	60 ± 11
ACQ score	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.7 ± 0.8

	UCSF Healthy control	UCSF cohort 1 Mild-moderate asthma, no ICS	UCSF cohort 2 Moderate asthma, on ICS	UCSF cohort 3 Moderate asthma, on ICS	Leicester Healthy control	Leicester Mod-severe asthma, on ICS	BOBCAT Mod-severe asthma, on ICS
Daily ICS dose (μg FPI equivalent*)	0	0	250	N.D.	0	500 (50-1500)**	≥ 1000***

N.D., not determined
Values presented as mean ± SD or median (range)
* FPI, fluticasone dipropionate. Equivalent ratio of fluticasone:budesonide = 1:1.6
** of these, 6 subjects were also on systemic corticosteroids, receiving between 4-20 mg prednisolone equivalents/day
*** of these, 7 subjects were also on systemic corticosteroids, receiving between 5-40 mg prednisolone equivalents/day

[0346] We used pre-specified cutoff values consistent with previous studies of 3% for sputum eosinophils (Green, R.H., et al., Lancet 360, 1715-1721 (2002); Haldar, P., et al., N Engl J Med 360, 973-984 (2009)) and 22 eosinophils/mm2 total biopsy area (Miranda, C., A. et al., J Allergy Clin Immunol 113, 101-108 (2004); Silkoff, P.E., et al., J Allergy Clin Immunol 116, 1249-1255 (2005)). Serum periostin levels were very stable within individual subjects across the three visits spanning up to 5 weeks (data not shown). Mean periostin levels were significantly higher in "eosinophil high" compared to "eosinophil low" subjects as defined by sputum or tissue eosinophil measurements (data not shown). Subjects stratified by a composite score of 0 for neither, 1 for either, or 2 for both sputum and tissue eosinophilia exhibited a highly significant trend for increasing serum periostin with increasing eosinophil scores (data not shown). Furthermore, non-eosinophilic asthmatics across all cohorts consistently had serum periostin levels below 25 ng/ml using the E4 Assay described above. Using 25 ng/ml serum periostin as a cutoff, "eosinophil-low" and "eosinophil-high" subjects in BOBCAT were effectively differentiated with a positive predictive value of 93% (Table 6). Tissue neutrophil counts were positively correlated with tissue eosinophils and serum periostin levels (data not shown). Taken together, these data show that serum periostin is a systemic biomarker of persistent airway eosinophilia in moderate-to-severe asthmatics despite steroid treatment.

Table 6. Contingency table for cutoff values of serum periostin (N=57) and FE_{NO} (N=56) vs. composite airway eosinophil status in BOBCAT.

Test		**Serum Periostin ≥ 25 ng/ml**		**FE_{NO} ≥ 35 ppb**	
Airway phenotype		Eosinophil low	Eosinophil high	Eosinophil low	Eosinophil high
Test result	-	11	19	12	26
	+	2	25	1	17

Sensitivity	0.57	0.40
Specificity	0.85	0.92
PPV	0.93	0.94
NPV	0.37	0.32
p-value	0.011	0.042

Eosinophil low: sputum eosinophils < 3% AND biopsy eosinophils < 22/mm2
Eosinophil high: sputum eosinophils > 3% OR biopsy eosinophils > 22/mm2
PPV, positive predictive value
NPV, negative predictive value
p-value is Fisher's exact test (2-tailed)

Comparison of serum periostin to fractional exhaled nitric oxide (FE_{NO}), peripheral blood eosinophils, serum IgE, and serum YKL-40 as asthma biomarkers

[0347] In recent years, other non-invasive biomarkers of asthma severity and airway inflammation have been described. Four markers of particular interest are fractional exhaled nitric oxide (FE_{NO}), an exhaled gas produced by the action of the enzyme iNOS (inducible nitric oxide synthase) in inflamed bronchial mucosa (Pavord, I.D. et al., J Asthma 45, 523-531 (2008)); peripheral blood eosinophils; serum IgE; and YKL-40, a chitinase-like protein detectable in peripheral blood (Chupp, G.L., et al., N Engl J Med 357, 2016-2027 (2007)). We measured these biomarkers in our asthmatic cohorts and compared values with airway eosinophilia and other biomarkers.

[0348] Neither periostin, FE_{NO}, IgE, nor blood eosinophils was significantly correlated with ACQ, FEV1, age, gender, or body mass index (BMI) in BOBCAT. In BOBCAT, FE_{NO} levels, like serum periostin levels, were generally consistent across multiple visits, although FE_{NO} varied more at higher levels (data not shown) whereas blood eosinophils were considerably more variable (r2=0.18 for blood eosinophils between visits 1 and 3, not shown, as compared to r2=0.65 for serum periostin between visits 1 and 3). As shown in Fig. 14 A-F, serum periostin levels at visits 1, 2, and 3 were highly correlated with each other and with the mean periostin level across all visits in BOBCAT.

[0349] Stratifying for sputum and biopsy eosinophil status as indicated in Table 6, FE_{NO} levels were significantly higher in eosinophilic asthmatics compared to noneosinophilic asthmatics. However, while both FE_{NO} and periostin had a high degree of specificity, exhibiting consistently low values for "eosinophil-low" subjects, FE_{NO} detected fewer subjects with tissue eosinophilia and exhibited greater overlap between "eosinophil-low" and "eosinophil-high" subjects according to each metric employed (Figs. 15A-D). We fit a logistic regression model incorporating age, sex, BMI, blood eosinophils, serum IgE, FE_{NO},

and serum periostin (Table 7), and found that periostin was the most significant single predictor of composite airway eosinophil status (p=0.007).

Table 7. Logistic regression model of biomarkers vs. eosinophil status in the BOBCAT study (N=59).

	Estimate	Std. Error	z-score	p-value
Age	-0.0396	0.039	-1.015	0.31
Sex (male)	-0.2031	0.889	-0.229	0.82
Body mass index	-0.1004	0.066	-1.527	0.13
Blood eosinophils	1.7482	3.621	0.483	0.63
Serum IgE	-0.0002	0.001	-0.100	0.92
FE_{NO}	0.0476	0.038	1.238	0.22
Serum periostin	0.2491	0.092	2.719	***0.007***

[0350] Using a cutoff value of 35 ppb as previously described (Dweik, R.A., et al., Am J Respir Crit Care Med 181, 1033-1041 (2010)), FE_{NO} differentiated "eosinophil-low" and "eosinophil-high" asthmatics with comparable specificity to but lower sensitivity than a periostin cutoff of 25 ng/ml (Table 6). Peripheral blood eosinophils trended higher in "eosinophil-high" asthmatics but did not reach statistical significance (data not shown). To assess the relative performance of each marker on a continuous basis, we performed receiver operating characteristic (ROC) analysis of periostin, FE_{NO}, blood eosinophils, and serum IgE vs. composite airway eosinophil status and found that periostin performed favorably to FE_{NO} (AUCs of 0.84 and 0.79 respectively), while blood eosinophils and serum IgE performed substantially less well (Fig. 15 E).

[0351] YKL-40 showed no significant correlations with periostin nor with any measures of airway or peripheral eosinophilia in any cohort (data not shown). Consistent with these findings of exhaled and blood biomarker levels, we found that bronchial epithelial gene expression levels of periostin and NOS2 (the gene that encodes iNOS) were significantly correlated with each other and with bronchial mucosal expression levels of IL-13 and IL-5 while expression of CHI3L1 (the gene that encodes YKL-40) was not correlated with periostin, IL-13, nor IL-5 (Table 8). Taken together, these data suggest that peripheral blood periostin is a more reliable indicator of airway Th2/eosinophilic inflammation than FE_{NO}, blood eosinophils, serum IgE, or YKL-40 in asthmatics across a range of severity and steroid treatment.

Table 8. Correlation matrix between expression levels of genes encoding biomarkers and Th2 cytokines.

	POSTN_210809_s_at	NOS2_210037_s_at	CHI3L1_209395_at
IL-13	***0.42 (0.014)***	***0.37 (0.029)***	-0.23 (0.198)
IL-5	***0.42 (0.013)***	***0.34 (0.048)***	-0.13 (0.450)
POSTN_210809_s_at	-	***0.72 (<0.001)***	-0.24 (0.136)
NOS2_210037_s_at	-	-	***-0.34 (0.027)***

Values given as Spearman's rank correlation (p-value)
IL-13 and IL-5 expression levels determined from endobronchial biopsies by qPCR
Periostin (POSTN_210809_s_at), NOS2 (NOS2_210037_s_at), and CHI3L1 (CHI3L1_209395_at), the gene encoding YKL-40, determined from bronchial epithelial microarray described in Truyen, E., L. et al. *Thorax* **61**, 202-208 (2006).

Discussion

[0352] While asthma is traditionally regarded as an allergic disease mediated by Th2-driven inflammation(1), there is emerging evidence of pathophysiological heterogeneity(3-8). We have recently shown that, in mild-to-moderate asthmatics not on steroid treatment, only about half the subjects have evidence of Th2 inflammation in their airways. The "Th2-high" subset is distinguished by elevated markers of allergy, eosinophilic airway inflammation, bronchial fibrosis, and sensitivity to ICS(13). As antagonists of the Th2 cytokines IL-4, IL-5, and IL-13 are under active development as asthma therapeutics(35-37), it will become important to identify asthmatics most likely to benefit from these targeted therapies. While bronchoscopy, induced sputum sampling, and measurement of exhaled gases enable the direct characterization of inflammatory pathways in the airways, these modalities can be time consuming, expensive, invasive, and/or are not widely available in primary care settings. Furthermore, assay procedures are not standardized across the relatively few centers equipped to analyze airway samples, which makes implementation in multi-center clinical trials challenging. Thus, to select patients with evidence of Th2-driven eosinophilic inflammation in their airways for targeted therapies, it will be beneficial to develop noninvasive biomarkers of Th2-driven eosinophilic airway inflammation widely available on accessible assay platforms. To address this need, we have used gene expression profiling in asthmatic airway samples to enable the discovery and characterization of clinically useful peripheral biomarkers of Th2-driven eosinophilic airway inflammation.

[0353] Periostin is a secreted matricellular protein associated with fibrosis whose expression is upregulated by recombinant IL-4 and IL-13 in cultured bronchial epithelial cells(21, 38) and bronchial fibroblasts(39). It is expressed at elevated levels in vivo in a mouse model of asthma(40), a rhesus model of asthma (unpublished data), and in bronchial

epithelial cells(21) and the subepithelial bronchial layer(39) of human asthmatics. In human asthmatics, periostin expression levels correlate with reticular basement membrane thickness, an indicator of subepithelial fibrosis(23). Periostin is also overexpressed in nasal polyps associated with aspirin-sensitive asthma(41, 42) and in the esophageal epithelium of patients with eosinophilic esophagitis in an IL-13 dependent manner(43) and thus may play a role in the tissue infiltration of eosinophils in Th2-driven disease processes(44). Elevated periostin expression has also been observed in several types of epithelial derived cancers(45-49), and elevated levels of soluble periostin have been reported in the serum of some cancer patients(24-26, 45, 46). Whether the local and systemic expression of periostin in asthma or other conditions is due to the direct or indirect actions of IL-13 is as yet unclear and will best be addressed by assessments comparing periostin expression before and after therapeutic blockade of IL-13.

[0354] Periostin is detectable at considerable systemic concentrations in the peripheral blood of non-asthmatic subjects but is elevated in the peripheral blood of a subset of asthmatics not on ICS treatment. Its expression in bronchial epithelium is suppressed by ICS treatment(13, 21) and its systemic levels are generally lower in moderate asthmatics relatively well-controlled on ICS compared to asthmatics not on ICS, although with considerable heterogeneity. Given that ICS primarily exert their effects locally in the airway and systemic periostin levels (as we previously showed, see e.g., Intn'l Patent Pub. No. WO2009/124090) are suppressed in asthmatics after undergoing ICS treatment, one may conclude that a substantial fraction of systemic periostin originates from the airways in asthmatics and thus differences in systemic periostin levels of 10-20% are clinically meaningful with respect to airway inflammation.

