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(54) Title: ASSAY TO DETECT HUMAN DPP-4

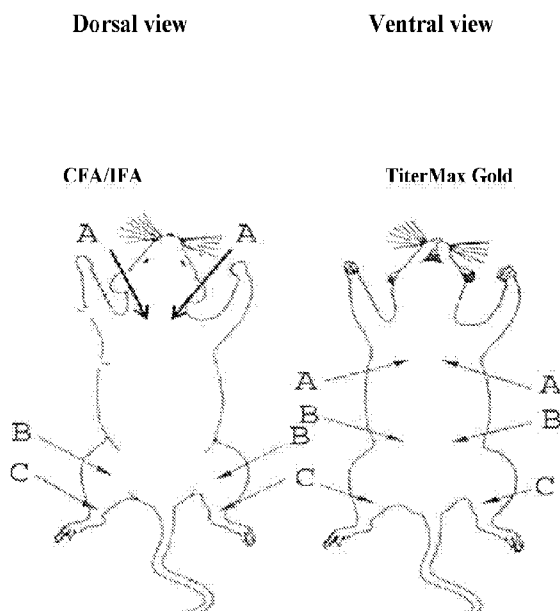


FIG. 1

(57) Abstract: This disclosure provides a robust, sensitive, and specific assay for the detection and measurement of DPP-4 levels in samples obtained from human patients. The disclosure further provides novel anti-DPP-4 monoclonal antibodies that recognize human DPP-4, and assay kits comprising one or more of these antibodies.



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ASSAY TO DETECT HUMAN DPP-4

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing in ASCII text file (Name DPP4-100P1_sequence_listing.txt; Size: 26,741 bytes; and Date of Creation: December 15, 2014) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Dipeptidyl Peptidase IV (DPP-4) (also known as CD26 or adenosine deaminase binding protein) is a type II transmembrane serine protease in the prolyl oligopeptidase family that catalyzes the hydrolysis of N-terminal dipeptides from the N-terminus of polypeptides having a proline or alanine in position 2 (Enzyme Commission (EC) Number 3.4.14.5 (BRENDA | IUBMB)). A number of chemokine and peptide hormones including GLP-1, GLP-2, gastric inhibitory polypeptide (GIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and neuropeptide Y are cleaved and inactivated by DPP-4. *See, e.g.,* Yaron A., Naider F. Crit. Rev. Biochem. Mol. Biol. 28, 31–81 (1993); Mentlein R. Regul Pept. 85(1):9-24 (1999). As a result, DPP-4 regulates glucose metabolism, appetite and pain regulation through its ability to inhibit chemokine and peptide hormones.

[0003] In addition to its role as a regulatory protease, DPP-4 also binds several molecules and induces intracellular signal transduction. In particular, DPP-4 induces T-cell co-stimulation / proliferation and lymphocyte-epithelial cell adhesion by binding to several ligands including adenosine deaminase (ADA). *See, e.g.,* Gines et al., Biochem. J. 361:203-209 (2002). DPP-4 has also been reported to enhance T-cell maturation and migration, cytokine secretion, antibody production, immunoglobulin isotype switching of B cells, and activation of cytotoxic T cells. Ohnuma *et. al.*, Front Biosci. 13:2299-310 (2008).

[0004] DPP-4 is a 110 kDa glycoprotein, encoded by a gene located on chromosome 2 (2q24.3) (Abbott *et al.* Immunogenetics. 40(5):331-8 (1994)), and functions as a homodimer consisting of 766 amino acids. Each monomer consists of two domains: an alpha/beta hydrolase domain and an eight-blade beta-propeller domain. DPP-4 is widely expressed in several tissues

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including liver, lung, kidney, epithelial cells and lymphocytes. Heike *et al.* Clin Exp Immunol. 74:431–434 91988); Gorrell *et al.*, Scand J Immunol. 54:249–264 (2001). Upon T cell activation, DPP-4 expression is up-regulated on resting T cells. A soluble, active form of DPP-4 containing most of the extracellular domain (residues 39-766) including the key catalytic domain, has also been observed. Ikushima H, *et al.* Cell Immunol. 215(1):106-10 (2002).

- [0005] DPP-4 gene knock out mice show improved glucose tolerance with oral glucose loading, increased insulin and GLP-1 activity; resistance to diet-induced obesity; and increased insulin sensitivity following high-fat diets. Marguet *et al.*, Proc Natl Acad Sci U S A. 97(12):6874-9 (2000); Conarello *et al.*, Proc Natl Acad Sci U S A. 100(11):6825-30 (2003). In addition to its role in metabolic disorders and glycemic control, DPP-4 has also been implicated in controlling immune function, cell migration, entry of viruses into cells, cancer metastasis and inflammation. *See, e.g.*, Aytac *et al.*, Curr Drug Targets Immune Endocr Metabol Disord 4(1):11-8 (2004).
- [0006] More recently, DPP-4 expression has been reported to be highly induced by Interleukin-13 (IL-13). *See, e.g.*, Zhang *et al.*, Am J Respir Crit Care Med 189:A4875 (2014); Shiobara *et al.*, Am J Respir Crit Care Med 189:A4239 (2014); Brightling *et al.*, Am J Respir Crit Care Med 189:A6670 (2014); U.S. Provisional Application No. 61/931,878, filed January 27, 2014; and U.S. Provisional Application No. 61/990,932, filed May 9, 2014, each herein incorporated by reference in its entirety for all purposes. IL-13 is a 114 amino acid cytokine with an unmodified molecular mass of approximately 12 kDa. McKenzie, A. N., *et al.* J Immunol, 1993. 150:5436-44; Minty, A., *et al.* Nature, 1993. 362:248-50. IL-13 levels have been shown to correlate with disease severity in a number of diseases or disorders including, but not limited to, asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and atopic dermatitis. For example, in asthmatics and rodent models of allergic inflammation elevated IL-13 levels have been reported to correlate with disease severity (*see* U.S. Pat. Appl. Publ. No. 2012-0052060, published March 1, 2012, and incorporated herein by reference in its entirety).
- [0007] Chronic obstructive pulmonary disease (COPD) includes patient populations with varying degrees of chronic bronchitis, small airway disease, and emphysema, and is characterized by progressive irreversible lung function decline that responds poorly to current asthma based therapy. Zheng *et al* (J Clin Invest, 2000. 106:1081-93) demonstrated that overexpression of

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IL-13 in the mouse lung caused emphysema, elevated mucus production, and inflammation, reflecting aspects of human COPD. The signs are therefore that IL-13 plays an important role in the pathogenesis of COPD, particularly in patients with asthma-like features.

- [0008] IL-13 can also play a role in the pathogenesis of inflammatory bowel disease, and has been associated with fibrotic conditions, such as idiopathic pulmonary fibrosis (IPF). *See, e.g.*, Jovani, M., *et al.* Curr Drug Targets. 2013.12:1444-52; and Rafii, R., *et al.* J Thorac Dis. 2013. 1:48-73
- [0009] Atopic dermatitis is a common chronic inflammatory skin disease that is often associated with other atopic disorders such as allergic rhinitis and asthma (Bieber, New England Journal of Medicine, 2008, 358: 1483-1494). Upregulation of IL-13 mRNA has been observed in subacute and chronic lesions of atopic dermatitis (Tazawa et al., Arch. Dermatol. Res., 2004, 295:459-464; Purwar et al., J. Invest. Derm., 2006, 126, 1043-1051; Oh et al., J Immunol., 2011, 186:7232-42).
- [0010] Elevated DPP-4 levels have been observed in asthma, COPD and AD patients (*see, e.g.*, U.S. Provisional Application No. 61/931,878, filed January 27, 2014; and U.S. Provisional Application No. 61/990,932, filed May 9, 2014, each incorporated herein by reference in its entirety). In addition, in a phase 2B clinical study involving asthma patients, high serum DPP-4 levels predicted improved response rates in patients treated with an IL-13 antibody antagonist (tralokinumab) identifying DPP-4 as a predictive biomarker for IL-13-mediated disease or disorders including an IL-13-mediated pulmonary disease or disorder (*e.g.*, asthma, IPF or COPD) or an IL-13-mediated chronic inflammatory skin disease or disorder (*e.g.*, atopic dermatitis). *See* Brightling *et al.*, Am J Respir Crit Care Med 189:A6670 (2014); and U.S. Provisional Application No. 61/931,878, filed January 27, 2014; and U.S. Provisional Application No. 61/990,932, filed May 9, 2014, each herein incorporated by reference in its entirety. Thus, while increased DPP-4 levels are known to correlate with certain IL-13-mediated diseases or disorders and DPP-4 serum levels are known to predict patient response to anti-IL-13 therapy, there remains a need for specific and sensitive assays to measure the amount and/or determine changes in DPP-4 levels in patients, including, but not limited to, patients suffering from an IL-13-mediated disease or disorder. Although there are commercially available reagents which could be used to measure serum DPP-4 levels in

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patients, these commercially available reagents or kits rely on polyclonal antibodies, which not only introduce assay variations due to lot to lot differences and/or are not very sensitive. Accordingly, there still remains a need for specific and sensitive antibodies, reagents and/or immunoassays to measure the amount and/or determine changes in DPP-4 levels in patients.

BRIEF SUMMARY

- [0011]** This disclosure provides anti-dipeptidyl peptidase-4 (DPP-4) antibodies that can be used, e.g., in diagnostic assays to determine DPP-4 levels in a subject. Exemplary anti-DPP-4 antibodies of the disclosure include: (1) mouse monoclonal antibody m3B7.6 produced by a hybridoma deposited on January 8, 2015 at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedures, and assigned ATCC accession number PTA-121870; (2) mouse monoclonal antibody m5B7.7 produced by a hybridoma deposited on January 8, 2015 at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedures, and assigned ATCC accession number PTA-121871; (3) rat monoclonal antibody R11A2.15 produced by a hybridoma deposited on January 8, 2015 at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedures, and assigned ATCC accession number PTA-121872; and (4) rat monoclonal antibody R11A9.11 produced by a hybridoma deposited on January 8, 2015 at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedures, and assigned ATCC accession number PTA-121873.
- [0012]** In certain aspects the disclosure provides an isolated antibody or antigen-binding fragment, variant, or derivative thereof, or two or more such antibodies, where the antibody or antibodies competitively inhibit binding of and/or bind to the same DPP-4 epitope as: (1) mouse

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monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, (2) mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, (3) rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or (4) rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873, or any combination thereof.

[0013] In certain aspects, the disclosure provides an isolated antibody or antigen-binding fragment, variant, or derivative thereof that binds DPP-4, which includes a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, where the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[0014] In certain aspects, the antibody or antigen-binding fragment, variant, or derivative thereof provided by this disclosure can include a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[0015] In certain aspects the disclosure provides an antigen-binding antibody fragment as described above. In certain aspects the antibody fragment can be a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, or a single chain antibody molecule.

[0016] The disclosure further provides a hybridoma deposited at the ATCC under Deposit No. 121870, a hybridoma deposited at the ATCC under Deposit No. 121871, a hybridoma

deposited at the ATCC under Deposit No. 121872, a hybridoma deposited at the ATCC under Deposit No. 121873, and/or a combination thereof. In certain aspects the disclosure provides an antibody-producing cell culture that includes a hybridoma deposited at the ATCC under Deposit No. 121870, a hybridoma deposited at the ATCC under Deposit No. 121871, a hybridoma deposited at the ATCC under Deposit No. 121872, a hybridoma deposited at the ATCC under Deposit No. 121873, and/or a combination thereof. In another aspect the disclosure provides an isolated antibody or antigen-binding fragment, variant, or derivative thereof produced by the hybridoma as provided herein or the antibody-producing cell culture as provided herein.

- [0017]** In certain aspects, the antibody, or fragment, variant, or derivative thereof provided by the disclosure, or the antibody produced by the hybridoma or the cell culture provided by the disclosure, or a fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, or R11A9.11), further includes a heterologous polypeptide fused thereto. For example, in certain aspects, the heterologous polypeptide is a stabilizing polypeptide, a tag, a label, or a combination thereof.
- [0018]** In certain aspects, the antibody, or fragment, variant, or derivative thereof provided by the disclosure, or the antibody produced by the hybridoma or the cell culture provided by the disclosure, or a fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, or R11A9.11), is conjugated to a heterologous moiety. In certain aspects, the heterologous moiety includes one or more of: a peptide, a protein, an enzyme, a lipid, a heterologous antibody or fragment thereof, a detectable label, or polyethylene glycol (PEG). In certain aspects, the heterologous moiety is, *e.g.*, biotin, or a ruthenium chelate.
- [0019]** The disclosure further provides a composition that includes the antibody, or fragment, variant, or derivative thereof as provided by the disclosure, and/or the antibody produced by the hybridoma or the cell culture provided by the disclosure, or a fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, and/or R11A9.11). In certain aspects, the composition includes a combination of at least two such antibodies.
- [0020]** In another aspect, the disclosure provides an isolated polynucleotide that includes a nucleic acid molecule encoding an antibody, or a subunit, fragment, variant, or derivative thereof as provided by the disclosure, or the antibody or fragment thereof produced by the hybridoma or

the cell culture provided by the disclosure, or a subunit, fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, or R11A9.11). The disclosure further provides a vector that includes the polynucleotide as provided.

[0021] In certain aspects the disclosure provides a composition that includes two or more nucleic acid molecules encoding the antibody, or a fragment, variant, or derivative thereof as provided by the disclosure, or the antibody or fragment thereof produced by the hybridoma or the cell culture provided by the disclosure, or a fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, or R11A9.11). In certain aspects the two or more nucleic acid molecules are situated in the same vector. The disclosure further provides the vector that includes the two or more nucleic acid molecules as provided, situated in the same vector. In certain aspects, the two or more nucleic acid molecules are situated in at least two separate vectors. The disclosure further provides the two separate vectors.

[0022] The disclosure further provides an isolated host cell that includes the provided vector, or the two or more provided vectors. The disclosure further provides a method of making an anti-DPP-4 antibody, or a subunit, fragment, variant, or derivative thereof as provided by the disclosure, or the antibody produced by the hybridoma or the cell culture provided by the disclosure, or a subunit, fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, and/or R11A9.11), where the method includes (a) culturing the provided host cell, and (b) recovering the antibody, subunit, fragment, or derivative thereof.

[0023] In another aspect, the disclosure provides a kit for measuring DPP-4 levels in a sample, where the kit includes the antibody, or fragment, variant, or derivative thereof as provided by the disclosure, or the antibody produced by the hybridoma or the cell culture provided by the disclosure, or a fragment, variant, or derivative thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, or R11A9.11). In certain aspects, the kit includes at least two such antibodies, or fragments, variants, or derivatives thereof. The kit can further include a solid support and/or detection reagents. In certain aspects, one of the at least two antibodies or fragments, variants, or derivatives thereof can be a capture antibody, or fragment, variant, or derivative thereof, and one of the at least two antibodies or fragments, variants, or derivatives thereof can be a detection antibody, or fragment, variant, or derivative thereof. In certain aspects the detection antibody can include a detectable label, *e.g.*, the detectable label can be

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biotin and the kit can include detection reagents such as a streptavidin-horse radish peroxidase (HRP) conjugate and a colorimetric substrate for HRP, or the detectable label can be a ruthenium chelate. In certain aspects:

- [0024]** The capture antibody can be mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;
- [0025]** The capture antibody can be mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- [0026]** The capture antibody can be mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;
- [0027]** The capture antibody can be mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- [0028]** The capture antibody can be rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof;
- [0029]** The capture antibody can be rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment,

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variant, or derivative thereof and the detection antibody can be mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof;

[0030] The capture antibody can be rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof; and/or

[0031] The capture antibody can be rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody can be mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[0032] The disclosure further provides an immunoassay for detecting DPP-4 levels in one or more samples, where the immunoassay includes the use of at least two anti-DPP-4 antibodies or antigen-binding fragments, variants, or derivatives thereof, where one of the anti-DPP-4 antibodies can be an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of and/or binds to the same DPP-4 epitope as mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and where one of the anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of and/or binds to the same DPP-4 epitope as rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[0033] In certain aspects, the immunoassay includes the use of at least two anti-DPP-4 antibodies or antigen-binding fragments, variants, or derivatives thereof, where one of the anti-DPP-4 antibodies can be an isolated antibody or antigen-binding fragment, variant, or derivative thereof that includes VH with three heavy chain CDRs VHCDR1, VHCDR2 and VHCDR3,

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and VL with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, where the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and where one of the two or more anti-DPP-4 antibodies can be an isolated antibody or antigen-binding fragment, variant, or derivative thereof that includes a VH with three heavy chain CDRs VHCDR1, VHCDR2 and VHCDR3, and VL with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, where the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[0034] In certain aspects, the immunoassay includes the use of at least two anti-DPP-4 antibodies or antigen-binding fragments, variants, or derivatives thereof, where one of the two or more anti-DPP-4 antibodies can be an isolated antibody or antigen-binding fragment, variant, or derivative thereof that includes a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and where one of the two or more anti-DPP-4 antibodies can be an isolated antibody or antigen-binding fragment, variant, or derivative thereof that includes a VH and a VL identical to the VH and VL of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[0035] In certain aspects, the immunoassay can be a sandwich immunoassay that includes use of a first anti-DPP-4 "capture" antibody or antigen-binding fragment thereof attached to a solid support, and a second anti-DPP-4 "detection" antibody or antigen-binding fragment thereof (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, and/or R11A9.11). For example, the immunoassay can include attaching a capture antibody or antigen-binding fragment thereof to a solid support; applying the test sample or a control sample under conditions sufficient to

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allow DPP-4, if present in the sample, to bind to the capture antibody or antigen-binding fragment thereof; applying the detection antibody or antigen-binding fragment thereof under conditions sufficient to allow binding to DPP-4 already bound to the capture antibody or antigen-binding fragment thereof; and measuring the amount of detection antibody or antigen-binding fragment thereof bound to DPP-4. In certain aspects the detection antibody can include a detectable label, e.g., the detectable label can be biotin and the kit can include detection reagents such as a streptavidin-horse radish peroxidase (HRP) conjugate and a colorimetric substrate for HRP, or the detectable label can be a ruthenium chelate.

- [0036] In certain aspects the immunoassay can include the use of a capture antibody and a detection antibody where:
- [0037] The capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;
- [0038] The capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- [0039] The capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;
- [0040] The capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;

- [0041] The capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof;
- [0042] The capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof;
- [0043] The capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof; and/or
- [0044] The capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.
- [0045] In certain aspects, the disclosure provides a method of measuring the amount of DPP-4 in a sample obtained from a subject, where the method includes assaying the sample using the provided immunoassay or kit, and/or the provided antibody, or fragment, variant, or derivative thereof, or a combination of two or more such antibodies (including, *e.g.*, antibodies m3B7.6, m5B7.7, R11A2.15, and/or R11A9.11). In certain aspects, the sample can be one or more of whole blood, serum, plasma, saliva, urine, sputum, bronchoalveolar lavage fluid, lung epithelial cells, or nasal polyps, or skin. In certain aspects, the subject has a disease or condition selected from the group consisting of: an IL-13-mediated disease or disorder, a pulmonary disease or disorder, and a chronic inflammatory skin disease or disorder. For example, the disease or condition can be asthma, atopic asthma, corticosteroid naive asthma, chronic

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asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, asthma due to smoking, asthma uncontrolled on corticosteroids, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), ulcerative colitis (UC), atopic dermatitis (AD), allergic rhinitis, chronic rhinosinusitis, skin fibrosis, allergic contact dermatitis, eczema and/or psoriasis. In certain aspects, the sample can be obtained from the subject and can be submitted for measurement of the DPP-4 level in the sample. In certain aspects, the subject can be an asthma patient, and the sample taken from the patient can be serum.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0046] Figure 1:** Draining lymph node injection sites for RIMMS protocol. Mouse and rat monoclonal antibodies specific for human DPP-4 were produced using the Repetitive Immunization at Multiple Sites (RIMMS) protocol (Kilpatrick, K., et al., Hybridoma 16:381-389 (2009)). Briefly, two Wistar rats were immunized with 30, 15, 7, 3, 3, and 3 µg CD26 on days 0, 2, 5, 7, 9, and 13, respectively, into draining lymph node sites A, B, and C as shown in Figure 1. Mouse immunization followed the same schedule but with half the amount of antigen at each time point.
- [0047] Figures 2A-D:** Anti-DPP-4 mouse and rat antibodies are specific for human DPP-4 as shown using a direct binding ELISA. Direct binding ELISA against human DPP-4 (r-DPP-4-his) (**A**) or human gp130-his (**B**) using pre-immune (left side) or day 13 test-bleed (right side) sera from two rats (R9472 and R9473) immunized with human DPP-4. Day 13 rat sera specifically binds to human DPP4, while no specific binding is seen using pre-immune sera. Direct binding ELISA against human DPP-4 (r-DPP-4-his) (**C**) or human gp130-his (**D**) using pre-immune (left side) and 13 day test-bleed (right side) sera from five mice (ms9447, ms9448, ms9449, ms9450 and ms9451) immunized with human DPP-4-. Day 13 mouse sera specifically binds to human DPP-4, while no specific binding is seen using pre-immune sera.
- [0048] Figures 3A-B:** Detection of human DPP-4 using m3B7.6 antibody (**A**) or m5B7.7 (**B**) as the capture antibody in an immunoassay. **A.** DPP-4 was added to PBS or in 1% normal human serum (NHS) using m3B7.6 as the capture antibody and R11A2.15, R11A9.11, or control antibody R222113 (R&D Systems Catalog No. MAB1180) as the detection antibody, in a sandwich ELISA assay. m3B7.6 + R11A2.15 or m3B7.6 + R11A9.11 provided the best

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sensitivity in measuring human DPP-4 in a sandwich ELISA assay. **B.** DPP-4 was added to PBS or in 1% normal human serum (NHS) using m5B7.7 as the capture antibody and R11A2.15, R11A9.11, or control antibody R222113 (R&D Systems Catalog No. MAB1180) as the detection antibody, in a sandwich ELISA assay. m5B7.7 + R11A2.15 or m5B7.7 + R11A9.11 provided the best sensitivity in measuring human DPP-4 in a sandwich ELISA assay. These results, in combination with the results reported in Figure 2, demonstrate that antibodies m3B7.6, m5B7.7, R11A2.15, and R11A9.11 detect both endogenous and recombinant human DPP4. In addition, the commercially available antibody (R222113) failed to detect DPP4 in these immunoassays, compared to antibodies m3B7.6, m5B7.7, R11A2.15, and R11A9.11 which detected DPP-4.