[0355] FE_{NO} is associated with airway inflammation and predicts ICS responsiveness in asthmatics of varying severity(11, 34). However, FE_{NO} levels do not reliably reflect airway eosinophilia in severe, steroid-dependent asthma and there are discrepancies between sputum and mucosal eosinophil quantification with respect to FE_{NO} (29, 50). Titrating ICS treatment to suppress sputum eosinophil count reduces the the rate of severe asthma exacerbations(12), but titrating ICS treatment to FE_{NO} levels does not(51). Serum YKL-40 has been described as a marker of asthmatic airway inflammation, but its levels were not correlated with measures of Th2 inflammation such as IgE or eosinophils(33). Accordingly, in the present study, we found that bronchial epithelial gene expression levels of periostin and NOS2 but not CHI3L1 were correlated with bronchial IL-13 and IL-5 expression (Table 8). While we observed

relatively strong positive correlations between bronchial or systemic eosinophilia and serum periostin levels, the correlations between eosinophilia and FE_{NO} were weaker and we failed to observe a correlation between serum YKL-40 and eosinophilic inflammation in the asthmatics studied. Sputum and blood eosinophil counts and FE_{NO} are subject to significant temporal variability depending on allergen exposure, exacerbations, and steroid treatment (50, 52, 53). While the half-life of circulating periostin is presently unknown, it is possible that, if periostin is relatively long-lived in blood, systemic periostin levels may reflect an integration of total airway Th2 inflammation over an extended period of time. Consistent with this possibility, we observed relatively little intra-subject variability in serum periostin in 3 measurements over the course of up to 5 weeks (data not shown). Future studies should be directed at comparatively assessing longitudinal intra-subject variability in serum periostin, airway eosinophilia, FE_{NO}, and other candidate biomarkers of Th2 inflammation over longer periods of time.

[0356] The standard of care for bronchial asthma that is not well controlled on symptomatic therapy (e.g. β-agonists) is inhaled corticosteroids (ICS). In mild-to-moderate asthmatics with elevated levels of IL-13 in the airway(19) and eosinophilic esophagitis patients with elevated expression levels of IL-13 in esophageal tissue(43), ICS treatment substantially reduces the level of IL-13 and IL-13-induced genes in the affected tissues. In the airway epithelium of asthmatics after one week of ICS treatment and in cultured bronchial epithelial cells, we have shown that corticosteroid treatment substantially reduces IL-13-induced expression levels of Th2 signature genes(13, 21). This downregulation could be the result of ICS-mediated reduction of IL-13 levels, ICS-mediated reduction of target gene expression, or a combination of the two. In severe asthmatics refractory to ICS treatment, a similar fraction of subjects (approximately 40%) was found to have detectable sputum IL-13 levels to those seen in mild, ICS-naïve asthmatics(19), which is comparable to the proportion of subjects with the bronchial epithelial Th2 signature we have described (13). Analogous observations have been reported for persistence in steroid-refractory asthmatics of IL-4 and IL-5 expressing cells in BAL(54) and eosinophilic inflammation in bronchial biopsies and sputum (8). These observations suggest that, although Th2-driven eosinophilic inflammation is suppressed by ICS treatment in moderate asthmatics, it reappears in a subset of severe asthmatics incompletely controlled by steroid treatment. An additional complication is brought on by incomplete adherence to prescribed ICS therapy, which may underlie poor control in some severe asthmatics. Hence, future studies should be directed at assessing blood

periostin levels in the context of ICS treatment status, ICS dose, intrinsic steroid sensitivity, and adherence to ICS therapy in controlled and uncontrolled asthmatics.

[0357] Currently, there are numerous biological therapeutics in clinical development targeting IL-13 and related factors driving Th2 inflammation in asthma(35-37), including those described herein. It is important that these treatments are targeted to patients with relevant molecular pathology, otherwise important treatment effects may be underestimated; studies of anti-IL5 therapy highlight this point(14, 15, 55). Our findings suggest that approximately half of steroid-naïve mild-to-moderate asthmatics may exhibit activity of the Th2 pathway in their airways, and a similar fraction of moderate-to-severe, steroid-refractory asthmatics exhibits activity of this pathway. Therefore, as described herein, biomarkers that identify asthmatics likely to have Th2 driven inflammation in their airways may aid in the identification and selection of patients most likely to respond to these experimental targeted therapies.

References (Example 5)

1. Galli, S.J., M. Tsai and A.M. Piliponsky, The development of allergic inflammation. *Nature* **454**, 445-454 (2008)
2. Haldar, P. and I.D. Pavord, Noneosinophilic asthma: a distinct clinical and pathologic phenotype. *J Allergy Clin Immunol* **119**, 1043-1052; quiz 1053-1044 (2007)
3. Anderson, G.P., Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* **372**, 1107-1119 (2008)
4. Moore, W.C., D.A. Meyers, S.E. Wenzel, W.G. Teague, H. Li, X. Li, R. D'Agostino, Jr., M. Castro, D. Curran-Everett, A.M. Fitzpatrick, B. Gaston, N.N. Jarjour, R. Sorkness, W.J. Calhoun, K.F. Chung, S.A. Comhair, R.A. Dweik, E. Israel, S.P. Peters, W.W. Busse, S.C. Erzurum and E.R. Bleecker, Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* **181**, 315-323 (2010)
5. Siddiqui, S. and C.E. Brightling, Airways disease: phenotyping heterogeneity using measures of airway inflammation. *Allergy Asthma Clin Immunol* **3**, 60-69 (2007)
6. Simpson, J.L., R. Scott, M.J. Boyle and P.G. Gibson, Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* **11**, 54-61 (2006)
7. Wardlaw, A.J., M. Silverman, R. Siva, I.D. Pavord and R. Green, Multi-dimensional phenotyping: towards a new taxonomy for airway disease. *Clin Exp Allergy* **35**, 1254-1262 (2005)
8. Wenzel, S.E., S.J. Szefler, D.Y. Leung, S.I. Sloan, M.D. Rex and R.J. Martin, Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* **156**, 737-743 (1997)
9. Fahy, J.V., Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. *Proc Am Thorac Soc* **6**, 256-259 (2009)
10. Fahy, J.V., Identifying clinical phenotypes of asthma: steps in the right direction. *Am J Respir Crit Care Med* **181**, 296-297 (2010)

11. Cowan, D.C., J.O. Cowan, R. Palmay, A. Williamson and D.R. Taylor, Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* **65**, 384-390 (2010)
12. Green, R.H., C.E. Brightling, S. McKenna, B. Hargadon, D. Parker, P. Bradding, A.J. Wardlaw and I.D. Pavord, Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* **360**, 1715-1721 (2002)
13. Woodruff, P.G., B. Modrek, D.F. Choy, G. Jia, A.R. Abbas, A. Ellwanger, L.L. Koth, J.R. Arron and J.V. Fahy, T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* **180**, 388-395 (2009)
14. Haldar, P., C.E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R.P. Marshall, P. Bradding, R.H. Green, A.J. Wardlaw and I.D. Pavord, Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* **360**, 973-984 (2009)
15. Nair, P., M.M. Pizzichini, M. Kjarsgaard, M.D. Inman, A. Efthimiadis, E. Pizzichini, F.E. Hargreave and P.M. O'Byrne, Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* **360**, 985-993 (2009)
16. Hershey, G.K., IL-13 receptors and signaling pathways: an evolving web. *J Allergy Clin Immunol* **111**, 677-690; quiz 691 (2003)
17. Berry, M.A., D. Parker, N. Neale, L. Woodman, A. Morgan, P. Monk, P. Bradding, A.J. Wardlaw, I.D. Pavord and C.E. Brightling, Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* **114**, 1106-1109 (2004)
18. Humbert, M., S.R. Durham, P. Kimmitt, N. Powell, B. Assoufi, R. Pfister, G. Menz, A.B. Kay and C.J. Corrigan, Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol* **99**, 657-665 (1997)
19. Saha, S.K., M.A. Berry, D. Parker, S. Siddiqui, A. Morgan, R. May, P. Monk, P. Bradding, A.J. Wardlaw, I.D. Pavord and C.E. Brightling, Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J Allergy Clin Immunol* **121**, 685-691 (2008)
20. Truyen, E., L. Coteur, E. Dilissen, L. Overbergh, L.J. Dupont, J.L. Ceuppens and D.M. Bullens, Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. *Thorax* **61**, 202-208 (2006)
21. Woodruff, P.G., H.A. Boushey, G.M. Dolganov, C.S. Barker, Y.H. Yang, S. Donnelly, A. Ellwanger, S.S. Sidhu, T.P. Dao-Pick, C. Pantoja, D.J. Erle, K.R. Yamamoto and J.V. Fahy, Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* **104**, 15858-15863 (2007)
22. Winpenny, J.P., L.L. Marsey and D.W. Sexton, The CLCA gene family: putative therapeutic target for respiratory diseases. *Inflamm Allergy Drug Targets* **8**, 146-160 (2009)
23. Sidhu, S.S., S. Yuan, A.L. Innes, S. Kerr, P.G. Woodruff, L. Hou, S.J. Muller and J.V. Fahy, Roles of epithelial cell-derived periostin in TGF-{beta} activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* (2010)
24. Sasaki, H., M. Dai, D. Auclair, I. Fukai, M. Kiriyama, Y. Yamakawa, Y. Fujii and L.B. Chen, Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in nonsmall cell lung carcinomas. *Cancer* **92**, 843-848 (2001)
25. Sasaki, H., K.M. Lo, L.B. Chen, D. Auclair, Y. Nakashima, S. Moriyama, I. Fukai, C. Tam, M. Loda and Y. Fujii, Expression of Periostin, homologous with an insect cell

adhesion molecule, as a prognostic marker in non-small cell lung cancers. *Jpn J Cancer Res* **92**, 869-873 (2001)
26. Sasaki, H., C.Y. Yu, M. Dai, C. Tam, M. Loda, D. Auclair, L.B. Chen and A. Elias, Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer. *Breast Cancer Res Treat* **77**, 245-252 (2003)
27. Gibson, P.G., Inflammatory phenotypes in adult asthma: clinical applications. *Clin Respir J* **3**, 198-206 (2009)
28. Chanez, P., S.E. Wenzel, G.P. Anderson, J.M. Anto, E.H. Bel, L.P. Boulet, C.E. Brightling, W.W. Busse, M. Castro, B. Dahlen, S.E. Dahlen, L.M. Fabbri, S.T. Holgate, M. Humbert, M. Gaga, G.F. Joos, B. Levy, K.F. Rabe, P.J. Sterk, S.J. Wilson and I. Vachier, Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol* **119**, 1337-1348 (2007)
29. Lemiere, C., P. Ernst, R. Olivenstein, Y. Yamauchi, K. Govindaraju, M.S. Ludwig, J.G. Martin and Q. Hamid, Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. *J Allergy Clin Immunol* **118**, 1033-1039 (2006)
30. Miranda, C., A. Busacker, S. Balzar, J. Trudeau and S.E. Wenzel, Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol* **113**, 101-108 (2004)
31. Silkoff, P.E., A.M. Lent, A.A. Busacker, R.K. Katial, S. Balzar, M. Strand and S.E. Wenzel, Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol* **116**, 1249-1255 (2005)
32. Pavord, I.D. and D. Shaw, The use of exhaled nitric oxide in the management of asthma. *J Asthma* **45**, 523-531 (2008)
33. Chupp, G.L., C.G. Lee, N. Jarjour, Y.M. Shim, C.T. Holm, S. He, J.D. Dziura, J. Reed, A.J. Coyle, P. Kiener, M. Cullen, M. Grandsaigne, M.C. Dombret, M. Aubier, M. Pretolani and J.A. Elias, A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* **357**, 2016-2027 (2007)
34. Dweik, R.A., R.L. Sorkness, S. Wenzel, J. Hammel, D. Curran-Everett, S.A. Comhair, E. Bleecker, W. Busse, W.J. Calhoun, M. Castro, K.F. Chung, E. Israel, N. Jarjour, W. Moore, S. Peters, G. Teague, B. Gaston and S.C. Erzurum, Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* **181**, 1033-1041 (2010)
35. Barnes, P.J., The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* **118**, 3546-3556 (2008)
36. Holgate, S.T. and R. Polosa, Treatment strategies for allergy and asthma. *Nat Rev Immunol* **8**, 218-230 (2008)
37. Walsh, G.M., Emerging drugs for asthma. *Expert Opin Emerg Drugs* **13**, 643-653 (2008)
38. Yuyama, N., D.E. Davies, M. Akaiwa, K. Matsui, Y. Hamasaki, Y. Suminami, N.L. Yoshida, M. Maeda, A. Pandit, J.L. Lordan, Y. Kamogawa, K. Arima, F. Nagumo, M. Sugimachi, A. Berger, I. Richards, S.L. Roberds, T. Yamashita, F. Kishi, H. Kato, K. Arai, K. Ohshima, J. Tadano, N. Hamasaki, S. Miyatake, Y. Sugita, S.T. Holgate and K. Izuhara, Analysis of novel disease-related genes in bronchial asthma. *Cytokine* **19**, 287-296 (2002)
39. Takayama, G., K. Arima, T. Kanaji, S. Toda, H. Tanaka, S. Shoji, A.N. McKenzie, H. Nagai, T. Hotokebuchi and K. Izuhara, Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* **118**, 98-104 (2006)

40. Hayashi, N., T. Yoshimoto, K. Izuhara, K. Matsui, T. Tanaka and K. Nakanishi, T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN-gamma and IL-13 production. *Proc Natl Acad Sci U S A* **104**, 14765-14770 (2007)
41. Plager, D.A., J.C. Kahl, Y.W. Asmann, A.E. Nilson, J.F. Pallanch, O. Friedman and H. Kita, Gene transcription changes in asthmatic chronic rhinosinusitis with nasal polyps and comparison to those in atopic dermatitis. *PLoS One* **5**, e11450 (2010)
42. Stankovic, K.M., H. Goldsztein, D.D. Reh, M.P. Platt and R. Metson, Gene expression profiling of nasal polyps associated with chronic sinusitis and aspirin-sensitive asthma. *Laryngoscope* **118**, 881-889 (2008)
43. Blanchard, C., M.K. Mingler, M. Vicario, J.P. Abonia, Y.Y. Wu, T.X. Lu, M.H. Collins, P.E. Putnam, S.I. Wells and M.E. Rothenberg, IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* **120**, 1292-1300 (2007)
44. Blanchard, C., M.K. Mingler, M. McBride, P.E. Putnam, M.H. Collins, G. Chang, K. Stringer, J.P. Abonia, J.D. Molkentin and M.E. Rothenberg, Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol* **1**, 289-296 (2008)
45. Baril, P., R. Gangeswaran, P.C. Mahon, K. Caulee, H.M. Kocher, T. Harada, M. Zhu, H. Kalthoff, T. Crnogorac-Jurcevic and N.R. Lemoine, Periostin promotes invasiveness and resistance of pancreatic cancer cells to hypoxia-induced cell death: role of the beta4 integrin and the PI3k pathway. *Oncogene* **26**, 2082-2094 (2007)
46. Ben, Q.W., Z. Zhao, S.F. Ge, J. Zhou, F. Yuan and Y.Z. Yuan, Circulating levels of periostin may help identify patients with more aggressive colorectal cancer. *Int J Oncol* **34**, 821-828 (2009)
47. Kudo, Y., I. Ogawa, S. Kitajima, M. Kitagawa, H. Kawai, P.M. Gaffney, M. Miyauchi and T. Takata, Periostin promotes invasion and anchorage-independent growth in the metastatic process of head and neck cancer. *Cancer Res* **66**, 6928-6935 (2006)
48. Puglisi, F., C. Puppin, E. Pegolo, C. Andreetta, G. Pascoletti, F. D'Aurizio, M. Pandolfi, G. Fasola, A. Piga, G. Damante and C. Di Loreto, Expression of periostin in human breast cancer. *J Clin Pathol* **61**, 494-498 (2008)
49. Siriwardena, B.S., Y. Kudo, I. Ogawa, M. Kitagawa, S. Kitajima, H. Hatano, W.M. Tilakaratne, M. Miyauchi and T. Takata, Periostin is frequently overexpressed and enhances invasion and angiogenesis in oral cancer. *Br J Cancer* **95**, 1396-1403 (2006)
50. Nair, P., M. Kjarsgaard, S. Armstrong, A. Efthimiadis, P.M. O'Byrne and F.E. Hargreave, Nitric oxide in exhaled breath is poorly correlated to sputum eosinophils in patients with prednisone-dependent asthma. *J Allergy Clin Immunol* (2010)
51. Shaw, D.E., M.A. Berry, M. Thomas, R.H. Green, C.E. Brightling, A.J. Wardlaw and I.D. Pavord, The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med* **176**, 231-237 (2007)
52. Pavord, I.D., P.K. Jeffery, Y. Qiu, J. Zhu, D. Parker, A. Carlsheimer, I. Naya and N.C. Barnes, Airway inflammation in patients with asthma with high-fixed or low-fixed plus as-needed budesonide/formoterol. *J Allergy Clin Immunol* **123**, 1083-1089, 1089 e1081-1087 (2009)
53. D'Silva, L., R.J. Cook, C.J. Allen, F.E. Hargreave and K. Parameswaran, Changing pattern of sputum cell counts during successive exacerbations of airway disease. *Respir Med* **101**, 2217-2220 (2007)

54. Leung, D.Y., R.J. Martin, S.J. Szefler, E.R. Sher, S. Ying, A.B. Kay and Q. Hamid, Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. *J Exp Med* **181**, 33-40 (1995)
55. Flood-Page, P., C. Swenson, I. Faiferman, J. Matthews, M. Williams, L. Brannick, D. Robinson, S. Wenzel, W. Busse, T.T. Hansel and N.C. Barnes, A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* **176**, 1062-1071 (2007).

EXAMPLE 6 – Asthma Patient Study III (to assess safety, tolerability and efficacy)

[0358] The study will be a randomized, multicenter, double-blind, placebo-controlled, parallel-group study of lebrikizumab in patients with severe asthma that remains uncontrolled despite daily therapy with ICS (500 – 2000 μg/day fluticasone propionate dry powder inhaler [DPI] or equivalent) plus a second controller medication, such as a long-acting β-agonist (LABA), leukotriene receptor antagonist (LTRA), long acting muscarinic antagonist (LAMA), or theophylline. This study will also assess the diagnostic value of baseline levels of serum periostin ≥ 50 ng/mL (as measured by the Elecsys® assay). While continuing their standard of care therapy, which must include ICS and a second controller medication, patients will be randomly assigned to one of three doses of lebrikizumab or placebo for a 52-week placebo-controlled period.

[0359] During a 2-week run-in period, also referred to as a screening period, (Visit 1 through Visit 3), patients will be assessed for compliance with their current asthma therapy and their ability to use the equipment necessary for monthly clinic visits throughout the study as well as the degree of asthma control provided by their standard-of-care medications. Patients reporting an Asthma Control Questionnaire (ACQ-5) score ≥ 1.5 and one or more symptoms of asthma that is not controlled (night time awakening ≥ 1 time/week, symptoms >2 days/week, SABA use > 2 days/week, and/or interference with daily activities) will be considered uncontrolled. Patients whose symptoms remain uncontrolled at Visit 1 or 2 and Visit 3 despite adherence with controller medicines will be eligible to participate. The screening period may be extended by 1 week in the case that adherence data are incomplete and for patients whose ACQ-5 is < 1.5 at Visit 3, if the investigator's experience with this patient suggests this week was atypical for their disease. Patients may be eligible for rescreening for selected reasons up to two additional times.

[0360] At the end of the run-in (screening) period, eligible patients will be randomly allocated (1:1:1:1) to study drug (either placebo or lebrikizumab 37.5 mg SC monthly, 125 mg SC monthly, or 250 mg SC every 4 weeks). Randomization will be stratified by baseline

serum periostin as measured using the Elecsys® assay (< 42 ng/mL, ≥ 42 to < 50 ng/mL, ≥ 50 to < 58 ng/mL, ≥ 58 ng/mL), history of asthma exacerbations in the last 12 months (0, 1–2, ≥ 3 events), baseline asthma medications (ICS dose ≥ 1000 μg/day of fluticasone propionate DPI or equivalent plus LABA [yes, no]), and geographical region (United States/Canada, Europe, Asia, rest of world). Patients will continue on stable doses of their standard-of-care therapy, which must include ICS (500 2000 μg/day of fluticasone propionate DPI or equivalent) and a second controller medication, in addition to receiving double-blind study treatment for 52 weeks.

[0361] The first SC injection of study treatment will occur on the same day as randomization (Visit 3 [Day 1]), and dosing will be repeated once every 4 weeks over the 52-week placebo controlled period (for a total of 13 study treatment doses). Safety, efficacy, and patient reported outcome (PRO) measures will be assessed throughout the 52 week placebo-controlled period. The primary efficacy endpoint is the rate of asthma exacerbations and will be assessed over the 52-week placebo-controlled period.

[0362] Patients who complete the 52-week placebo-controlled period (i.e., patients who have not prematurely discontinued study treatment) will continue into a 52-week active-treatment extension.

[0363] All patients who continue into the 52-week active-treatment extension study will receive double blind SC lebrikizumab at a dose of either 125 mg or 250 mg every 4 weeks. Patients assigned to receive either 125 mg or 250 mg lebrikizumab during the 52-week placebo controlled period will remain on their assigned lebrikizumab dose. Patients assigned to receive either placebo or lebrikizumab 37.5 mg SC every 4 weeks during the 52-week placebo controlled period will be randomized in a 1:1 ratio to either SC lebrikizumab 125 mg or 250 mg every 4 weeks for the 52-week active-treatment extension, with randomization stratified by baseline periostin level and prior treatment assignment.

[0364] At Week 76 of the 52-week active-treatment extension, each patient will be assessed for asthma control using data from the three most recent visits (Weeks 68, 72, and 76). Patients whose asthma symptoms have been controlled for the 12 consecutive weeks (ACQ 5 ≤ 0.75 on each assessment) and who have had no exacerbations within the first half of the active-treatment extension (Weeks 52–76) will discontinue lebrikizumab therapy and enter the follow up period. During follow-up, patients will be followed for safety for 24 weeks after the last dose of study drug. Patients who remain symptomatic (ACQ-5 > 0.75) at Week 76 or who have experienced an exacerbation event during the first half of the active-

treatment extension (Weeks 52–76) will continue on lebrikizumab treatment for an additional 28 weeks. Safety, efficacy, and PRO measures will be assessed throughout the 52-week active-treatment extension.

[0365] In the follow-up period, all patients will be followed for safety for 24 weeks (> 5 half-lives of the drug) after the last dose of study treatment whenever this may occur, either as scheduled in the protocol or in the event of early discontinuation from study treatment. For patients who complete the study through Visit 23 (Week 76), are well-controlled, and discontinued from study treatment, Visit 23 will replace Safety Follow-up Visit 1. For patients who complete the entire study through Visit 30 (Week 104), Visit 30 will replace Safety Follow-up Visit 1. In both of these cases, the next visit in the follow-up period will be Safety Follow-up Visit 2 at Week 12. For all other patients, the first follow up visit will be Safety Follow-up Visit 1 at Week 4 (approximately 4 weeks after the last dose of study treatment) of the safety follow up period.

[0366] Total participation in the study, from randomization at Visit 3 (Day 1), including the 52-week placebo-controlled period, the 52-week active-treatment extension and the safety follow-up period, may be as long as 124 weeks.

[0367] Approximately 1400 patients (175 patients per treatment group [SC lebrikizumab 250 mg, 125 mg, 37.5 mg, or placebo] per periostin group [periostin high ≥ 50 ng/mL, periostin low < 50 ng/mL]) will be enrolled in the study at approximately 250 sites located globally. A minimum of 650 patients will be enrolled in the periostin high group (≥ 50 ng/mL). A minimum of approximately 450 patients who are on ICS fluticasone ≥ 1000 µg/day DPI or equivalent plus LABA will be enrolled.

[0368] The key inclusion criteria include the following: Asthma diagnosis ≥12 months prior to screening; Bronchodilator response/reversibility: Patients must have bronchodilator response ≥12% in response to four puffs of short-acting β-agonist (e.g., albuterol or salbutamol) during the screening period; Pre-bronchodilator FEV1 40%–80% of predicted at both Visit 2 and Visit 3; On ICS ≥ 500 (e.g., 500 – 2000) µg of fluticasone propionate DPI or equivalent (total daily dose) for ≥6 months prior to screening; On second controller medication (e.g., LABA, LAMA, LTRA, or theophylline within prescribed dosing range) for 6 months prior to screening; Uncontrolled asthma demonstrated both during the screening period (i.e., Visit 1 [Day –14] or Visit 2 [Day –7]) and at the time of randomization (Visit 3 [Day 1]), defined as follows: ACQ-5 score ≥ 1.5 and at least one of the following symptoms of asthma that is not controlled based upon the National Heart, Lung, and Blood Institute and

National Asthma Education and Prevention Program Expert Panel Report 3 (2007) and the Global Initiative for Asthma (2010) guidelines:

- Symptoms > 2 days/week
- Night-time awakenings ≥ 1 time/week
- Use of SABA as rescue medication > 2 days/week
- Interference with normal daily activities;

Chest X-ray or computed tomography (CT) scan obtained within 12 months prior to Visit 1 or chest X-ray during the screening period confirming the absence of other lung disease; and demonstrated adherence with controller medication of ≥ 70% during the screening period (Adherence is defined as patients responding affirmatively that they have taken their asthma controller therapy ≥ 70% of days during the screening period (Visit 1 to Visit 3) as recorded in their peak flow meter device).