[0049] Figures 4A-E: Mouse monoclonal antibodies m3B7.6 and m5B7.7 compete with each other, while the rat monoclonal antibodies R11A2.15 and R11A9.11 compete with each other. **A.** Schematic of the OCTET assay used to determine relative binding specificities and competition profiles of m3B7.6, m5B7.7, R11A2.15, and R11A9.11 as shown in **Figures 4B-E**. Briefly, the testing antibody was first biotinylated and then captured on a streptavidin biosensor at concentration of 20 µg/ml in 200 µl for 5min. The biosensor was then washed with 200 µL of PBS buffer for 1 min, and then incubated with recombinant human DPP-4 at 10 µg/mL for 5 min and was washed with 200 µL of PBS buffer for 1 min. The competitor antibodies were mixed with testing antibody in 1:1 ratio and at a final concentration of 20 µg/ml each and loaded onto the biosensor in 200 µl for 5min. Competitor antibodies displaying additional bindings to the testing antibody were deemed to have different epitopes. Otherwise they were considered to share the same epitope. **B.** OCTET assay results using m3B7.6 (a) as the testing antibody. m5B7.7 (b) showed the same binding profile as m3B7.6 (a) while R11A2.15 (c) and R11A9.11 (d) showed different binding profiles than m3B7.6 (a). On the basis of these results, antibodies m3B7.6 and m5B7.7 share the same or overlapping epitope. **C.** OCTET assay results using m5B7.7 (b) as the testing antibody. m3B7.6 (a) showed the same binding profile as m5B7.7 (b), while R11A2.15 (c) and R11A9.11 (d) showed different binding profiles than m5B7.7 (b). On the basis of these results, antibodies m5B7.7 and m3B7.6 share the same or overlapping epitope. **D.** OCTET assay results using R11A2.15 (c) as the testing antibody. R11A9.11 (d) showed the same binding profile as R11A2.15 (c), while

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m3B7.6 (a) and m5B7.7 (b) showed different binding profiles than R11A2.15 (c). On the basis of these results, antibodies R11A2.15 and R11A9.11 share the same or overlapping epitope E. OCTET assay results using R11A9.11 (d) as the testing antibody., R11A2.15(c) showed the same binding profile as R11A9.11 (d), while m3B7.6 (a) and m5B7.7 (b) showed different binding profiles than R11A9.11 (d). On the basis of these results, antibodies R11A9.11 and R11A2.15 share the same or overlapping epitope.

DETAILED DESCRIPTION

- [0050]** The present disclosure provides anti-DPP-4 monoclonal antibodies, assay kits comprising one or more of these antibodies, and robust, sensitive, and specific immunoassays using one or more of these antibodies for the detection and measurement of DPP-4 levels in samples obtained from human patients.
- [0051]** In this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein.
- [0052]** Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).
- [0053]** Wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.
- [0054]** The term "about" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is $\pm 10\%$.
- [0055]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show,

2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

- [0056]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.
- [0057]** An "isolated" substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, e.g., isolated biological material, is a substance that is not in its natural milieu. No particular level of purification is required. For example, an isolated antibody is an antibody that is not produced or situated in its native or natural environment. Recombinantly produced biological materials are considered isolated as disclosed herein, as are materials that are produced in a non-native cell, such as a hybridoma. A substance, e.g., biological material, is also considered "isolated" if it has been separated, fractionated, or partially or substantially purified by any suitable technique. In certain aspects, an isolated substance, e.g., isolated biological material, can be "non-naturally occurring."
- [0058]** As used herein, the term "non-naturally occurring" substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being "naturally-occurring," or that are, or might be at any time, determined or interpreted by a judge or an administrative agency such as the United States Patent and Trademark Office, or judicial body to be, "naturally-occurring." For example, the term "a non-naturally occurring antibody explicitly excludes those antibodies that exist in nature, e.g., an antibody that would naturally be present in the immune system of a mouse exposed to a normal milieu of antigenic stimulus, or an antibody

finally determined by an administrative body, *e.g.*, the United States Patent and Trademark Office, or a judicial body, *e.g.*, a federal court, to be “naturally-occurring.”

[0059] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and their analogs. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0060] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, transporter molecule provided herein. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into one or more mRNAs, and the translation of such mRNAs into one or more polypeptides. If the final product is a biochemical, expression includes the creation of that biochemical and any precursors.

[0061] An “expression product” can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide. Expression products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0062] The term “vector” or “expression vector” is used herein to mean vectors used as a vehicle for introducing into and expressing an expression product of interest in a host cell. As known to those skilled in the art, such vectors can easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors can comprise a selection marker, appropriate restriction sites to facilitate cloning of a particular nucleic acid and the ability to enter and/or replicate in eukaryotic or prokaryotic cells. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or

RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

- [0063]** The term “host cell” refers to a cell that harbors a vector constructed using recombinant DNA techniques and encoding at least one expression product. In descriptions of processes for the isolation of an expression product from recombinant hosts, the terms “cell” and “cell culture” are used interchangeably to denote the source of the expression product unless it is clearly specified otherwise, *i.e.*, recovery of the expression product from the “cells” means either recovery from spun down whole cells, or recovery from the cell culture containing both the medium and the suspended cells.
- [0064]** The terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and non-amino acids can interrupt it. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure are based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains.
- [0065]** The term “DPP-4” as used herein refers to the dipeptidyl peptidase IV protein (EC 3.4.14.5; Uniprot: P27487 (membrane bound form: SEQ ID NO: 5; soluble form SEQ ID NO: 6) encoded by the DPP-4 gene (cDNA: SEQ ID NO: 7). DPP-4 is also known as DPP-IV, adenosine deaminase complexing protein 2, or CD26 (cluster of differentiation 26). DPP-4 is related to attractin, FAP, DPP8 and DPP9. DPP-4 is a highly conserved multifunctional type II transmembrane glycoprotein, which is present both in circulation (plasma) and on the surface of several cell types, including epithelial, endothelial and lymphoid cells. DPP-4 is part of the serine protease family that is involved in T-cell costimulation, chemokine biology, type II diabetes, and tumor biology (Zhong *et al.*, *Atherosclerosis* 2013;226:305-314). The endogenous substrates of DPP-4 include a wide variety of proline-containing peptides such as

growth factors, chemokines, neuropeptides and vasoactive peptides (Gorrell, M., Clin. Sci. 108, 277-292, 2005; McIntosh, C. H. S., *et al.* Int. J. Biochem. Cell Biol. 38, 860-872, 2006). A role for DPP-4 in inflammatory respiratory diseases like asthma is suggested by Giovannini-Chami (Giovannini-Chami *et al.*, European Respiratory Journal. 2012 May;39(5):1197-205), who found elevated DPP-4 transcripts (and other Th2 signature genes) in the nasal epithelia of children with dust mite allergic rhinitis, associated with uncontrolled asthma. The term DPP-4 also includes fragments, variants (*e.g.*, the K1R, V7I, S437I, T557I, D663E variants known in the arts), and derivatives thereof (*e.g.*, glycosylated or aglycosylated protein forms of the DPP-4 protein, or otherwise chemically modified forms of the protein).

- [0066] The term "level" or "amount", *e.g.*, as in "DPP-4 level" or "amount of DPP-4" refers to a measurement that is made using an analytical method for detecting presence or expression of DPP-4 (protein expression) in a biological sample and that indicates the presence, absence, absolute amount or concentration, relative amount or concentration, titer, expression level, ratio of measured levels, or the like, of, for, or corresponding to DPP-4 in the biological sample. The exact nature of the "value" or "level" depends on the specific designs and components of the particular analytical method employed to detect DPP-4 (*e.g.*, immunoassays as provided herein,.). See, *e.g.*, U.S. 2010/00221752.
- [0067] As used herein with reference to DPP-4, the terms "elevated DPP-4," "high DPP-4," "elevated DPP-4 level," or "high DPP-4 level" refer to a level in a biological sample (*e.g.*, blood serum) that is higher than a normal level or range. The normal level or range for DPP-4 is defined in accordance with standard practice. Thus, the level measured in a particular biological sample can be compared with level or range of levels determined in similar normal samples. The level of DPP-4 is said to be elevated where the DPP-4 is present in the test sample at a higher level or range than in a normal sample.
- [0068] As used herein, the term "antibody" (or a fragment, variant, or derivative thereof) refers to at least the minimal portion of an antibody which is capable of binding to antigen, *e.g.*, at least the variable domain of a heavy chain (VH) and the variable domain of a light chain (VL) in the context of a typical antibody produced by a B cell. Basic antibody structures in vertebrate systems are relatively well understood. See, *e.g.*, Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise noted, an

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antibody "fragment," "variant," or "derivative" refers to an antigen-binding "fragment," "variant," or "derivative."

[0069] The terms "fragment," "variant," "derivative" and "analog" when referring to an antibody as disclosed herein can include any antibody that retains at least some of the activity, *e.g.*, antigen-binding activity, of the reference antibody, but which is structurally different. Fragments of antibodies include, for example, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments. Variants include fragments as described above, and also antibodies with altered amino acid sequences, *e.g.*, in the variable domains, due to amino acid substitutions, deletions, or insertions. Variants can occur spontaneously or be intentionally constructed. Intentionally constructed variants can be produced using art-known mutagenesis techniques. Variant antibodies can comprise conservative or non-conservative amino acid substitutions, deletions or additions. The variations are limited only by the constraint that the antibody maintain a function of the reference antibody, *e.g.*, binding to the same epitope as the reference antibody, or competitively inhibiting the reference antibody. Derivatives are antibodies that have been altered so as to exhibit additional features not found on the native antibody. Examples include fusion proteins comprising an antigen-binding domain of the antibody, or conjugated antibodies. A "derivative" antibody can also comprise one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those antibodies that contain one or more standard or synthetic amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0070] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like.