[0369] Key Exclusion criteria include the following: History of a severe allergic reaction or anaphylactic reaction to a biologic agent or known hypersensitivity to any component of lebrikizumab injection; Maintenance oral corticosteroid therapy, defined as daily or alternate day oral corticosteroid maintenance therapy within the 3 months prior to Visit 1; Asthma exacerbation during the 4 weeks prior to screening (Visit 1) or at anytime during screening that required systemic (oral, intravenous (IV) or intramuscular (IM)) corticosteroids for any reason including an acute exacerbation event; A major episode of infection requiring any of the following:

1. Hospitalization for ≥ 24 hrs within the 4 weeks prior to screening (Visit 1)
2. Treatment with IV antibiotics within the 4 weeks of screening (Visit 1)
3. Oral antibiotics within the 2 weeks prior to screening (Visit 1);

[0370] Active parasitic infection within the 6 months prior to Visit 1; tuberculosis requiring treatment within the 12 months prior to screening (patients treated for tuberculosis with no recurrence in the 12 months after completing treatment are permitted); Known immunodeficiency, including but not limited to HIV infection; Current use of an immunomodulatory therapy; Known current malignancy or current evaluation for a potential malignancy; Evidence of active hepatitis B/C or unstable liver disease; Active parasitic infection within the 6 months prior to screening; AST/ALT elevation ≥2.0 the upper limit of normal; History of cystic fibrosis, chronic obstructive pulmonary disease, and/or other clinically significant lung disease other than asthma; Current smoker or history of smoking >10 pack-years; Use of a biologic therapy at any time during the 6 months prior to screening;

Female patients of reproductive potential who are not willing to use a highly effective method of contraception for the duration of the study, or who are pregnant or lactating; Other unstable medical disease; Body mass index >38 kg/m^2; Body weight <40 kg.

Phase III Dosing Rationale

Population PK Analysis

[0371] A preliminary population PK model was developed to describe the PK profile of lebrikizumab in adult patients. A total of 4914 serum concentration–time samples from 333 lebrikizumab-treated patients in the studies described above were used to develop the model. No apparent difference was observed across studies, as evidenced by good agreement between the observed mean PK profile in each study and the profile predicted from historical data.

[0372] A two-compartmental model with first-order absorption and elimination kinetics adequately described the serum lebrikizumab concentration–time data. The structural model parameters included clearance (CL), volume of distribution of the central compartment (V_1), volume of the peripheral compartment (V_2), inter compartmental clearance (Q), as well as first-order rate of absorption (K_a) and bioavailability (F) following SC administration. All parameters, except for V2 and Q (which were fixed at population mean values), were assumed to be log normally distributed.

[0373] The population mean CL and V_1 were estimated to be 0.18 L/day and 3.7 L, respectively. The population mean bioavailability was estimated as 76%. The inter-individual variability of PK parameters was modest, ranging from 15% to 27%. Body weight was found to be a significant covariate for the CL and volumes of distribution of lebrikizumab, with higher weights associated with higher clearances and higher volumes of distribution. Approximately 19% of the inter-individual variability in CL and 11% of inter-individual variability in V_1 were explained by body weight. Estimated population PK parameters are summarized in Table 13. The population PK analysis indicated that the PK characteristics of lebrikizumab are consistent with those typical of an IgG monoclonal antibody.

[0374] The effect of periostin on PK parameters was assessed by comparing the individual post hoc estimates of PK parameters between patients with baseline periostin levels above and below the median in the Example 2 study and in the Example 3 study. The differences were insignificant ($p > 0.05$) in both studies for CL (p=0.54, 0.11), V_1 (p=0.83, 0.79), and F (p=0.74, 0.37).

Table 13. Lebrikizumab Population PK Parameters.

Parameter Description	Population Mean Estimate (% SE)	Inter-Individual Variability (% SE)
Clearance, CL (L/day)	0.176 (3.2%)	24.5% (23.3%)
Effect of BW on CL	0.864 (10.6%)	
Volume of distribution in central compartment, V_1 (L)	3.74 (3.4%)	24.6% (39.6%)
Effect of BW on V_1	0.890 (12.1%)	
Volume of distribution in peripheral compartment, V_2 (L)	2.01 (4.9%)	
Effect of BW on V_2	0.316 (34.8%)	
Distribution clearance, Q (L/day)	0.443 (10.2%)	
First-order rate of absorption, K_a (1/day)	0.213 (5.0%)	26.9% (39.4%)
Bioavailability, F (%)	75.6 (3.7%)	14.9% (70.9%)
Proportional residual error	13.6% (3.6%)	
Additive residual error (µg/mL)	442 (15.6%)	

BW=body weight; CL=clearance; PK=pharmacokinetic; Q=inter-compartmental clearance; SE=standard error; V_1=volume of distribution of the central compartment; V_2=volume of distribution of the peripheral compartment.

Note: BW effect was modeled as power function, $TVP_i = \theta 1 * (BW_i / BW_{Ref})^{\theta 2}$, for which TVP is a typical value of the PK parameter; BW_i refers to body weight for ith individual, BW_{Ref} refers to reference body weight, which is the median body weight of all patients included in the population PK model (82 kg), θ1 refers to the population mean estimate of the PK parameter, and θ2 refers to the effect of body weight on the PK parameter.

Rationale for Flat Dosing

[0375] Flat dosing was used in the Phase II studies described above. Given the effect of body weight on the PK profile of lebrikizumab, heavier patients generally had lower drug exposure. However, no correlation was found between body weight and the proportional change in FEV1 from baseline to Week 12 in individual patients treated with lebrikizumab, indicating that the effect of body weight on exposure did not have an impact on efficacy (see Figure 20). Furthermore, the data from these studies indicated no safety concern with flat dosing within the body weight range tested (53–135 kg).

[0376] To evaluate the impact of flat dosing on the overall variability of exposure to lebrikizumab, population PK simulations were performed to compare flat dosing and the equivalent body weight–based (i.e., mg/kg) dosing regimens, assuming a body weight distribution similar to that seen in the Phase II studies described above. The predicted proportion of patients with steady-state trough concentrations above various levels was similar between flat and body weight–based dosing regimens (see Figure 21), suggesting no clear advantage for dosing based on body weight. Consequently, flat dosing was chosen for

the Phase III study given its advantage for reducing the risk of dosing error and operational complexity (e.g., drug preparation) compared with individualized body weight–based dosing.

Rationale for Target Concentrations

[0377] Exposure–response analyses using the Phase II data were performed to derive target lebrikizumab concentrations to inform dose selection for the Phase III studies. In both Phase II studies described above, no apparent correlation was found between the placebo-corrected change in FEV1 from baseline and serum trough drug concentration at Week 12 (data not shown) in individual patients treated with lebrikizumab, suggesting that the effect of lebrikizumab on FEV1 is saturated at the range of exposure tested in both studies. Moreover, assessments of the correlation between the change in PD biomarkers (FeNO, IgE, CCL17, and CCL13) and serum trough drug concentrations in the Phase II studies suggested saturation of these PD responses during the treatment period in both studies. The results were similar in the periostin high group. On the basis of these results, a target serum steady-state trough concentration of 10 μg/mL was proposed to bracket the lower end of the observed $C_{trough,wk12}$ range in both studies (5th–95th percentile: 9.6–50 μg/mL in Example 2 study; 9.4–73 μg/mL in Example 3 study). Furthermore, a serum concentration of 10 μg/mL is expected to maintain a sufficiently high drug level in the lung to neutralize IL 13 in asthma patients, given the assumed serum-to-lung partitioning of lebrikizumab (1:500) and IL-13 levels in the lung based on available data in literature. Therefore, it is anticipated that an effective dose in Phase III will maintain a serum steady-state trough concentration >10 μg/mL.

[0378] To select a partially effective dose in Phase III, a lower target steady state trough concentration of 5 μg/mL was proposed to ensure no overlap with the observed $C_{trough,wk12}$ range in both Example 2 and Example 3 studies, in which efficacy was observed. Additionally, in both studies, the effect of lebrikizumab on serum CCL17 (TARC) levels returned toward baseline during drug washout (see Figure 22; (A) Example 2 study, (B) Example 3 study), at mean serum lebrikizumab concentrations >5 μg/mL. Although the effect of lebrikizumab on serum CCL17 (TARC) did not directly correlate with efficacy, the recovery of this biomarker suggests suboptimal suppression of IL-13 activity in certain biologic pathways at this concentration. Therefore, a partially effective dose is proposed to maintain a serum steady-state trough concentration <5 μg/mL.

Rationale for the Proposed Phase III Doses

[0379] Given the lack of a clear dose response in the Example 3 study as described above (i.e. that the doses 125 mg, 250 mg, and 500 mg appeared equally efficacious), the Phase III doses were selected to demonstrate a dose response of lebrikizumab by including both effective and partially effective dose levels. To fulfill this objective, the proposed doses of lebrikizumab for the Phase III program include 250 mg, 125 mg, and 37.5 mg given by SC injection every four weeks (q4wk). Figure 23 shows the simulated population PK profiles at these doses, assuming a body weight distribution similar to that seen in the Phase II studies.

[0380] The highest dose of 250 mg (two 1-mL SC injections) q4wk is anticipated to demonstrate clinical efficacy in Phase III. It is the only dose regimen studied in the Phase II proof-of-concept study (Example 2) and showed efficacy in reducing the rate of severe asthma exacerbations in patients whose asthma was uncontrolled despite ICS therapy, the patient population intended for treatment. Example 2 patients had uncontrolled asthma despite treatment with ICS with or without another controller, and ≥90% of patients in Example 2 who were on ICS ≥500 µg/day were also taking a LABA medication. At this dose regimen, almost all (99%) patients are predicted to achieve the target $C_{trough,ss}$ of 10 µg/mL on the basis of the population PK simulations (see Table 14), which means that maximum efficacy is expected. Therefore, this dose will be tested to replicate the clinical efficacy seen in Phase II.

Table 14. Predicted Percentage of Patients with Steady-State Trough Concentration above Target at the Doses Proposed for Phase III.

Dose (mg every 4 weeks)	Target Steady-State Trough Concentration	
	>5 µg/mL	>10 µg/mL
250	100%	99%
125	99%	78%
37.5	28%	0.6%

[0381] A middle dose of 125 mg (one 1-mL SC injection) q4wk is proposed on the basis of the similar magnitudes of FEV1 improvement observed at 125 and 250 mg q4wk in the Phase II dose-ranging study (Example 3). It is reasonable to expect that the same dose–response relationship holds true in the population of patients with severe asthma for the exacerbation endpoint and thus, the 125 mg dose is anticipated to show efficacy in Phase III. This expectation is further supported by the population PK simulations, which suggest that a

majority (78%) of patients will achieve the target $C_{trough,ss}$ of 10 μg/mL with this dose regimen (see Table 14).

[0382] The dose of 37.5 mg (one 0.3-mL SC injection) q4wk is proposed in order to demonstrate a partially effective or clinically ineffective dose of lebrikizumab that is important to establish a dose–response relationship. This dose regimen is chosen taking into account the following considerations:

- Ability to maintain $C_{trough,ss}$ below 5 μg/mL in the majority (72%) of patients and below 10 μg/mL in almost all (99%) patients (see Table 14)
- Reasonable (3.3-fold) separation from the middle dose
- Minimal overlap in the simulated range of serum exposure to lebrikizumab with the middle dose (see Table 14) in order to demonstrate differential clinical responses
- A convenient injection volume (a multiple of 0.1 mL) to reduce possible dosing errors

[0383] The various outcome measures of this Phase III trial are described below.

OUTCOME MEASURES

[0384] Each of the following endpoints will be assessed separately in periostin high and periostin low patients. The trial will be considered positive if the primary endpoint is met in the periostin-high group when the 250 mg lebrikizumab group is compared with the placebo group.

PRIMARY EFFICACY OUTCOME MEASURE

[0385] The primary efficacy outcome measure is the rate of asthma exacerbations during the 52-week placebo-controlled period. For this trial, asthma exacerbations will be defined as new or increased asthma symptoms (including, for example, wheeze, cough, dyspnea, or chest tightness or nocturnal awakenings due to these symptoms) that lead to treatment with systemic corticosteroids or to hospitalization. Here, treatment with systemic corticosteroids is defined as treatment with oral (i.e. OCS) or parenteral corticosteroids for ≥3 days or an emergency department visit with one or more doses of parenteral (IV or IM) corticosteroids.

SECONDARY EFFICACY OUTCOME MEASURES

[0386] The secondary efficacy outcome measures at Week 52 are as follows: Relative change in pre-bronchodilator FEV1 from baseline to Week 52; Time to first asthma exacerbation during the 52-week treatment period; Change in fractional excretion of nitric oxide (FE_{NO}) from baseline to Week 52; Change in asthma-specific health-related quality of life, assessed by the Overall Score of the Standardized Version of the Asthma Quality of Life

Questionnaire, (AQLQ(S)) from baseline to Week 52; Change in rescue medication use (measured by number of puffs per day of rescue medication or nebulized rescue medication (i.e., SABA)) from baseline to Week 52; Rate of urgent asthma-related health care utilization (i.e., hospitalizations, emergency department visits, and acute care visits) during the 52-week placebo-controlled period.