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- [0071] As indicated above, the variable region allows the binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or a subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three-dimensional antigen-binding site. This quaternary binding molecule structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VL chains.
- [0072] In antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (see, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference in their entireties).
- [0073] In the cases where there are two or more definitions of a term that is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human

Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein.

- [0074] Antibodies or antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules encompassed by this disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.
- [0075] By "specifically binds," it is generally meant that an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope via its antigen-binding domain more readily than it would bind to a random, unrelated epitope.
- [0076] An antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen-binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen-binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen-binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.
- [0077] Antibodies or antigen-binding fragments, variants, or derivatives thereof disclosed herein can be described or specified in terms of the epitope(s) or portion(s) of an antigen, *e.g.*, a target polysaccharide that they recognize or specifically bind. For example, the portion of human

DPP-4 that specifically interacts with the antigen-binding domain of an antibody provided in this disclosure is an "epitope."

- [0078] As used herein, the term "IL-13-mediated disease or disorder" refers to any pathology caused by (alone or in association with other mediators), exacerbated by, associated with, or prolonged by abnormal levels of IL-13 in the subject having the disorder. Non-limiting examples of IL-13-mediated diseases or disorders include asthma, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), ulcerative colitis (UC), atopic dermatitis (AD), allergic rhinitis, or chronic rhinosinusitis.
- [0079] As used herein, the term "pulmonary disease or disorder" refers to any pathology affecting at least in part the lungs or respiratory system. Non-limiting examples include asthma, atopic asthma, corticosteroid naive asthma, chronic asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, asthma due to smoking, asthma uncontrolled on corticosteroids, IPF, COPD, allergic rhinitis, or chronic rhinosinusitis. In certain aspects, the pulmonary disease or disorder is IL-13-mediated.
- [0080] As used herein, the term "chronic inflammatory skin disease or disorder" refers to any pathology affecting at least in part the skin. Non-limiting examples include atopic dermatitis, skin fibrosis, allergic contact dermatitis, eczema or psoriasis. In certain aspects, the chronic inflammatory skin disease or disorder is IL-13-mediated.
- [0081] The term "asthma" refers to diseases that present as reversible airflow obstruction and/or bronchial hyper-responsiveness in some instances is associated with underlying inflammation. Examples of asthma include allergic asthma, atopic asthma, corticosteroid naive asthma, chronic asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, asthma due to smoking, asthma uncontrolled on corticosteroids and other asthmas as mentioned, *e.g.*, in the Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma, National Asthma Education and Prevention Program (2007) ("NAEPP Guidelines"), incorporated herein by reference in its entirety.
- [0082] The term "COPD" as used herein refers to chronic obstructive pulmonary disease. The term "COPD" includes two main conditions: emphysema and chronic obstructive bronchitis.
- [0083] The term "Idiopathic Pulmonary Fibrosis" (IPF) refers to a disease characterized by progressive scarring, or fibrosis, of the lungs. It is a specific type of interstitial lung disease in

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which the alveoli gradually become replaced by fibrotic tissue. With IPF, progressive scarring causes the normally thin and pliable tissue to thicken and become stiff, making it more difficult for the lungs to expand, preventing oxygen from readily getting into the bloodstream. See, *e.g.*, Am. J. Respir. Crit. Care Med. 2000. 161:646-664.

[0084] The term "Ulcerative colitis" (UC) refers to an inflammatory disorder of the gastrointestinal (GI) tract that affects the colorectum which includes characteristic ulcers, or open sores. UC is an intermittent disease, with periods of exacerbated symptoms, and periods that are relatively symptom-free. Symptom of active disease include constant diarrhea mixed with blood that persists for an extended period (weeks), weight loss, chronic loss of blood from the GI tract, anemia, abdominal pain, and mild discomfort to painful bowel movements or painful abdominal cramping with bowel movements. See, *e.g.*, Danese, *et al.* N Engl J Med. 2011 365(18):1713-25.

[0085] As used herein, the term "atopic dermatitis" refers to a chronic inflammatory, relapsing, non-contagious and itchy skin disorder that is often associated with other atopic disorders such as allergic rhinitis and asthma (Bieber, New England Journal of Medicine, 2008, 358: 1483-1494). The term "atopic dermatitis" is equivalent to "neurodermatitis", "atopic eczema" or "endogenous eczema". Particular forms of atopic dermatitis, which get their names from the place where they occur or from their appearance or from the stress factors which provoke them, are, according to the present disclosure also comprised by the term "atopic dermatitis". These include, but are not limited to, eczema flexurarum, eczema mulluscatum, eczema verrucatum, eczema vaccinatum, eczema dyskoides, dyshydrotic eczema, microbial eczema, nummular eczema, seborrhobic eczema and other forms of eczema; perioral dermatitis and periorbital dermatitis. As used herein, the term atopic dermatitis also comprises the frequently occurring bacterial secondary infections such as those due to *e.g.* Staphylococcus aureus infections, pyoderms such as impetigo contagiosa and its derivatives as well as the follicularis barbae or viral secondary infections. IL-13 is involved in the pathogenesis of the disease and is an important in vivo inducer. See, *e.g.*, Oh et al., J. Immunol. 186:7232-42 (2011); Tazawa et al., Arch. Dermatol. Res. 295:459-464 (2004); Metwally et al. Egypt J. Immunol. 11:171-7 (2004).

- [0086] The terms "subject" or "patient" as used herein refer to any subject, particularly a mammalian subject, including any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, bears, chickens, amphibians, reptiles, *etc.*.
- [0087] In some aspects of the present disclosure, a subject is a naïve subject. A naïve subject is a subject that has not been administered a therapy, for example a therapeutic agent. In some aspects, a naïve subject has not been treated with a therapeutic agent prior to being diagnosed as having an IL-13-mediated disease or disorder, for example, asthma, IFP, COPD, AD, or UC. In another aspect, a subject has received therapy and/or one or more doses of a therapeutic agent (*e.g.*, a therapeutic agent capable of modulating an inflammatory response associated with an IL-13-mediated disease or disorder, a pulmonary disease or disorder, a chronic inflammatory skin disease or disorder or an inflammatory bowel disease or disorder) prior to being diagnosed as having an IL-13-mediated disease or disorder..
- [0088] As used herein, the term "IL-13 antagonist" refers to any agent, which can affect the expression, activity, or half-life of IL-13 either *in vitro* or *in vivo*, or symptoms, pathology, or sequelae caused by or exacerbated by IL-13 in a subject with an IL-13-mediated disease or disorder. An IL-13 antagonist can be any "therapeutic agent" as defined below, which either directly or indirectly can inhibit, lessen, or neutralize IL-13 activity, inhibit or reduce IL-13 expression, reduce IL-13 half-life, or can prevent exacerbation of symptoms due to IL-13. In certain aspects, an IL-13 antagonist is an anti-IL-13 monoclonal antibody, *e.g.*, tralokinumab (SEQ ID NOs 3 and 4), or other anti-IL-13 monoclonal antibodies described in U.S. Pat. Appl. Publ. No. 2012-0052060, published March 1, 2012, herein incorporated by reference in its entirety. In other aspects, the IL-13 antagonists include, without limitation: (a) an anti-human-IL-13 antibody, for example, Lebrikizumab (SEQ ID NOs 1 and 2) (MILR1444A / RG3637, Roche/Genentech), ABT-308 (Abbott), GSK679586 (GlaxoSmithKline) or QAX576 (Novartis); (b) an anti-human-IL-13R α antibody, for example, Merck MK6105; (c) an IL-13-toxin conjugate such as IL-13-PE38QQR (NeoPharm, Inc.); (d) an IL-4 mutein Aerovant™ (Aerovance, Inc.); (e) an anti-IL-4R α antibody such as dupilumab/REGN668 (Regeneron); (f) a double-stranded oligonucleotide directed against IL-4R α such as AIR645(Isis); or (g) an IL-4 / IL-13 bispecific antibody such as GSK2434735 (Glaxo SmithKline).

- [0089] The term "therapeutic agent" as used herein refers to any therapeutically active substance that is administered to a subject to produce a desired, usually beneficial, effect. The term therapeutic agent includes, *e.g.*, classical low molecular weight therapeutic agents commonly referred to as small molecule drugs and biologics including but not limited to: antibodies or active fragments thereof, peptides, lipids, protein drugs, protein conjugate drugs, enzymes, oligonucleotides, ribozymes, genetic material, prions, virus, bacteria, and eukaryotic cells. A therapeutic agent can also be a pro-drug, which metabolizes into the desired therapeutically active substance when administered to a subject. In some aspects, the therapeutic agent is a prophylactic agent. In addition, a therapeutic agent can be pharmaceutically formulated. A therapeutic agent can also be a radioactive isotope or agent activated by some other form of energy such as light or ultrasonic energy, or by other circulating molecules that can be systemically administered.
- [0090] The term "sample" as used herein includes any biological fluid or issue, such as whole blood, serum, muscle, saliva obtained from a subject. Samples include any biological fluid or tissue, such as whole blood, serum, muscle, saliva, urine, synovial fluid, bone marrow, cerebrospinal fluid, nasal secretions, sputum, amniotic fluid, bronchoalveolar lavage fluid, lung tissue, peripheral blood mononuclear cells, total white blood cells, lymph node cells, spleen cells, tonsil cells, or skin. In some specific aspects, that sample is blood or a fraction thereof, muscle, skin, or a combination thereof. Samples can be obtained by any means known in the art.
- [0091] In order to apply the methods and systems of the disclosure, samples from a patient can be obtained at any time. In some cases, successive samples can be obtained from the patient after therapy has commenced or after therapy has ceased. Samples can, for example, be requested by a healthcare provider (*e.g.*, a doctor) or healthcare benefits provider, obtained and/or processed by the same or a different healthcare provider (*e.g.*, a nurse, a hospital) or a clinical laboratory, and after processing, the results can be forwarded to yet another healthcare provider, healthcare benefits provider or the patient. Similarly, the measuring/determination of one or more scores, comparisons between scores, evaluation of the scores and treatment decisions can be performed by one or more healthcare providers, healthcare benefits providers, and/or clinical laboratories.