EXPLORATORY OUTCOME MEASURES

[0387] Exploratory outcome measures will include the following: Proportion of patients who do not experience a protocol-defined asthma exacerbation during the 52-week placebo-controlled period; Change in morning post-bronchodilator peak flow value (L/min) from baseline to Week 52; Change in post-bronchodilator FEV1 from baseline to Week 52; Time to a 150-mL improvement in pre-bronchodilator FEV_1 during the 52-week placebo-controlled period; Relative change in pre-bronchodilator FEV_1 (liters) from baseline averaged over Weeks 4 to 52; Relative change in forced vital capacity from baseline to Week 52; rate of exacerbations that are associated with lung function decline, defined as an exacerbation resulting in PEF or FEV1 <60% of the highest value during screening period (Visits 1–3) that requires treatment with systemic corticosteroids; Change in work, school and activity impairment as assessed by the Work Productivity and Activity Impairment–Asthma Questionnaire (WPAI–Asthma) from baseline to Week 52; Change in Asthma Control Questionnaire-5 (ACQ-5) score from baseline to Week 52; Change in asthma symptoms, as measured by the Asthma Symptom Utility Index (ASUI) from baseline to Week 52; Change in health utilities, as assessed by the EQ-5D, from baseline to Week 52; Change in the Global Evaluation of Treatment Effectiveness (GETE) from baseline to Week 52.

[0388] These exploratory outcome measures may also be assessed during the 52-week active-treatment extension and the 24-week follow-up period. Additional exploratory outcomes measures may include frequency and severity of adverse events in patients exposed to lebrikizumab for > 52 weeks; change in interleukin-13 (IL-13)/asthma-related PD biomarkers during the 52-week active-treatment extension or 24-week follow-up period; and serum lebrikizumab concentrations during the 52-week active-treatment extension or 24-week follow-up period.

PATIENT-REPORTED OUTCOMES

Asthma Quality of Life Questionnaire – Standardized (AQLQ(S))

[0389] The AQLQ(S) will be used to assess the patients' asthma-specific health-related quality of life (Juniper 2005). The questionnaire contains four domains including activity limitations, symptoms, emotional function, and environmental stimuli. The AQLQ(S) has

been validated for use in this study population. The AQLQ(S) has a recall specification of 2 weeks. The AQLQ(S) will be administered to the patient prior to all other non-PRO assessments and before the patient receives any disease status information or study drug during that assessment.

Work, Productivity, and Activity Impairment-Asthma (WPAI–Asthma)

[0390] To assess impairment at work, school, and with activities, the WPAI–Asthma questionnaire will be administered (Reilly et al. 1993, 1996). Questionnaire items are adapted from the WPAI–Allergy Specific (WPAI–AS) questionnaire, substituting all occurrences of the term allergy with asthma. The WPAI–AS will be administered to the patient prior to all other non-PRO assessments and before the patient receives any disease status information or study drug during that assessment.

Euro-QOL 5D Questionnaire (EQ-5D)

[0391] The EQ-5D is generic preference-based health-related quality of life questionnaire that provides a single index value for health status (The EuroQol Group 1990). The EQ-5D is designed for self-completion by patients. The Eq-5D will be administered to the patient prior to all other non-PRO assessments and before the patient receives any disease-status information or study drug during that assessment.

Asthma Symptom Utility Index (ASUI)

[0392] The ASUI (Revicki 1998) is an asthma specific symptom questionnaire measuring cough, wheezing, shortness of breath and night time awakening. The ASUI will be administered to the patient prior to all other non-PRO assessments and before the patient receives any disease-status information or study drug during that assessment.

EXAMPLE 7 - Elecsys® periostin assay

[0393] The quantitative detection of periostin is assessed in an automated Roche cobas e601 Elecsys® analyzer (Roche Diagnostics GmbH). The test is carried out in the sandwich format wherein the analyte periostin is sandwiched between two monoclonal antibodies binding to two different epitopes on periostin. One antibody is biotinylated and enables the capture of the immuno complex to streptavidin-coated magnetic beads. The second antibody bears a complexed ruthenium cation as the signaling moiety that allows a voltage dependent electrochemi¬luminescent detection of the bound immuno complex.

[0394] In detail, reagents are used as follows:

-Beads (M): Streptavidin-coated magnetic microparticles 0.72 mg/mL; preservative.

-Reagent 1 (R1): Anti-periostin-antibody~biotin:

This purified mouse monoclonal-antibody corresponds to the coating antibody 25D4 according to example 4 and is used in biotinylated form >1.0 mg/L; TRIS buffer >100 mmol/L, pH 7.0; preservative.

-Reagent 2 (R2): Anti-periostin-antibody~Ru(bpy) :

This purified mouse monoclonal anti-periostin antibody corresponds to the detection antibody 23B9 according to example 4 and is used in labeled form (labeled with a (Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) complex) >1.0 mg/L; TRIS buffer >100 mmol/L, pH 7.0; preservative.

[0395] The immunoassay is carried out using two incubations. In the first incubation of about 9 minutes periostin in 20 µL of sample and the biotinylated monoclonal anti-periostin antibody (R1) form a complex. In the second incubation step for further 9 minutes ruthenylated monoclonal anti-periostin antibody (R2) and streptavidin-coated microparticles (M) are added to the vial of the first incubation so that a 3-membered sandwich complex is formed and becomes bound to the solid phase (microparticles) via the interaction of biotin and streptavidin.

[0396] The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of a platinum electrode. Unbound substances are washed away and the cell flushed with ProCell, a reagent containing Tripropylamine. Application of a voltage to the electrode then induces a chemi¬luminescent emission which is measured by a photomultiplier.

[0397] Results are determined via an instrument-specific calibration curve which is generated by 2-point calibration and a master curve provided via the reagent barcode. Calibrator 1 is analyte free, whereas calibrator 2 contains 50 ng/mL recombinant human periostin in a buffered matrix. To verify calibration, two controls with approximately 30 and 80 ng/mL periostin are employed.

EXAMPLE 8 – Comparison of E4 Assay and Elecsys® Periostin Assay

[0398] The periostin levels were measured in patient serum samples from the clinical trials described in each of Example 2 and Example 3 using methods similar to the E4 Assay (Example 4) and to the Elecsys® periostin assay (Example 7). Results from samples from both trials showed that the periostin values overlapped. The variability and spread was comparable across the assays. In general, at the median, the Elecsys® assay results were

typically = or > 2 fold higher than the E4 Assay results. See Figure 17, i.e., the median was 50-51ng/ml for the Elecsys® periostin assay and the median was 23ng/ml for the E4 Assay.

[0399] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

SEQUENCE LISTING KEY

SEQ ID NO:	Sequence
1	QVHLQQSGAELAKPGASVHMSCKASGYTFTTYWMHWVKQRPGQGLE WIGYINPNTGYADYNQKFRDKATLTADKSSSTAYMQLSSLTSEDSTVYF CARRRTGTSYFDYWGQGTTLTVSSTKTTPPSV
2	QTVLSQSPAILSASPGEKVTMTCRASSSVTYMHWYQQKPGSSPKPWIFA TSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPLTFG AGTK
3	QVQLQQSGAELARPGASVKLSCKASGYSFTHYWMQWVKQRPGQGLE WIGAIYPGDGDTRYTQRLKGKATLTADKSSSTAYMELSSLASEDSAVYY CAREGEGNSAMDYWGQGTSVTVSSAKTTPPSV
4	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGSSVAWFQQKPGQSPKTLI YSASYRDSGVPDRFTGSGSGTDFTLTITNVQSEDLTDYFCLQYGTYPYTF GGGTR
5	MIPFLPMFSL LLLLIVNPIN ANNHYDKILA HSRIRGRDQG PNVCALQQIL GTKKKYFSTCKNWYKKSICG QKTTVLYECC PGYMRMEGMK GCPAVLPIDH VYGTLGIVGA TTTQRYSDAS KLREEIEGKG SFTYFAPSNE AWDNLDSDIR RGLESNVNVE LLNALHSHMI NKRMLTKDLK NGMIIPSMYN NLGLFINHYP NGVVTVNCAR IIHGNQIATN GVVHVIDRVL TQIGTSIQDF IEAEDDLSSF RAAAITSDIL EALGRDGHFT LFAPTNEAFE KLPRGVLERI MGDKVASEALMKYHILNTLQ CSESIMGGAV FETLEGNTIE IGCDGDSITV NGIKMVNKKD IVTNNGVIHLIDQVLIPDSA KQVIELAGKQ QTTFTDLVAQ LGLASALRPD GEYTLLAPVN NAFSDDTLSMDQRLLKLILQ NHILKVKVGL NELYNGQILE TIGGKQLRVF VYRTAVCIEN SCMEKGSKQGRNGAIHIFRE IIKPAEKSLH EKLKQDKRFS TFLSLLEAAD LKELLTQPGD WTLFVPTNDAFKGMTSEEKE ILIRDKNALQ NIILYHLTPG VFIGKGFEPG VTNILKTTQG SKIFLKEVNDTLLVNELKSK ESDIMTTNGV IHVVDKLLYP ADTPVGNDQL LEILNKLIKY IQIKFVRGST FKEIPVTVYT TKIITKVVEP KIKVIEGSLQ PIIKTEGPTL TKVKIEGEPE FRLIKEGETITEVIHGEPII KKYTKIIDGV PVEITEKETR EERIITGPEI KYTRISTGGG ETEETLKKLLQEEVTKVTKF IEGGDGHLFE *DEEIKRLLQG DTPVRKLQAN KKVQGSRRRL REGRSQ*
6	MIPFLPMFSL LLLLIVNPIN ANNHYDKILA HSRIRGRDQG PNVCALQQIL GTKKKYFSTCKNWYKKSICG QKTTVLYECC PGYMRMEGMK GCPAVLPIDH VYGTLGIVGA TTTQRYSDAS KLREEIEGKG SFTYFAPSNE AWDNLDSDIR RGLESNVNVE LLNALHSHMI NKRMLTKDLKNGMIIPSMYN NLGLFINHYP NGVVTVNCAR IIHGNQIATN GVVHVIDRVL TQIGTSIQDF IEAEDDLSSF RAAAITSDIL EALGRDGHFT LFAPTNEAFE KLPRGVLERI MGDKVASEALMKYHILNTLQ CSESIMGGAV FETLEGNTIE IGCDGDSITV NGIKMVNKKD IVTNNGVIHLIDQVLIPDSA KQVIELAGKQ QTTFTDLVAQ LGLASALRPD GEYTLLAPVN NAFSDDTLSMDQRLLKLILQ NHILKVKVGL NELYNGQILE TIGGKQLRVF VYRTAVCIEN SCMEKGSKQGRNGAIHIFRE IIKPAEKSLH EKLKQDKRFS TFLSLLEAAD LKELLTQPGD WTLFVPTNDAFKGMTSEEKE ILIRDKNALQ NIILYHLTPG VFIGKGFEPG VTNILKTTQG SKIFLKEVNDTLLVNELKSK ESDIMTTNGV IHVVDKLLYP ADTPVGNDQL LEILNKLIKY IQIKFVRGSTFKEIPVTVYK PIIKKYTKII DGVPVEITEK ETREERIITG PEIKYTRIST GGGETEETLK KLLQEEVTKV TKFIEGGDGH LFEDEEIKRL LQGDTPVRKL QANKKVQGSR RRLREGRSQ
7	MIPFLPMFSL LLLLIVNPIN ANNHYDKILA HSRIRGRDQG PNVCALQQIL GTKKKYFSTCKNWYKKSICG QKTTVLYECC PGYMRMEGMK GCPAVLPIDH VYGTLGIVGA TTTQRYSDAS KLREEIEGKG SFTYFAPSNE AWDNLDSDIR RGLESNVNVE LLNALHSHMI NKRMLTKDLKNGMIIPSMYN NLGLFINHYP NGVVTVNCAR IIHGNQIATN GVVHVIDRVL TQIGTSIQDF IEAEDDLSSF RAAAITSDIL EALGRDGHFT LFAPTNEAFE KLPRGVLERI MGDKVASEALMKYHILNTLQ CSESIMGGAV FETLEGNTIE IGCDGDSITV NGIKMVNKKD IVTNNGVIHLIDQVLIPDSA KQVIELAGKQ QTTFTDLVAQ LGLASALRPD GEYTLLAPVN NAFSDDTLSMDQRLLKLILQ NHILKVKVGL NELYNGQILE TIGGKQLRVF VYRTAVCIEN SCMEKGSKQGRNGAIHIFRE IIKPAEKSLH EKLKQDKRFS TFLSLLEAAD LKELLTQPGD WTLFVPTNDAFKGMTSEEKE ILIRDKNALQ NIILYHLTPG VFIGKGFEPG VTNILKTTQG SKIFLKEVNDTLLVNELKSK ESDIMTTNGV IHVVDKLLYP ADTPVGNDQL LEILNKLIKY IQIKFVRGSTFKEIPVTVYR PTLTKVKIEG EPEFRLIKEG ETITEVIHGE PIIKKYTKII DGVPVEITEK ETREERIITG PEIKYTRIST GGGETEETLK KLLQEDTPVR