- [0092]** As used herein, the term "healthcare provider" refers to individuals or institutions that directly interact and administer to living subjects, *e.g.*, human patients. Non-limiting examples of healthcare providers include doctors, nurses, technicians, therapist, pharmacists, counselors, alternative medicine practitioners, medical facilities, doctor's offices, hospitals, emergency rooms, clinics, urgent care centers, alternative medicine clinics/facilities, and any other entity providing general and/or specialized treatment, assessment, maintenance, therapy, medication, and/or advice relating to all, or any portion of, a patient's state of health, including but not limited to general medical, specialized medical, surgical, and/or any other type of treatment, assessment, maintenance, therapy, medication and/or advice.
- [0093]** As used herein, the term "clinical laboratory" refers to a facility for the examination or processing of materials derived from a living subject, *e.g.*, a human being. Non-limiting examples of processing include biological, biochemical, serological, chemical, immunohematological, hematological, biophysical, cytological, pathological, genetic, or other examination of materials derived from the human body for the purpose of providing information, *e.g.*, for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of living subjects, *e.g.*, human beings. These examinations can also include procedures to collect or otherwise obtain a sample, prepare, determine, measure, or otherwise describe the presence or absence of various substances in the body of a living subject, *e.g.*, a human being, or a sample obtained from the body of a living subject, *e.g.*, a human being.
- [0094]** As used herein, the term "healthcare benefits provider" encompasses individual parties, organizations, or groups providing, presenting, offering, paying for in whole or in part, or being otherwise associated with giving a patient access to one or more healthcare benefits, benefit plans, health insurance, and/or healthcare expense account programs.
- [0095]** In some aspects, a healthcare provider can administer or instruct another healthcare provider to administer or use any of the immunoassays or kits disclosed herein to measure DPP-4. A healthcare provider can implement or instruct another healthcare provider or patient to perform the following actions: obtain a sample, process a sample, submit a sample, receive a sample, transfer a sample, analyze or measure a sample, quantify a sample, provide the results obtained after analyzing/measuring/quantifying a sample, receive the results obtained after

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analyzing/measuring/quantifying a sample, compare/score the results obtained after analyzing/measuring/quantifying one or more samples, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples, administer a therapy (*e.g.*, a therapeutic agent that treats an IL-13-mediated disease or disorder such as asthma, IPF, COPD, AD, or UC), commence the administration of a therapy, cease the administration of a therapy, continue the administration of a therapy, temporarily interrupt the administration of a therapy, increase the amount of an administered therapeutic agent, decrease the amount of an administered therapeutic agent, continue the administration of an amount of a therapeutic agent, increase the frequency of administration of a therapeutic agent, decrease the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

[0096] In some aspects, a healthcare benefits provider can authorize or deny, for example, collection of a sample, processing of a sample, submission of a sample, receipt of a sample, transfer of a sample, analysis or measurement a sample, quantification a sample, provision of results obtained after analyzing/measuring/quantifying a sample, transfer of results obtained after analyzing/measuring/quantifying a sample, comparison/scoring of results obtained after analyzing/measuring/quantifying one or more samples, transfer of the comparison/score from one or more samples, administration of a therapy or therapeutic agent, commencement of the administration of a therapy or therapeutic agent, cessation of the administration of a therapy or therapeutic agent, continuation of the administration of a therapy or therapeutic agent, temporary interruption of the administration of a therapy or therapeutic agent, increase of the amount of administered therapeutic agent, decrease of the amount of administered therapeutic agent, continuation of the administration of an amount of a therapeutic agent, increase in the frequency of administration of a therapeutic agent, decrease in the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, or combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

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- [0097] In addition a healthcare benefits provides can, *e.g.*, authorize or deny the prescription of a therapy, authorize or deny coverage for therapy, authorize or deny reimbursement for the cost of therapy, determine or deny eligibility for therapy, etc.
- [0098] In some aspects, a clinical laboratory can, for example, collect or obtain a sample, process a sample, submit a sample, receive a sample, transfer a sample, analyze or measure a sample, quantify a sample, provide the results obtained after analyzing/measuring/quantifying a sample, receive the results obtained after analyzing/measuring/quantifying a sample, compare/score the results obtained after analyzing/measuring/quantifying one or more samples, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples, or other related activities.

DPP-4 as a Biomarker

- [0099] Elevated DPP-4 levels have been observed in asthma, COPD and AD patients (*see, e.g.*, U.S. Provisional Application No. 61/931,878, filed January 27, 2014; and U.S. Provisional Application No. 61/990,932, filed May 9, 2014, each incorporated herein by reference in its entirety). In addition, in a phase 2B clinical study of asthma patients, high serum DPP-4 levels predicted improved response rates in patients treated with an IL-13 antibody antagonist (tralokinumab) identifying DPP-4 as a predictive biomarker for IL-13-mediated disease or disorders including an IL-13-mediated pulmonary disease or disorder (*e.g.*, asthma, IPF or COPD) or an IL-13-mediated chronic inflammatory skin disease or disorder (*e.g.*, atopic dermatitis). *See* Brightling *et al.*, Am J Respir Crit Care Med 189:A6670 (2014); and U.S. Provisional Application No. 61/931,878, filed January 27, 2014; and U.S. Provisional Application No. 61/990,932, filed May 9, 2014, each incorporated herein by reference in its entirety.
- [00100] Accordingly, an elevated DPP-4 level in patients with asthma, IPF, COPD, AD and UC, as well as other inflammatory diseases, pulmonary diseases or disorders, or chronic inflammatory skin diseases or disorders can be used to identify those patients who can benefit from particular therapies, including, but not limited to, therapies that neutralize IL-13 activity. Anti-DPP-4 antibodies and immunoassays and kits using the anti-DPP4 antibodies disclosed herein useful to measure DPP-4 levels in patients are provided.

Anti-DPP-4 Antibodies

- [00101]** This disclosure provides isolated anti-DPP-4 antibodies and antigen-binding fragments thereof. In certain aspects, the anti-DPP-4 antibodies and antigen-binding fragments provided herein can bind to human DPP-4.
- [00102]** The disclosure provides, in particular, two mouse monoclonal antibodies and two rat monoclonal antibodies that bind to human DPP-4. These antibodies were produced by standard hybridoma technology, and the hybridomas producing these antibodies have been deposited under the Budapest Treaty at the American Type Culture Collection, Manassas, VA on January 8, 2015. The mouse anti-DPP-4 antibodies are referred to herein as m3B7.6 and m5B7.7, and the rat anti-DPP-4 antibodies are referred to herein as R11A2.15, and R11A9.11. Also provided are antigen-binding fragments, variants, and/or derivatives of these antibodies. Also provided are antibodies that are related to these antibodies in that they bind to the same epitope, or they are capable of competitively inhibiting one or more of m3B7.6, m5B7.7, R11A2.15, and R11A9.11. Mouse monoclonal antibody m3B7.6 is produced from a hybridoma deposited at the American Type Culture Collection, Manassas, VA (the ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 is produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 is produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and rat monoclonal antibody R11A9.11 is produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.
- [00103]** In certain aspects, an isolated antibody or antigen-binding fragment, variant, or derivative thereof is provided, where the antibody binds to the same DPP-4 epitope as mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.
- [00104]** In certain aspects, an isolated antibody or antigen-binding fragment, variant, or derivative thereof is provided, where the antibody competitively inhibits binding of mouse monoclonal

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antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 to DPP-4, *e.g.*, human DPP-4. For example, monoclonal antibodies m3B7.6 and m5B7.7 are capable of competitively inhibiting each other for binding to human DPP-4, and monoclonal antibodies R11A9.11 and R11A2.15 are capable of competitively inhibiting each other for binding to human DPP-4.

[00105] In certain aspects, an isolated anti-DPP-4 antibody or fragment, variant, or derivative thereof is provided, where the antibody comprises a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, where the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[00106] In certain aspects, an isolated anti-DPP-4 antibody or fragment, variant, or derivative thereof is provided, where the antibody comprises a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

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- [00107] A person of ordinary skill in the art, upon obtaining one or more of the antibodies from one or more of the deposited hybridomas can isolate, clone, and sequence the expressed antibodies to determine the VH, VL, and CDR regions, without undue experimentation.
- [00108] In certain aspects, a hybridoma is provided, where the hybridoma comprises the hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. 121870, the hybridoma deposited at the ATCC under Deposit No. 121871, the hybridoma deposited at the ATCC under Deposit No. 121872, the hybridoma deposited at the ATCC under Deposit No. 121873, or a combination thereof.
- [00109] In certain aspects, an antibody-producing cell culture is provided, where the cell culture can be used to express an anti-DPP-4 antibody or fragment, variant, or derivative thereof as provided herein. In certain aspects, the cell culture comprises a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. 121870, a hybridoma deposited at the ATCC under Deposit No. 121871, a hybridoma deposited at the ATCC under Deposit No. 121872, a hybridoma deposited at the ATCC under Deposit No. 121873, or a combination thereof.
- [00110] Any anti-DPP-4 antibody or fragments, variants or derivatives thereof provided by this disclosure can further include additional polypeptides, *e.g.*, a signal peptide to direct secretion. Additionally, anti-DPP-4 antibody or fragments, variants or derivatives thereof provided by this disclosure can be, for example, fusion polypeptides, Fab fragments, scFvs, or other derivatives, as described herein.
- [00111] In certain aspects, an anti-DPP-4 antibody, or fragment, variant, or derivative thereof provided by this disclosure can be part of a fusion protein, that is, the antibody or antigen-binding fragment thereof can be fused to a heterologous polypeptide. The term "heterologous polypeptide" as used herein means that the polypeptide is derived from a distinct entity from the anti-DPP-4 antibody, or fragment, variant, or derivative thereof. In a non-limiting example, a "heterologous polypeptide" to be fused to an antibody or an antigen-binding fragment, variant, or derivative thereof can be derived from a non-immunoglobulin polypeptide of the same species, or an immunoglobulin or non-immunoglobulin heterologous polypeptide. In some aspects, the heterologous polypeptide can be, for example, a stabilizing polypeptide, a tag, a label, or a combination thereof.

[00112] In certain aspects, an anti-DPP-4 antibody or fragment, variant or derivative thereof provided by this disclosure can comprise a heterologous amino acid sequence or one or more other moieties not normally associated with an antibody (*e.g.*, a peptide, a protein, an enzyme, a lipid, a heterologous antibody, or fragment, variant, or derivative thereof, a detectable label, polyethylene glycol (PEG), or a combination of two or more of any said agents). In further aspects, an anti-DPP-4 antibody or fragment, variant or derivative thereof provided by this disclosure can comprise a detectable label selected from the group consisting of an enzyme, a fluorescent label, a chemiluminescent label, a bioluminescent label, a radioactive label, or a combination of two or more of any said detectable labels. In certain aspects, the detectable label is biotin, which can interact with streptavidin conjugated, *e.g.*, to an enzyme, *e.g.*, horseradish peroxidase (HRP). In certain aspects, the detectable label is a ruthenium chelate, which can emit light upon exposure to electrical current. Other detectable labels are well-known to those of ordinary skill in the art.