	KLQANKKVQG SRRRLREGRSQ
8	MIPFLPMFSL LLLLIVNPIN ANNHYDKILA HSRIRGRDQG PNVCALQQIL GTKKKYFSTCKNWYKKSICG QKTTVLYECC PGYMRMEGMK GCPAVLPIDH VYGTLGIVGA TTTQRYSDAS KLREEIEGKG SFTYFAPSNE AWDNLDSDIR RGLESNVNVE LLNALHSHMI NKRMLTKDLKNGMIIPSMYN NLGLFINHYP NGVVTVNCAR IIHGNQIATN GVVHVIDRVL TQIGTSIQDF IEAEDDLSSF RAAAITSDIL EALGRDGHFT LFAPTNEAFE KLPRGVLERI MGDKVASEALMKYHILNTLQ CSESIMGGAV FETLEGNTIE IGCDGDSITV NGIKMVNKKD IVTNNGVIHLIDQVLIPDSA KQVIELAGKQ QTTFTDLVAQ LGLASALRPD GEYTLLAPVN NAFSDDTLSMDQRLLKLILQ NHILKVKVGL NELYNGQILE TIGGKQLRVF VYRTAVCIEN SCMEKGSKQGRNGAIHIFRE IIKPAEKSLH EKLKQDKRFS TFLSLLEAAD LKELLTQPGD WTLFVPTNDAFKGMTSEEKE ILIRDKNALQ NIILYHLTPG VFIGKGFEPG VTNILKTTQG SKIFLKEVNDTLLVNELKSK ESDIMTTNGV IHVVDKLLYP ADTPVGNDQL LEILNKLIKY IQIKFVRGST FKEIPVTVYK PIIKKYTKII DGVPVEITEK ETREERIITG PEIKYTRIST GGGETEETLKKLLQEDTPVR KLQANKKVQG SRRRLREGRS Q
9	VTLRESGPALVKPTQTLTLTCTVSGFSLSAYSVNWIRQPPGKALEWLAMI WGDGKIVYNSALKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCAGD GYYPYAMDNWGQGSLVTVSS
10	DIVMTQSPDSLSVSLGERATINCRASKSVDSYGNSFMHWYQQKPGQPP KLLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQNNED PRTFGGGTKVEIK
11	AYSVNW
12	MIWGDGKIVYNSALKS
13	DGYYPYAMDN
14	RASKSVDSYGNSFMH
15	LASNLES
16	QQNNEDPRT
17	Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val
18	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Asn Pro Ser Val Lys Gly Arg Ile Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly Gln Gly
19	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQG PNVCALQQILGTKKKYFSTCKNWYKKSICGQKTTVLYE CCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQR YSDASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLES NVNVELLNALHSHMINKRMLTKDLKNGMIIPSMYNNLG LFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQI GTSIQDFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAP TNEAFEKLPRGVLERIMGDKVASEALMKYHILNTLQCSE SIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVT NNGVIHLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLG LASALRPDGEYTLLAPVNNAFSDDTLSMDQRLLKLILQN HILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIEN SCMEKGSKQGRNGAIHIFREIIKPAEKSLHEKLKQDKRFS TFLSLLEAADLKELLTQPGDWTLFVPTNDAFKGMTSEEK EILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQG SKIFLKEVNDTLLVNELKSKESDIMTTNGVIHVVDKLLY PADTPVGNDQLLEILNKLIKYIQIKFVRGSTFKEIPVTVYT

	TKIITKVVEPKIKVIEGSLQPIIKTEGPTLTKVKIEGEPEFR LIKEGETITEVIHGEPIIKKYTKIIDGVPVEITEKETREERII TGPEIKYTRISTGGGETEETLKKLLQEEVTKVTKFIEGGD GHLFEDEEIKRLLQGDTPVRKLQANKKVQGSRRRLREG RSQ
20	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQG PNVCALQQILGTKKKYFSTCKNWYKKSICGQKTTVLYE CCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQR YSDASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLES NVNVELLNALHSHMINKRMLTKDLKNGMIIPSMYNNLG LFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQI GTSIQDFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAP TNEAFEKLPRGVLERIMGDKVASEALMKYHILNTLQCSE SIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVT NNGVIHLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLG LASALRPDGEYTLLAPVNNAFSDDTLSMDQRLLKLILQN HILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIEN SCMEKGSKQGRNGAIHIFREIIKPAEKSLHEKLKQDKRFS TFLSLLEAADLKELLTQPGDWTLFVPTNDAFKGMTSEEK EILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQG SKIFLKEVNDTLLVNELKSKESDIMTTNGVIHVVDKLLY PADTPVGNDQLLEILNKLIKYIQIKFVRGSTFKEIPVTVY KPIIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGG GETEETLKKLLQEEVTKVTKFIEGGDGHLFEDEEIKRLLQ GDTPVRKLQANKKVQGSRRRLREGRSQ
21	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQG PNVCALQQILGTKKKYFSTCKNWYKKSICGQKTTVLYE CCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQR YSDASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLES NVNVELLNALHSHMINKRMLTKDLKNGMIIPSMYNNLG LFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQI GTSIQDFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAP TNEAFEKLPRGVLERIMGDKVASEALMKYHILNTLQCSE SIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVT NNGVIHLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLG LASALRPDGEYTLLAPVNNAFSDDTLSMDQRLLKLILQN HILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIEN SCMEKGSKQGRNGAIHIFREIIKPAEKSLHEKLKQDKRFS TFLSLLEAADLKELLTQPGDWTLFVPTNDAFKGMTSEEK EILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQG SKIFLKEVNDTLLVNELKSKESDIMTTNGVIHVVDKLLY PADTPVGNDQLLEILNKLIKYIQIKFVRGSTFKEIPVTVYR PTLTKVKIEGEPEFRLIKEGETITEVIHGEPIIKKYTKIIDG VPVEITEKETREERIITGPEIKYTRISTGGGETEETLKKLL QEDTPVRKLQANKKVQGSRRRLREGRSQ
22	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQG PNVCALQQILGTKKKYFSTCKNWYKKSICGQKTTVLYE CCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQR YSDASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLES NVNVELLNALHSHMINKRMLTKDLKNGMIIPSMYNNLG LFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQI GTSIQDFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAP TNEAFEKLPRGVLERIMGDKVASEALMKYHILNTLQCSE SIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVT NNGVIHLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLG LASALRPDGEYTLLAPVNNAFSDDTLSMDQRLLKLILQN HILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIEN SCMEKGSKQGRNGAIHIFREIIKPAEKSLHEKLKQDKR FSTFLSLLEAADLKELLTQPGDWTLFVPTNDAFKGMTSE

	EKEILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTT QGSKIFLKEVNDTLLVNELKSKESDIMTTNGVIHVVDKL LYPADTPVGNDQLLEILNKLIKYIQIKFVRGSTFKEIPVTV YKPIIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTG GGETEETLKKLLQEDTPVRKLQANKKVQGSRRRLREGR SQ
23	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQG PNVCALQQILGTKKKYFSTCKNWYKKSICGQKTTVLYE CCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQR YSDASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLES NVNVELLNALHSHMINKRMLTKDLKNGMIIPSMYNNLG LFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQI GTSIQDFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAP TNEAFEKLPRGVLERIMGDKVASEALMKYHILNTLQCSE SIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVT NNGVIHLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLG LASALRPDGEYTLLAPVNNAFSDDTLSMDQRLLKLILQN HILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIEN SCMEKGSKQGRNGAIHIFREIIKPAEKSLHEKLKQDKRFS TFLSLLEAADLKELLTQPGDWTLFVPTNDAFKGMTSEEK EILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQG SKIFLKEVNDTLLVNELKSKESDIMTTNGVIHVVDKLLY PADTPVGNDQLLEILNKLIKYIQIKFVRGSTFKEIPVTVYS PEIKYTRISTGGGETEETLKKLLQE
24	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYGIAWVR QAPGKGLEWVAFISDLAYTIYYADTVTGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDNWDAMDYWGQGTLV TVSS
25	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHNNANTYLH WYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCSQNTLVPWTFGQGTKVEIK

CLAIMS:

1. A method of identifying an asthma patient or a respiratory disorder patient who is likely to be responsive to treatment with a TH2 Pathway Inhibitor comprising determining whether the patient is Eosinophilic Inflammation Positive (EIP) using an Eosinophilic Inflammation Diagnostic Assay (EIDA), wherein the EIP status indicates that the patient is likely to be responsive to treatment with the TH2 Pathway Inhibitor.

2. A method of identifying an asthma patient or a respiratory disorder patient who is likely to suffer from severe exacerbations comprising determining whether the patient is Eosinophilic Inflammation Positive (EIP) using an Eosinophilic Inflammation Diagnostic Assay (EIDA), wherein the EIP status indicates that the patient is likely to suffer from an increase in severe exacerbations.

3. A method of identifying an asthma patient or a respiratory disorder patient who is less likely to be responsive to treatment with a TH2 Pathway Inhibitor comprising determining whether the patient is Eosinophilic Inflammation Negative (EIN) using an Eosinophilic Inflammation Diagnostic Assay (EIDA), wherein the EIN status indicates that the patient is less likely to be responsive to treatment with the TH2 Pathway Inhibitor.

4. A method of monitoring an asthma patient being treated with a TH2 Pathway inhibitor, comprising determining whether the patient is Eosinophilic Inflammation Positive (EIP) or Eosinophilic Inflammation Negative (EIN) using an Eosinophilic Inflammation Diagnostic Assay (EIDA).

5. The method of claim 4, further comprising determining a treatment regimen for the TH2 Pathway Inhibitor.

6. The method of claim 5, wherein the determination of EIP indicates continuing therapy with the TH2 Pathway Inhibitor and the determination of EIN indicates discontinuing therapy with the TH2 Pathway Inhibitor.

7. The method according to claims 1-4, wherein the EIDA comprises the steps of:

a) determining the amount of Total Periostin in a sample obtained from an asthma patient;

b) comparing the amount of Total Periostin determined in step a) to a reference amount; and

c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step b).

8. The method according to claim 7, wherein the Total Periostin is serum periostin, which periostin is measured using an immunoassay.

9. The method according to claim 8, wherein the immunoassay is a sandwich immunoassay.

10. The method according to claim 9, wherein the sandwich immunoassay is an E4 Assay or is performed by an Elecsys® analyzer.

11. The method according to claim 7, wherein the reference amount for EIP is 23ng/ml or greater when using an E4 Assay in step (a).

12. The method according to claim 7, wherein the reference amount for EIP is 50ng/ml or greater when using an Elecsys® analyzer in step (a).

13. The method according to claim 7, wherein the reference amount for EIN is 21 ng/ml or lower when using an E4 Assay in step (a).

14. The method according to claim 7, wherein the reference amount for EIN is 48 ng/ml or lower when using an Elecsys® analyzer in step (a).

15. The method according to any one of claims 1-14, wherein the patient is suffering from moderate to severe asthma.

16. The method according to any one of claims 1-14, wherein the asthma or respiratory disorder is uncontrolled on a corticosteroid.

17. The method according to claim 16, wherein the corticosteroid is an inhaled corticosteroid.

18. The method according to claim 17, wherein the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®.

19. The method according to any one of claims 1-14, wherein the patient is also being treated with a second controller.

20. The method according to claim 19, wherein the second controller is a long acting bronchial dilator (LABD).

21. The method according to claim 20, wherein the LABD is a long-acting beta-2 agonist (LABA), leukotriene receptor antagonist (LTRA), long-acting muscarinic antagonist (LAMA), theophylline, or oral corticosteroids (OCS).

22. The method according to claim 20, wherein the LABD is Symbicort®, Advair®, Brovana®, Foradil®, Perforomist™ or Serevent®.

23. The method according to any one of claims 1-19, wherein the TH2 Pathway Inhibitor inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR®, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935), or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8).

24. The method according to any one of claim 1-19, wherein the TH2 Pathway Inhibitor is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent.

25. The method according to any one of claims 1-19, wherein the TH2 Pathway Inhibitor is an anti-IL-13 antibody.

26. The method according to claim 25, wherein the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, wherein the respective HVRs have the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab.

27. The method according to claim 25, wherein the anti-IL-13 antibody is a bispecific antibody that also binds IL-4.

28. The method according to any one of claims 1-19, wherein the TH2 Pathway Inhibitor is an anti-IgE antibody.

29. The method according to claim 28, wherein the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti- M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25 [SQNTLVPWT].