[00113] Also provided herein is a composition comprising one or more of the anti-DPP-4 antibodies or fragments thereof as noted above. In certain aspects, a composition includes a "capture" antibody and a "detection" antibody, as described elsewhere herein. Compositions as provided herein can include without limitation buffers, carriers, and preservatives. Preservatives, stabilizers, buffers, antioxidants and/or other additives can include buffers such as phosphate, citrate, and other organic acids; antioxidants, such as 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 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 dextrans; 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 TWEEN™, PLURONIC™, or polyethylene glycol (PEG). Compositions as provided

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herein can be mixed in a single vial or receptacle, or can be provided in two or more vials or receptacles, or as part of a kit, as described elsewhere herein.

Polynucleotides

- [00114]** This disclosure provides polynucleotides encoding any anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein. In certain aspects an isolated polynucleotide or a polynucleotide composition comprising two or more polynucleotides is provided, which singly or collectively encodes an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein.
- [00115]** In certain aspects, this disclosure provides an isolated polynucleotide comprising a nucleic acid that encodes an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof, where the antibody comprises a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, where the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.
- [00116]** The disclosure also provides an isolated polynucleotide comprising a nucleic acid that encodes an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof, where the antibody comprises a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma

deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

- [00117]** The disclosure further provides a composition comprising two or more polynucleotides that singly or collectively encodes an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein.
- [00118]** In certain aspects, a vector, or two or more vectors are provided, to facilitate display, screening, isolation, cloning, and/or expression of an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein. In certain aspects the vector or vectors is/are expression vectors.
- [00119]** In certain aspects, two or more nucleic acid molecules of a polynucleotide composition can be situated in the same vector. In certain aspects, the two or more nucleic acid molecules of the polynucleotide composition can be situated in at least two separate vectors.
- [00120]** Expression vectors are used express isolated polynucleotide(s) encoding an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of a transporter molecule, operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated.
- [00121]** In certain aspects an isolated polynucleotide or composition comprising two or more isolated polynucleotides is provided, comprising a nucleic acid molecule that is operably associated with a promoter, or the two or more nucleic acid molecules that are operably associated with two or more promoters, where the promoters can be the same or different.

- [00122]** Nucleic acid regions are “operably associated” when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably associated with DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably associated with a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably associated with a coding sequence if it is positioned so as to permit translation.
- [00123]** The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.
- [00124]** In certain aspects an isolated host cell is provided that comprises a polynucleotide as provided herein. In certain aspects one or more isolated host cells are provided that comprise the two or more polynucleotides of the polynucleotide composition provided herein.
- [00125]** Suitable host cells for expression of transporter molecules provided herein include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed. Additional information regarding methods of protein production, including antibody production, can be found, *e.g.*, in U.S. Patent Publication No. 2008/0187954, U.S. Patent Nos. 6,413,746 and 6,660,501, and International Patent Publication No. WO 04009823, each of which is hereby incorporated by reference herein in its entirety.
- [00126]** Various mammalian or insect cell culture systems can also be employed to express an anti-DPP-4 antibody or a subunit (*e.g.*, the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein. Examples of suitable mammalian host cell lines include HEK-293 and HEK-293T, the COS-7 lines of monkey kidney cells, described by Gluzman (Cell 23:175, 1981), and other cell lines including, for example, L cells, C127, 3T3, Chinese

hamster ovary (CHO), NS0, HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer operably associated with the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *BioTechnology* 6:47 (1988).

[00127] Host cells provided herein can be utilized in a method of making an anti-DPP-4 antibody or a subunit (e.g., the heavy chain or the light chain), fragment, variant, or derivative thereof provided herein, where the method includes (a) culturing the host cell and (b) isolating the antibody, fragment, or subunit expressed from the host cell.

Assays for Detecting DPP-4 Levels

[00128] This disclosure provides a method of measuring DPP-4 levels in a sample obtained from a subject comprising assaying the sample using an immunoassay employing at least one, *e.g.*, at least two anti-DPP-4 antibodies or antigen-binding fragments thereof that recognize distinct epitopes on human DPP-4. Exemplary antibodies for use in this method include one or more of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873, and/or antigen-binding fragments, variants, or derivatives thereof, as described herein.

[00129] The method involves the use of a specific and sensitive immunoassay for the detection of DPP-4 in samples obtained from a subject. The samples are assayed in an immunoassay employing at least one, *e.g.*, at least two anti-DPP-4 antibodies provided herein, *e.g.*, mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15

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produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873, and/or antigen-binding fragments, variants, or derivatives thereof, as described herein.

[00130] For example, the disclosure provides a method of measuring DPP-4 levels in a sample obtained from a subject, comprising assaying the sample in an immunoassay employing at least one, *e.g.*, at least two anti-DPP-4 antibodies or antigen-binding fragments, variants, or derivatives thereof that recognize distinct epitopes on human DPP-4. In one aspect, one of the at least two anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that binds to the same DPP-4 epitope as mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870 or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, and one of the at least two anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that binds to the same DPP-4 epitope as rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[00131] In another aspect, one of the at least two anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, and/or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 to DPP-4; and one of the at least two anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 to DPP-4.

[00132] In another aspect, one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a heavy chain variable

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domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, wherein the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, and/or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, wherein the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[00133] In another aspect, one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, and/or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a VH and a VL identical to the VH and VL of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

[00134] Any of these antibodies or fragments thereof can be fused to one or more heterologous polypeptides, *e.g.*, a stabilizing polypeptide, a tag, a label, or a combination thereof, or can be conjugated to a heterologous moiety, *e.g.*, a peptide, a protein, an enzyme, a lipid, a heterologous antibody or fragment thereof, a detectable label, polyethylene glycol (PEG), or a combination of two or more of any the agents. In certain aspects, the antibody comprises a

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detectable label such as biotin or a ruthenium chelate. Other detectable labels are well known to those of ordinary skill in the art and are included in this disclosure.

[00135] In certain aspects, the immunoassay comprises a sandwich immunoassay, *e.g.*, an enzyme-linked immunosorbent assay (ELISA) or a sandwich electrochemiluminescent (ECL) assay, in which a first anti-DPP-4 "capture" antibody or antigen-binding fragment thereof is attached to a solid support, antigen from a sample or standard is allowed to bind to the capture antibody, and then a second anti-DPP-4 "detection" antibody or antigen-binding fragment thereof is added, and detected either by an enzymatic reaction, an electrochemiluminescent reaction, radioactivity, or other detection method.

[00136] In certain aspects, the immunoassay comprises the following: first, the capture antibody, or fragment, variant, or derivative thereof is allowed to bind to a solid support, *e.g.*, a multi-well plate or other assay device known to those of ordinary skill in the art. The capture antibody is allowed to attach for a period of time, *e.g.*, overnight, and then unbound antibody is removed. The plate can then be washed to remove any unbound capture antibody. The plate can then be treated with a blocking solution to allow non-specific protein to bind to any unbound regions of the solid support. Typical blocking solutions include an unrelated protein, *e.g.*, nonfat dry milk or serum albumin. The plate can then again be washed to remove any unbound blocking solution. Next, a sample suspected of containing DPP-4 is added to the plate. Samples are typically serially diluted and plated in duplicate or triplicate. Controls, including standard amounts of DPP-4 or a suitable fragment thereof and various negative controls are also included. The antigen is allowed to bind to the capture antibody for a period of time, *e.g.*, one hour at room temperature. Following incubation, the plate can then be washed to remove any unbound antigen.

[00137] Next, a detection antibody is added. The detection antibody is typically an anti-DPP-4 antibody that binds to a different DPP-4 epitope than the capture antibody. The detection antibody can be labeled or unlabeled. Where the detection antibody is unlabeled, a labeled secondary antibody can be used for detection, as is well known by those of ordinary skill in the art. The detection antibody can be directly labeled with an enzyme, *e.g.*, horseradish peroxidase or alkaline phosphatase, or can be labeled with a tag that will allow an enzyme to bind. For example the detection antibody can be conjugated to biotin, and the enzyme attached

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in a subsequent step by allowing enzyme-conjugated streptavidin to bind to the biotin tag. Alternatively the detection antibody can be conjugated to a chemiluminescent, fluorescent, or electrochemiluminescent tag. An example of the latter is a ruthenium chelate. Following incubation, the plate can then be washed to remove any unbound detection antibody.

[00138] Detection of the detection antibody is accomplished by methods that will vary based on the type of detection antibody that is used. If the detection antibody is tagged with biotin, then enzyme-conjugated streptavidin is added, unbound streptavidin is washed away, and a substrate is added which provides a colorimetric reaction that can be read, *e.g.*, on a spectrophotometer. If the detection antibody is conjugated to a ruthenium chelate, the plate is subjected to electrical current, and light emission is measured.

[00139] In certain aspects, the method directly measures DPP-4 levels in a patient sample, where absolute levels are calculated by plotting the immunoassay results on a standard curve using, *e.g.*, purified full length DPP-4 or a DPP-4 fragment. The detected signal from the detection antibody can then be quantitated based on the various standards and controls included on the plate. By plotting the results on a standard curve, the absolute levels or amount of DPP-4 in the test samples can be calculated, *e.g.*, in ng/mL or pg DPP-4/mL protein.

[00140] In certain aspects of the immunoassay and method provided herein the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof, or mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, or rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00141] In certain aspects of the immunoassay and method provided herein the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, or rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC

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under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof, or mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00142] In certain aspects of the immunoassay and method provided herein the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00143] In certain aspects of the immunoassay and method provided herein the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00144] In certain aspects of the immunoassay and method provided herein the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC

under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00145] In certain aspects of the immunoassay and method provided herein the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the immunoassay and method provided herein the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00146] A variety of subject samples can be used in the methods presented herein. Exemplary, non-limiting examples of samples include one or more of whole blood, serum, plasma, saliva, sputum, nasal polyps, nasal mucus, bronchoalveolar lavage fluid, skin cells or lung tissue, *e.g.*, lung epithelial cells. In specific aspects, the sample is a serum sample, skin cells or lung tissue.

[00147] In particular aspects, the methods disclosed herein include informing the subject of a result of the DPP-4 assay and/or of a diagnosis based at least in part on the DPP-4 level. The patient can be informed verbally, in writing, and/or electronically. This diagnosis can also be recorded in a patient medical record.

[00148] The term "medical record" or "patient medical record" refers to an account of a patient's examination and/or treatment that typically includes one or more of the following: the patient's

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medical history and complaints, the physician's physical findings, the results of diagnostic tests and procedures, and patient medications and therapeutic procedures. A medical record is typically made by one or more physicians and/or physicians' assistants and it is a written, transcribed or otherwise recorded record and/or history of various illnesses or injuries requiring medical care, and/or inoculations, and/or allergies, and/or treatments, and/or prognosis, and/or frequently health information about parents, siblings, and/or occupation. The record can, in some instances, be reviewed by a physician in diagnosing the condition.