30. Use of a kit for detecting Total Periostin in a biological sample obtained from an asthma patient for stratifying/classifying asthma patients into likely responders and non-responders for therapeutic treatment with a TH2 Pathway Inhibitor.

31. The use according to claim 30, wherein the Total Periostin is detected using an EIDA, which EIDA comprises the steps of:

a) determining the amount of Total Periostin in a sample obtained from an asthma patient

b) comparing the amount of Total Periostin determined in step a) to a reference amount.

c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step b).

32. The use according to claim 30 or claim 31, wherein the Total Periostin is serum periostin, which periostin is a measured using an immunoassay.

33. The use according to claim 32, wherein the immunoassay is a sandwich immunoassay.

34. The use according to claim 33, wherein the sandwich immunoassay is performed by an Elecsys® analyzer.

35. The use according to claim 31, wherein the reference amount for a responder is 23ng/ml or greater when using the E4 Assay in step a.

36. The use according to claim 31, wherein the reference amount for a responder is 50ng/ml or greater when using the Elecsys® periostin assay in step a.

37. The use according to claims 30-36, wherein the patient is suffering from moderate to severe asthma.

38. The use according to claims 30-36, wherein the asthma or respiratory disorder is uncontrolled on a corticosteroid.

39. The method according to claim 38, wherein the corticosteroid is an inhaled corticosteroid

40. The method according to claim 39, wherein the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®.

41. The use according to claims 30-39, wherein the patient is also being treated with a second controller.

42. The use according to claim 41, wherein the second controller is a long acting bronchial dilator (LABD).

43. The use according to claim 42, wherein the LABD is a LABA, LTRA, LAMA, theophylline, or OCS.

44. The use according to claim 43, wherein the LABD is Symbicort®, Advair®, Brovana®, Foradil®, PerforomistTM or Serevent®.

45. The use according to claims 30-43, wherein the TH2 Pathway Inhibitor inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935) or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8).

46. The use according to claim 30-43, wherein the TH2 Pathway is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent.

47. The use according to claim 30-43, anti- anti-IL-13 antibody.

48. The use according to claim 47, wherein the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, wherein the respective HVRs have the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab.

49. The use according to claim 47, wherein the anti-IL-13 antibody is a bispecific antibody that also binds IL-4.

50. The use according to claim 30-43, wherein the TH2 Pathway Inhibitor is an anti-IgE antibody.

51. The use according to claim 50, wherein the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti- M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25. [SQNTLVPWT].

52. A kit for measuring the Total Periostin in a biological sample obtained from an asthma patient or a patient suffering from a respiratory disorder, wherein the kit comprises either (1) a first nucleic acid molecule that hybridizes to a second nucleic acid molecule, wherein the

second nucleic acid molecule encodes Total Periostin or a portion thereof, or (2) the kit comprises an antibody that binds to Total Periostin.

53. The kit according to claim 52, comprising a package insert containing information describing the uses according to any one of claims 30-51.

54. A kit for diagnosing an asthma subtype in a patient comprising:

(1) determining the levels of Total Periostin in a serum sample obtained from the patient and optionally the protein expressions levels in the serum sample for one or more proteins selected from TARC and MCP-4; and

(2) instructions for measuring the expression levels of the Total Periostin and optionally one or more proteins selected from TARC and MCP-4 in the serum sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype.

55. The kit according to claim 54, wherein the kit further comprises a package insert for determining whether an asthma patient or respiratory disorder patient is EIP or EIN.

56. The kit according to claim 55, wherein the kit further comprises a package insert for determining whether an asthma patient is likely to respond to a TH2 Pathway Inhibitor.

57. The kit according to claim 55, wherein kit further comprises a package insert containing information describing the uses according to any one of claims 30-51.

58. The kit according to claims 52-56, further comprising an empty container to hold a biological sample.

59. The kit according to claims 52-56, wherein the kit comprises two anti-periostin antibodies for use in an immunoassay for determining Total Periostin levels.

60. A method of treating an asthma or a respiratory disorder comprising administering an anti-IL-13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, wherein the respective HVRs have the amino acid sequence of SEQ ID NO.: 11, SEQ ID

NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 to a patient suffering from asthma or a respiratory disorder in a 125-500mg flat dose every 2-8 weeks.

61. The method according to claim 60, wherein the patient is suffering from moderate to severe asthma.

62. The method according to claim 60 or 61, wherein the asthma or respiratory disorder is uncontrolled on a corticosteroid.

63. The method according to claim 62, wherein the asthma or respiratory disorder is uncontrolled on an inhaled corticosteroid.

64. The method according to claim 62, wherein the asthma or respiratory disorder is uncontrolled on a total daily dose of at least 500 mcg fluticasone propionate (FP).

65. The method according to claim 63, wherein the corticosteroid is an inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair®, Azmacort®.

66. The method according to claim any one of claims 60-65, wherein the patient is being treated with a second controller.

67. The method according to any one of claims 60-66, wherein the patient is continuing to be treated with a corticosteroid, optionally an inhaled corticosteroid, during the treatment with the anti-IL13 antibody.

68. The method according to claim 67, wherein the patient is continuing to be treated with a second controller during the treatment with the anti-IL-13 antibody.

69. The method according to claim 66 or claim 68, wherein the second controller is a long acting bronchial dilator.

70. The method according to claim 69, wherein the long acting bronchial dilator is a LABA, LTRA, LAMA, theophylline, or OCS.

71. The method according to claim 70, wherein the patient has been determined to be EIP.

72. The method according to claim 70, wherein the patient has been determined to be EIP using the kit according to any one of claims 50-59.

73. The method according to claim 70, wherein the patient has been determined to be EIP using the method of any one of claims 7-12.

74. The method according to any one of claims 60-73, wherein the patient is administered a flat dose of 125 mg or 250 mg every four weeks.

75. The method according to claim 74, wherein the patient is 18 years or older.

76. The method according to claim 74, wherein the patient is 12-17 years old or 12 years old and older.

77. The method according to claim 74, wherein the patient is 6-11 years old or 6 years old and older.

78. The method according to any of claims 60-77, wherein the anti-IL-13 antibody is administered subcutaneously.

79. The method according to claim 78, wherein the anti-IL-13 antibody is administered using a prefilled syringe or autoinjector device.

80. The method according to claim 60, wherein the asthma patient to be treated is 18 years old or older and has serum periostin at $\geq$ 50 ng/mL and is uncontrolled on an inhaled corticosteroid and a second controller medication.

81. The method according to claim 78, wherein the serum periostin is measured using the method of any one of claims 7-10 and 12.

82. The method according to claim 78, wherein the serum periostin is measured using the kit of any one of claims 52-59.

83. The method according to any of claims 60-82, wherein the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and a VL comprising SEQ ID NO:10.

84. The method according to any of claims 60-82, wherein the anti-IL-13 antibody is lebrikizumab.

85. The method according to claim 76, wherein the asthma patient is 12 years old and above and uncontrolled on an inhaled corticosteroid and a second controller medication.

86. A method of treating asthma or a respiratory disease comprising administering a therapeutically effective amount of lebrikizumab to the patient, wherein the treatment results in a relative improvement in FEV1 of greater than 5% compared to before treatment with lebrikizumab.

87. The method according to claim 86, wherein the relative improvement in FEV1 is greater than 8% compared to before treatment with lebrikizumab.

88. A method of treating asthma or a respiratory disease comprising administering a therapeutically effective amount of lebrikizumab to the patient, wherein the treatment results in a reduction in severe exacerbations.

89. A method of treating of a patient suffering from asthma or a respiratory disease comprising administering a TH2 Pathway Inhibitor to the patient diagnosed as EIP.

90. The method according to claim 89 further comprising the step of diagnosing the patient as EIP using a Total Periostin Assay.

91. The method according to claim 89 or claim 90, further comprising the step of retreating the patient with the TH2 Pathway Inhibitor if the patient is determined to be EIP.

92. The method according to any one of claims 89-91, wherein serum from the patient is used to determine whether the patient is EIP.

93. The method according to any one of claims 89-91, wherein the EIP status is diagnosed using an EIDA in which the assay comprises the steps of:

a) determining the amount of Total Periostin in a sample obtained from the patient;

b) comparing the amount of Total Periostin determined in step a) to a reference amount; and

c) stratifying said patient into the category of responder or non-responder based on the comparison obtained in step (b).

94. The method according to claim 93, wherein the Total Periostin is serum periostin, which periostin is a measured using an immunoassay.

95. The method according to claim 94, wherein the immunoassay is a sandwich immunoassay.

96. The method according to claim 95, wherein the sandwich immunoassay is performed by an Elecsys® analyzer.

97. The method according to any one of claims 93-96, wherein the reference amount is 23ng/ml or greater when using the E4 Assay in step (a).

98. The method according to any one of claims 93-96, wherein the reference amount is 50ng/ml or greater when using the Elecsys® periostin assay in step a.

99. The method according to claims 89-98, wherein the patient is suffering from moderate to severe asthma.

100. The method according to claims 89-99, wherein the asthma or respiratory disorder is uncontrolled on a corticosteroid.

101. The method according to claim 100, wherein the corticosteroid is an inhaled corticosteroid.

102. The method according to claim 101, wherein the inhaled corticosteroid is Qvar®, Pulmicort®, Symbicort®, Aerobid®, Flovent®, Flonase®, Advair® or Azmacort®.

103. The method according to claims 89-101, wherein the patient is also being treated with a second controller.

104. The method according to claim 103, wherein the second controller is a long acting bronchial dilator (LABD).

105. The method according to claim 104, wherein the LABD is a LABA, LTRA, LAMA, theophylline, or OCS.

106. The method according to claim 104, wherein the LABD is Symbicort®, Advair®, Brovana®, Foradil®, PerforomistTM or Serevent®.

107. The method according to claim 88-101, wherein the TH2 Pathway Inhibitor inhibits the target ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL4/IL13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL4/IL13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343,

PF3526299); CCR4 (AMG-761), TLR9 (QAX-935) or is a multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8).

108. The method according to claim 88-106, wherein the TH2 Pathway is an anti-IL13/IL4 pathway inhibitor or an anti IgE binding agent.

109. The method according to claim 108, wherein the anti-IL-13/IL4 pathway inhibitor is an anti- anti-IL-13 antibody.

110. The method according to claim 109, wherein the anti-IL-13 antibody is an antibody comprising a VH comprising SEQ ID NO:9 and VL comprising SEQ ID NO:10, an anti-IL13 antibody comprising HVRH1, HVRH2, HVRH3, HVRL1, HVRL2, and HVRL3, wherein the respective HVRs have the amino acid sequence of SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, and SEQ ID NO.: 16 or lebrikizumab.

111. The method according to claim 109, wherein the anti-IL-13 antibody is a bispecific antibody that also binds IL-4.

112. The method according to claim 108, wherein the TH2 Pathway Inhibitor is an anti-IgE antibody.

113. The method according to claim 112, wherein the anti-IgE antibody is (i) the XOLAIR® antibody, (ii) anti-M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain is SEQ ID NO:24 and the variable light chain is SEQ ID NO:25 or (iii) an anti- M1' antibody comprising a variable heavy chain and a variable light chain, wherein the variable heavy chain further comprises an HVR-H1, HVR-H2 and HVR-H3, and the variable light chain further comprises and HVR-L1, HVR, L2 and HVR-L3 and: (a) the HVR-H1 is residues 26-35 of SEQ ID NO:24, [GFTFSDYGIA]; (b) the HVR-H2 is residues 49-66 of SEQ ID NO:24, [AFISDLAYTIYYADTVTG]; (c) the HVR-H3 is residues 97-106 of SEQ ID NO:24, [ARDNWDAMDY]; (d) the HVR-L1 is residues 24-39 of SEQ ID NO:25, [RSSQSLVHNNANTYLH]; (e) the HVR-L2 is residues 55-61 of SEQ ID NO:25, [KVSNRFS]; (f) the HVR-L3 is residues 94-102 of SEQ ID NO:25 [SQNTLVPWT].

114. A method for evaluating adverse events in a patient associated with treatment of asthma with lebrikizumab comprising the steps of monitoring the number and/or severity of events that are exacerbations, community-acquired pneumonia, anaphylaxis, musculoskeletal pains, musculoskeletal disorders, connective tissue pains or connective tissue disorders.

115. The method according to claim 114, wherein the musculoskeletal or connective tissue disorder is arthralgia, back pain, pain in extremity, myalgia, neck pain, arthritis, bone development abnormalities, bursitis, costochondritis, exostosis, flank pain, musculoskeletal chest pain, musculoskeletal pain, pain in jaw or tendinitis.