[00149] The medical record can be in paper form and/or can be maintained in a computer readable medium. The medical record can be maintained by a laboratory, physician's office, a hospital, a healthcare maintenance organization, an insurance company, and/or a personal medical record website. In some aspects, a diagnosis, based at least in part on the DPP-4 level, is recorded on or in a medical alert article such as a card, a worn article, and/or a radiofrequency identification (RFID) tag. As used herein, the term "worn article" refers to any article that can be worn on a subject's body, including, but not limited to, a tag, bracelet, necklace, arm band, or head band.

[00150] In certain aspects, the methods can entail ordering and/or performing one or more additional assays. For example, if the DPP-4 level is determined to be within a normal range (*i.e.*, not elevated), the DPP-4 assay can be repeated to rule out a false negative result, and/or one or more additional DPP-4 assays can be performed to monitor the subject's status. If the DPP-4 level is determined to be elevated, it can be desirable repeat the DPP-4 assay to rule out a false positive result.

DPP-4 Detection Methods, Assays, and Kits

[00151] This disclosure provides methods, assays, and kits to facilitate a determination or analysis of the DPP-4 level or amount of DPP-4 in the sample. In some aspects, the methods, assays, and kits disclosed herein are performed or used by a healthcare provider, a healthcare benefits provider, or a clinical laboratory to determine the DPP-4 level or amount of DPP-4 in the sample from the subject.

[00152] In certain aspects, the immunoassay is performed on a sample obtained from the patient, by the healthcare professional treating the patient, *e.g.*, using an immunoassay as described herein,

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formulated as a "point of care" diagnostic kit. In some aspects, a sample is obtained from the patient and is submitted, *e.g.*, to a clinical laboratory, for measurement of the DPP-4 level in the sample according to the healthcare professional's instructions, *e.g.*, using an immunoassay as described herein.

[00153] In certain aspects, the patient having, or suspected of having, an IL-13-mediated disease or disorder has been diagnosed with a pulmonary disease or disorder, an inflammatory bowel disease or disorder, or a chronic inflammatory skin condition. In certain aspects, the disease or disorder having or suspected of having IL-13-mediated pathology is asthma, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), ulcerative colitis (UC), atopic dermatitis, allergic rhinitis, or chronic rhinosinusitis.

[00154] This disclosure also provides kits for use in the practice of the immunoassays as disclosed herein. Such kits can comprise containers, each with one or more of the various reagents (*e.g.*, in concentrated form) utilized in the methods, including, for example, one or more anti-DPP-4 antibodies. One or more anti-DPP-4 antibodies, *e.g.*, capture antibodies can be provided already attached to a solid support, and one or more antibodies, *e.g.*, detection antibodies, can be provided already conjugated to a detectable label, *e.g.*, biotin or a ruthenium chelate. The kit can also provide reagents for coupling a detectable label to an antibody (as well as the label itself), buffers, and/or reagents and instrumentation to support the practice of the assays provided herein. In certain aspects, a labeled secondary antibody is provided that binds to the detection antibody. A kit provided according to this disclosure can further comprise suitable containers, plates and any other reagents or materials necessary to practice the assays provided herein.

[00155] A kit for measuring DPP-4 levels in a sample can comprise one or more of the anti-DPP-4 antibodies or fragments thereof provided herein, *e.g.*, mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under

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Deposit No. PTA-121873, and/or antigen-binding fragments, variants or derivatives thereof, or related antibodies or antigen-binding fragments thereof, also as described herein.

[00156] In certain aspects, a kit as provided herein comprises two isolated antibodies or antigen-binding fragments thereof, a capture antibody and a detection antibody. In certain aspects the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof, or mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, or rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00157] In certain aspects the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, or rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof, or mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00158] In certain aspects of the kits provided herein, the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the kits provided herein, the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof, and

the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00159] In certain aspects of the kits provided herein, the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the kits provided herein, the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof.

[00160] In certain aspects of the kits provided herein, the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof. In certain aspects of the kits provided herein, the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00161] In certain aspects of the kits provided herein, the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or

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derivative thereof. In certain aspects of the kits provided herein, the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof, and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

[00162] In certain aspects, the detection antibody is detectably labeled. In certain aspects, the detectable label is biotin and the detection reagents comprise a streptavidin-horse radish peroxidase (HRP) conjugate and a colorimetric substrate for HRP. In certain aspects the detectable label is a ruthenium chelate. Other antibodies, labels, and reagents as described elsewhere herein can also be used in kit as provided herein.

[00163] In certain aspects, this disclosure provides an immunoassay for detecting DPP-4 levels in one or more samples, comprising the use of at least one, *e.g.*, at least two anti-DPP-4 antibodies or antigen-binding fragments, variants, or derivatives thereof thereof, *e.g.*, mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, and/or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 as described herein, or antigen-binding fragments, variants or derivatives thereof, or related antibodies or antigen-binding fragments thereof, also as described herein.

[00164] In certain aspects, the immunoassay provided herein is a sandwich immunoassay, *e.g.*, an ELISA assay or an ECL assay, comprising a first anti-DPP-4 "capture" antibody or antigen-binding fragment thereof attached to a solid support, and a second anti-DPP-4 "detection" antibody or antigen-binding fragment thereof. The immunoassay is performed by methods provided herein or methods well known and understood by those of ordinary skill in the art. In one aspect the immunoassay comprises attaching a capture antibody, or fragment, variant, or derivative thereof to a solid support; applying the test sample or a control sample, allowing DPP-4, if present in the sample, to bind to the capture antibody, or fragment, variant, or

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derivative thereof; applying the detection antibody, or fragment, variant, or derivative thereof, which can bind to DPP-4 already bound to the capture antibody, or fragment, variant, or derivative thereof; and measuring the amount of detection antibody, or fragment, variant, or derivative thereof bound to DPP-4. In certain aspects, the assay can further include washing steps, blocking steps and incubation steps.

[00165] In certain aspects, the detection antibody, or fragment, variant, or derivative thereof further comprises a detectable label, *e.g.*, biotin or ruthenium chelate.

[00166] Aspects of the present disclosure can be further defined by reference to the following non-limiting examples, which describe in detail preparation of certain antibodies of the present disclosure and methods for using antibodies of the present disclosure. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

EXAMPLES

Example 1: Generation and Characterization of Rat and Mouse Monoclonal Antibodies Specific for Human DPP-4

[00167] Mouse and rat monoclonal antibodies specific for human DPP-4 were produced by the following method. Animals were immunized using the Repetitive Immunization at Multiple Sites (RIMMS) protocol (Kilpatrick, K., *et al.*, Hybridoma 16:381-389 (2009)). Briefly, five Balb/c mice and two Wistar rats, all at ages 4-6 weeks, were injected at 6 dorsal sites subcutaneously with r-human DPP4-His (R&D Systems Catalog No. 1180) mixed in Complete Freund's adjuvant (Sigma) (for 1st round followed by incomplete Freund's adjuvant for subsequent immunizations) and 6 ventral sites subcutaneously with antigen mixed in TiterMax (Sigma) adjuvant on each dosing date. In total, each animal received injections at twelve sites as shown in **Figure 1**. Test bleeds were collected on day 13, and both pre-bleed and immunized sera were tested by direct binding ELISA using DPP-4 (r-DPP-4-His, R&D Systems catalog number 1180) and negative control glycoprotein 130 (r-gp130-His) bound to the ELISA plates. Wells of high binding ELISA plates were coated with 50 μ L of a 2 μ g/mL solution of r-human DPP-4-His or gp130-His in PBS overnight at 4°C. The plates were then washed three times with 200 μ L/well wash buffer (PBS/0.1% TWEEN-20®). Following washing, 150 μ L/well

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blocking buffer (PBS/3% nonfat dry milk/0.1% TWEEN-20®) was added to each well, and the plates were incubated for one hour at room temperature. The plates were then washed three times as noted above.

[00168] Pre- and test- sera were serially diluted in PBS, pH7.2 buffer, from an initial 1:100 dilution to a final dilution of 1:218,600. Fifty μ L of each diluted sample was added to the plates and incubated for 1 hour at room temperature. The plates were washed three times as noted above. Following washing, 50 μ l of the detection antibody was added to each well. For the mouse group, Donkey anti-mouse (H+L):HRP (Jackson ImmunoResearch, Catalog No. 715-035-151) diluted 1:8000 in PBS was used. For the rat group, Donkey anti-rat(H+L):HRP (Jackson ImmunoResearch, Catalog No. 715-035-153), 1:8000 in PBS was used. The plates were incubated for 1 hour at room temperature. The plates were then washed three times and 50 μ L/well TMB substrate (KPL, Catalog No. 52-00-04) pre-warmed to room temperature was added. The plates were incubated at room temperature in the dark for 10 minutes, and 50 μ L of stop solution (1M HCl) was added to each well. The plates were read on a spectrophotometer at $\lambda = 450\text{nm}$.

[00169] Pre-bleed and immunized serum antibody titers for DPP-4 are shown in **Figures 2**. On day 16 and 19, lymph nodes were collected from the rats and mice showing robust serum titer, and hybridomas were generated as follows. On day 19 lymph nodes were collected from the animals, lymphocyte cells were extracted from the lymphoid tissues and filtered through 70 μ m cell strainers. Antigen specific B cells were isolated by using MACS, streptavidin microbeads (Miltenyi Biotec Catalog # 130-048-101) using the manufacturer suggested protocol. The DPP4 specific B cells were then fused with myeloma P3x/63Ag8.653 cells at 1:1 ratio following the PEG (Roche) fusion method. Fused cells were seeded at a density of 2.5×10^4 B cell/well in hybridoma growth media (Ex-Cell 610 + 10% Hi-FBS + 1% penicillin-streptomycin + 1X BM-condimed H1 hybridoma cloning supplement) supplemented with 1xHAT. Seven days after fusion, HAT containing medium was replaced with growth medium supplemented with 1xHT.

[00170] Hybridoma supernatants were screened for antibodies binding to human DPP-4, and four hybridoma-produced antibodies were selected for further investigation: mouse monoclonal antibody m3B7.6 (IgG1/ κ), mouse monoclonal antibody m5B7.7 (IgG1/ κ), rat monoclonal

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antibody R11A2.15 (IgG2a/ κ), and rat monoclonal antibody R11A9.11 (IgG2a/ κ). Hybridoma cell lines expressing these four monoclonal antibodies were deposited under the Budapest Treaty at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870 (m3B7.6), Deposit No. PTA-121871 (m5B7.7), Deposit No. PTA-121872 (R11A2.15), and Deposit No. PTA-121873 (R11A9.11) on January 8, 2015.

Example 2: Sandwich ELISA assays to detect DPP-4

Materials and Methods

A. DPP-4 sandwich ELISA protocol with HRP detection system.

[00171] HRP-based sandwich ELISA assays were performed as follows. Specific details such as anti-DPP-4 capture antibodies, samples to be tested, and anti-DPP-4 detection antibodies are noted for specific experiments in the various examples. Variations to standard ELISA protocols are well known to those of ordinary skill in the art, and can be used according to this disclosure.