116. An anti-periostin antibody comprising the HVR sequences of SEQ ID NO:1 and the HVR sequences of SEQ ID NO:2.

117. The antibody according to claim 116, wherein the antibody comprises the sequences of SEQ ID NO:1 and SEQ ID NO:2.

118. An anti-periostin antibody comprising the HVR sequences of SEQ ID NO:3 and the HVR sequences of SEQ ID NO:4.

119. The antibody according to claim 118, wherein the antibody comprises the sequences of SEQ ID NO:3 and SEQ ID NO:4.

120. A Total Periostin Assay comprising the use of the antibodies claims 116 and 118.

SEQUENCE LISTING

<110> GENENTECH, INC. ET AL.

<120> DIAGNOSIS AND TREATMENTS RELATING TO TH2 INHIBITION

<130> P4569R1-WO

<140>
<141>

<150> 61/557,295
<151> 2011-11-08

<150> 61/574,485
<151> 2011-08-02

<150> 61/484,650
<151> 2011-05-10

<150> 61/465,425
<151> 2011-03-18

<150> 61/459,760
<151> 2010-12-16

<160> 25

<170> PatentIn version 3.5

<210> 1
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 1

```
Gln Val His Leu Gln Gln Ser Gly Ala Glu Leu Ala Lys Pro Gly Ala
1               5                  10                  15

Ser Val His Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Asn Thr Gly Tyr Ala Asp Tyr Asn Gln Lys Phe
    50                  55                  60

Arg Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Thr Val Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Arg Thr Gly Thr Ser Tyr Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110
```

Thr Thr Leu Thr Val Ser Ser Thr Lys Thr Thr Pro Pro Ser Val
115 120 125

<210> 2
<211> 102
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 2
Gln Thr Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Thr Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Phe
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys
100

<210> 3
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 3
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr His Tyr
20 25 30

Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Tyr Pro Gly Asp Gly Asp Thr Arg Tyr Thr Gln Arg Leu
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Glu Gly Asn Ser Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
115 120 125

<210> 4
<211> 103
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 4
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Ser Ser
20 25 30

Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Thr Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Asp Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Thr Asp Tyr Phe Cys Leu Gln Tyr Gly Thr Tyr Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Arg
100

<210> 5
<211> 836
<212> PRT
<213> Homo sapiens

<400> 5
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1 5 10 15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
20 25 30

```
Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300
```

```
Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575
```

```
Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Thr Thr Lys
            660                 665                 670

Ile Ile Thr Lys Val Val Glu Pro Lys Ile Lys Val Ile Glu Gly Ser
        675                 680                 685

Leu Gln Pro Ile Ile Lys Thr Glu Gly Pro Thr Leu Thr Lys Val Lys
    690                 695                 700

Ile Glu Gly Glu Pro Glu Phe Arg Leu Ile Lys Glu Gly Glu Thr Ile
705                 710                 715                 720

Thr Glu Val Ile His Gly Glu Pro Ile Ile Lys Lys Tyr Thr Lys Ile
                725                 730                 735

Ile Asp Gly Val Pro Val Glu Ile Thr Glu Lys Glu Thr Arg Glu Glu
            740                 745                 750

Arg Ile Ile Thr Gly Pro Glu Ile Lys Tyr Thr Arg Ile Ser Thr Gly
        755                 760                 765

Gly Gly Glu Thr Glu Glu Thr Leu Lys Lys Leu Leu Gln Glu Glu Val
    770                 775                 780

Thr Lys Val Thr Lys Phe Ile Glu Gly Gly Asp Gly His Leu Phe Glu
785                 790                 795                 800

Asp Glu Glu Ile Lys Arg Leu Leu Gln Gly Asp Thr Pro Val Arg Lys
                805                 810                 815

Leu Gln Ala Asn Lys Lys Val Gln Gly Ser Arg Arg Arg Leu Arg Glu
            820                 825                 830

Gly Arg Ser Gln
        835
```

<210> 6
<211> 779
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                  10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255
```

```
Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525
```

```
Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Lys Pro Ile
            660                 665                 670

Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr
        675                 680                 685

Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys
    690                 695                 700

Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys
705                 710                 715                 720

Lys Leu Leu Gln Glu Glu Val Thr Lys Val Thr Lys Phe Ile Glu Gly
                725                 730                 735

Gly Asp Gly His Leu Phe Glu Asp Glu Glu Ile Lys Arg Leu Leu Gln
            740                 745                 750

Gly Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys Lys Val Gln Gly
        755                 760                 765

Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
    770                 775
```

<210> 7
<211> 781

<212> PRT
<213> Homo sapiens

<400> 7

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                  10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255
```

```
Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525
```

```
Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Arg Pro Thr
            660                 665                 670

Leu Thr Lys Val Lys Ile Glu Gly Glu Pro Glu Phe Arg Leu Ile Lys
        675                 680                 685

Glu Gly Glu Thr Ile Thr Glu Val Ile His Gly Glu Pro Ile Ile Lys
    690                 695                 700

Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr Glu Lys
705                 710                 715                 720

Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys Tyr Thr
                725                 730                 735

Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys Lys Leu
            740                 745                 750

Leu Gln Glu Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys Lys Val
        755                 760                 765

Gln Gly Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
    770                 775                 780
```

<210> 8
<211> 751
<212> PRT
<213> Homo sapiens

<400> 8

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                  10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270
```

```
Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540
```

```
Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Lys Pro Ile
            660                 665                 670

Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr
        675                 680                 685

Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys
    690                 695                 700

Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys
705                 710                 715                 720

Lys Leu Leu Gln Glu Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys
                725                 730                 735

Lys Val Gln Gly Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
            740                 745                 750


<210> 9
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 9
Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr
1               5                   10                  15
```

Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser
20 25 30

Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
35 40 45

Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
50 55 60

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
65 70 75 80

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly
85 90 95

Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 10
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

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

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95

Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 11
<211> 6

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 11
Ala Tyr Ser Val Asn Trp
1 5

<210> 12
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 12
Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
1 5 10 15

<210> 13
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 13
Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
1 5 10

<210> 14
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 14
Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
1 5 10 15

<210> 15
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 15
Leu Ala Ser Asn Leu Glu Ser
1 5

<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 16
Gln Gln Asn Asn Glu Asp Pro Arg Thr
1 5

<210> 17
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 17
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95

Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val

<210> 18
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 18
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

```
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30

Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45

Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Asn Pro Ser Val
    50                  55                  60

Lys Gly Arg Ile Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
            100                 105                 110

Gln Gly
```

<210> 19
<211> 836
<212> PRT
<213> Homo sapiens

<400> 19

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                   10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125
```

```
Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400
```

```
Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Thr Thr Lys
            660                 665                 670
```

```
Ile Ile Thr Lys Val Val Glu Pro Lys Ile Lys Val Ile Glu Gly Ser
        675                 680                 685

Leu Gln Pro Ile Ile Lys Thr Glu Gly Pro Thr Leu Thr Lys Val Lys
    690                 695                 700

Ile Glu Gly Glu Pro Glu Phe Arg Leu Ile Lys Glu Gly Glu Thr Ile
705                 710                 715                 720

Thr Glu Val Ile His Gly Glu Pro Ile Ile Lys Lys Tyr Thr Lys Ile
                725                 730                 735

Ile Asp Gly Val Pro Val Glu Ile Thr Glu Lys Glu Thr Arg Glu Glu
            740                 745                 750

Arg Ile Ile Thr Gly Pro Glu Ile Lys Tyr Thr Arg Ile Ser Thr Gly
        755                 760                 765

Gly Gly Glu Thr Glu Glu Thr Leu Lys Lys Leu Leu Gln Glu Glu Val
    770                 775                 780

Thr Lys Val Thr Lys Phe Ile Glu Gly Gly Asp Gly His Leu Phe Glu
785                 790                 795                 800

Asp Glu Glu Ile Lys Arg Leu Leu Gln Gly Asp Thr Pro Val Arg Lys
                805                 810                 815

Leu Gln Ala Asn Lys Lys Val Gln Gly Ser Arg Arg Arg Leu Arg Glu
            820                 825                 830

Gly Arg Ser Gln
        835


<210> 20
<211> 779
<212> PRT
<213> Homo sapiens

<400> 20
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                   10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80
```

```
Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350
```

```
Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620
```

```
Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Lys Pro Ile
            660                 665                 670

Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr
        675                 680                 685

Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys
    690                 695                 700

Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys
705                 710                 715                 720

Lys Leu Leu Gln Glu Glu Val Thr Lys Val Thr Lys Phe Ile Glu Gly
                725                 730                 735

Gly Asp Gly His Leu Phe Glu Asp Glu Glu Ile Lys Arg Leu Leu Gln
            740                 745                 750

Gly Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys Lys Val Gln Gly
        755                 760                 765

Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
    770                 775
```

<210> 21
<211> 781
<212> PRT
<213> Homo sapiens

<400> 21

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                   10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80
```

```
Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350
```

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
355 360 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
370 375 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385 390 395 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
405 410 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
420 425 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
435 440 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
450 455 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465 470 475 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
485 490 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
500 505 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
515 520 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
530 535 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545 550 555 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
565 570 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
580 585 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
595 600 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
610 615 620

```
Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Arg Pro Thr
            660                 665                 670

Leu Thr Lys Val Lys Ile Glu Gly Glu Pro Glu Phe Arg Leu Ile Lys
        675                 680                 685

Glu Gly Glu Thr Ile Thr Glu Val Ile His Gly Glu Pro Ile Ile Lys
    690                 695                 700

Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr Glu Lys
705                 710                 715                 720

Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys Tyr Thr
                725                 730                 735

Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys Lys Leu
            740                 745                 750

Leu Gln Glu Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys Lys Val
        755                 760                 765

Gln Gly Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
    770                 775                 780


<210> 22
<211> 751
<212> PRT
<213> Homo sapiens

<400> 22
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                   10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95
```

```
Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125

Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365
```

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
370 375 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385 390 395 400

Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
405 410 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
420 425 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
435 440 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
450 455 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465 470 475 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
485 490 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
500 505 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
515 520 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
530 535 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545 550 555 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
565 570 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
580 585 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
595 600 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
610 615 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625 630 635 640

```
Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Lys Pro Ile
            660                 665                 670

Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr
        675                 680                 685

Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys
    690                 695                 700

Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys
705                 710                 715                 720

Lys Leu Leu Gln Glu Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys
                725                 730                 735

Lys Val Gln Gly Ser Arg Arg Arg Leu Arg Glu Gly Arg Ser Gln
            740                 745                 750
```

<210> 23
<211> 695
<212> PRT
<213> Homo sapiens

<400> 23

```
Met Ile Pro Phe Leu Pro Met Phe Ser Leu Leu Leu Leu Leu Ile Val
1               5                   10                  15

Asn Pro Ile Asn Ala Asn Asn His Tyr Asp Lys Ile Leu Ala His Ser
            20                  25                  30

Arg Ile Arg Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu Gln Gln
        35                  40                  45

Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn Trp Tyr
    50                  55                  60

Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu Cys Cys
65                  70                  75                  80

Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala Val Leu
                85                  90                  95

Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala Thr Thr
            100                 105                 110

Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile Glu Gly
        115                 120                 125
```

```
Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp Asp Asn
    130                 135                 140

Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn Val Glu
145                 150                 155                 160

Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met Leu Thr
                165                 170                 175

Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn Asn Leu
            180                 185                 190

Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val Asn Cys
        195                 200                 205

Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val Val His
    210                 215                 220

Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe
225                 230                 235                 240

Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr
                245                 250                 255

Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe
            260                 265                 270

Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu
        275                 280                 285

Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His
    290                 295                 300

Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val
305                 310                 315                 320

Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp
                325                 330                 335

Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val
            340                 345                 350

Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp
        355                 360                 365

Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe
    370                 375                 380

Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp
385                 390                 395                 400
```

```
Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp
                405                 410                 415

Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln Asn His
            420                 425                 430

Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly Gln Ile
        435                 440                 445

Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr Arg Thr
    450                 455                 460

Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys Gln Gly
465                 470                 475                 480

Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro Ala Glu
                485                 490                 495

Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Ser Thr Phe
            500                 505                 510

Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr Gln Pro
        515                 520                 525

Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys Gly Met
    530                 535                 540

Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala Leu Gln
545                 550                 555                 560

Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly Lys Gly
                565                 570                 575

Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly Ser Lys
            580                 585                 590

Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu Leu Lys
        595                 600                 605

Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His Val Val
    610                 615                 620

Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp Gln Leu
625                 630                 635                 640

Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys Phe Val
                645                 650                 655

Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Ser Pro Glu
            660                 665                 670
```

Ile Lys Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr
675 680 685

Leu Lys Lys Leu Leu Gln Glu
690 695

<210> 24
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 24
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gly Ile Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Phe Ile Ser Asp Leu Ala Tyr Thr Ile Tyr Tyr Ala Asp Thr Val
50 55 60

Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Asn Trp Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 25
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Val His Asn
20 25 30

```
Asn Ala Asn Thr Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65                  70                  75                  80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Ser Gln Asn
                85                  90                  95

Thr Leu Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
```