[00172] High binding ELISA plates were coated with an anti-DPP-4 capture mAb, *e.g.*, m3B7.6 or m5B7.7 (5 μ g/mL in PBS; 50 μ L/well), and were incubated overnight at 4°C. The plates were washed three times with 200 μ L/well wash buffer (PBS/0.1% TWEEN-20®). Following washing, 150 μ L/well block buffer (PBS/3% nonfat dry milk/0.1% TWEEN-20®) was added to each well, and the plates were incubated for one hour at room temperature. The plates were then washed three times as noted above.

[00173] For the standard curve, DPP-4 standards (*e.g.*, standards available from R & D Systems Catalog # 1180-SE) were serially diluted in PBS, pH7.2 buffer or 1% normal human serum, *e.g.*, 2-fold dilutions from 500 ng/mL to 0.49 ng/mL. Fifty microliters (50 μ L) of each standard or diluted sample was added to the plates, and the plates were incubated for 1 hour at room temperature. Again, the plates were washed three times as noted above. Following washing, 50 μ L of detection mAb R11A2.15, R11A9.11 or 222113.11 (at 2 μ g/mL) was added to each well, and the plates were incubated for 1 hour at room temperature. Again, the plates were washed three times as noted above. Following washing, 50 μ L of Donkey anti-Rat(H+L)-HRP conjugate (Jackson ImmunoResearch catalog # 712-035-153), diluted 1:8,000 in PBS was added to each well, and the plates were incubated for one hour at room temperature. Again, the plates were washed three times as noted above. Following washing, 50 μ L/well TMB

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substrate (KPL, Catalog No. 52-00-04), pre-warmed to room temperature was added, the plates were incubated at room temperature in the dark for 10 minutes, and 50 μ L of TMB stop solution (1M HCl) was added to each well. Finally the plates were read on a spectrophotometer at $\lambda = 450\text{nm}$.

Results

[00174] The results using mouse m3B7.6 and m5B7.7 as the capture antibodies are shown in **Figures 3A** and **Figures 3B**, respectively. The two mouse and two rat mAbs (mAb R11A2.15 or R11A9.11) form four different pairs of detecting agents for human CD26 with similar lower limits of detection (LLOD) and provided high sensitivity in measuring human DPP-4 in a sandwich ELISA assay. In contrast, the commercially available rat mAb (R222113) (Human DPP-4/CD26 MAb (Clone 222113), Rat IgG2A Catalog No. MAB1180 from R&D Systems) detected less human CD26 and was significantly less sensitive in measuring human DPP-4 in a sandwich ELISA assay when paired with the two mouse mAbs m3B7.6 and m5B7.7. *See Figures 3A-B.* These results demonstrate that the antibodies provided herein (m3B7.6, m5B7.7, R11A2.15 and R11A9.11) are superior in detecting human CD26 in an immunoassay compared to the commercially available rat antibody R222113. In addition, the antibodies provided herein (m3B7.6, m5B7.7, R11A2.15 and R11A9.11) detect both endogenous and recombinant human CD26 making them useful as diagnostic reagents.

[00175] As shown in **Figure 3**, m3B7.6 + R11A2.15, m3B7.6 + R11A9.11, m5B7.7 + R11A2.15 and/or m5B7.7 + R11A9.11 are effective in measuring human DPP-4 in a sandwich ELISA assay. In addition, regardless of the choice of pairs, the serum concentration of DPP-4 in the study was between to 1-2 $\mu\text{g/ml}$.

Example 3: Anti-DPP-4 mAb epitope binning using OCTET.

[00176] Epitope binning for the four anti-DPP-4 antibodies (m3B7.6, m5B7.7, R11A2.15 and R11A9.11) was carried out by OCTET. Test antibody was biotinylated and captured on a streptavidin biosensor at concentration of 20 $\mu\text{g/ml}$ and in 200 μL /well for 5min and then washed with 200 μL of PBS buffer for 1 min. The biosensor was then incubated with recombinant human DPP4 at 10 $\mu\text{g/mL}$ for 5 min and was washed with 200 μL of PBS buffer

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for 1 min. The competitor antibodies were mixed in at a 1:1 ratio with the testing at a final concentration of 20 µg/ml and incubated with the biosensor in 200 µl for 5min.

[00177] A schematic of the assay is shown in

[00178] **Figures 4A**, and the results with the four different antibodies (m3B7.6, m5B7.7, R11A2.15 and R11A9.11) as the testing antibody are shown in

[00179] **Figures 4B-E**, respectively. The results demonstrated that the mouse monoclonal antibodies m3B7.6 and m5B7.7 compete with each other, and that the rat monoclonal antibodies R11A2.15 and R11A9.11 compete with each other. The mouse antibodies do not compete with the rat antibodies, and thus do not share an epitope. Based on this data, we conclude that the two mouse antibodies (m3B7.6 and m5B7.7) share the same or an overlapping epitope while the two rat antibodies (R11A2.15 and R11A9.11) share an epitope or an overlapping epitope that is different from the epitope shared by the mouse antibodies.

[00180] The foregoing description of the specific aspects will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[00181] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

[00182] All publications, patents, patent applications, and/or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated by reference for all purposes.

WHAT IS CLAIMED IS:

1. An isolated antibody or antigen-binding fragment, variant, or derivative thereof which competitively inhibits binding of and/or binds to the same dipeptidyl peptidase-4 (DPP-4) epitope as mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

2. An isolated antibody or antigen-binding fragment, variant, or derivative thereof that binds dipeptidyl peptidase-4 (DPP-4), comprising a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, wherein the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

3. The isolated antibody, or fragment, variant, or derivative thereof of claim 2, comprising a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870, mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871, rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

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4. The isolated antibody or fragment, variant, or derivative thereof any one of claims 1 to 3, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, or a single chain antibody molecule.

5. A hybridoma selected from the group consisting of the hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. 121870, the hybridoma deposited at the ATCC under Deposit No. 121871, the hybridoma deposited at the ATCC under Deposit No. 121872, the hybridoma deposited at the ATCC under Deposit No. 121873, and a combination thereof.

6. An antibody-producing cell culture comprising: a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. 121870, a hybridoma deposited at the ATCC under Deposit No. 121871, a hybridoma deposited at the ATCC under Deposit No. 121872, a hybridoma deposited at the ATCC under Deposit No. 121873, or a combination thereof.

7. An isolated antibody or antigen-binding fragment, variant, or derivative thereof produced by the hybridoma of claim 5 or the antibody-producing cell culture of claim 6.

8. The antibody, or fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7, or the antibody produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof, wherein the antibody or fragment, variant, or derivative thereof further comprises a heterologous polypeptide fused thereto.

9. The antibody, or fragment, variant, or derivative thereof of claim 8, wherein the heterologous polypeptide is a stabilizing polypeptide, a tag, a label, or a combination thereof.

10. The antibody, or fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7, or the antibody or fragment thereof produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof, wherein the antibody or fragment, variant, or derivative thereof is conjugated to a heterologous moiety.

11. The antibody, or fragment, variant, or derivative thereof of claim 10, wherein the heterologous moiety comprises one or more of: a peptide, a protein, an enzyme, a lipid, a heterologous antibody or fragment thereof, a detectable label, or polyethylene glycol (PEG).

12. The antibody, or fragment, variant, or derivative thereof of claim 11, wherein the heterologous moiety comprises biotin or a ruthenium chelate.

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13. A composition comprising an antibody, or fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof.

14. A composition comprising a combination of at least two antibodies or fragments thereof of any one of claims 1 to 4 or 7 to 12, or the antibody or fragment thereof produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof.

15. An isolated polynucleotide comprising a nucleic acid molecule encoding the antibody, or a subunit, fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody or fragment thereof produced by the hybridoma of claim 5 or the cell culture of claim 6, or a subunit, fragment, variant, or derivative thereof.

16. A composition comprising two or more nucleic acid molecules encoding the antibody, or a fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody or fragment thereof produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof.

17. The composition of claim 16, wherein the two or more nucleic acid molecules are situated in the same vector.

18. A vector comprising the isolated polynucleotide of claim 15 or the two or more nucleic acid molecules of any one of claims 16 or 17.

19. The composition of claim 16, wherein the two or more nucleic acid molecules are situated in at least two separate vectors.

20. The vectors of claim 19.

21. An isolated host cell comprising the vector of claim 18 or the vectors of claim 20.

22. A method of making the anti-DPP-4 antibody, or a subunit, fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody or fragment thereof produced by the hybridoma of claim 5 or the cell culture of claim 6, or a subunit, fragment, variant, or derivative thereof, comprising (a) culturing the host cell of claim 21, and (b) recovering the antibody, subunit, fragment, or derivative thereof.

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23. A kit for measuring dipeptidyl peptidase-4 (DPP-4) levels in a sample, comprising the antibody, or fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof.

24. A kit for measuring dipeptidyl peptidase-4 (DPP-4) levels in a sample, comprising at least two of the antibodies, or fragments, variants, or derivatives thereof of any one of claims 1 to 4 or 7 to 12, or the antibodies produced by the hybridoma of claim 5 or the cell culture of claim 6, or fragments, variants, or derivatives thereof.

25. The kit of claim 23 or claim 24, further comprising a solid support and detection reagents.

26. The kit of any one of claims 23 to 25, comprising a capture antibody, or fragment, variant, or derivative thereof and a detection antibody, or fragment, variant, or derivative thereof.

27. The kit of claim 26, wherein:

- (a) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or an antigen-binding fragment, variant, or derivative thereof;
- (b) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (c) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat

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monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;

- (d) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (e) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof;
- (f) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof;
- (g) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof; or
- (h) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-

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binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

28. The kit of any one of claims 23 to 27, wherein the detection antibody comprises a detectable label.

29. The kit of claim 28, wherein the detectable label is biotin and the detection reagents comprise a streptavidin-horse radish peroxidase (HRP) conjugate and a colorimetric substrate for HRP.

30. The kit of claim 29, wherein the detectable label is a ruthenium chelate.

31. A method of detecting dipeptidyl peptidase-4 (DPP-4) levels in one or more samples, comprising at least two anti-DPP-4 antibodies or antigen-binding fragments thereof, wherein one of the anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of and/or binds to the same DPP-4 epitope as mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the American Type Culture Collection (ATCC) under Deposit No. PTA-121870 or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and wherein one of the anti-DPP-4 antibodies comprises an isolated antibody or antigen-binding fragment, variant, or derivative thereof that competitively inhibits binding of and/or binds to the same DPP-4 epitope as rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

32. The method of claim 31, wherein one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, wherein the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or mouse

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monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and wherein one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a heavy chain variable domain (VH) with three heavy chain complementarity determining regions (CDRs) VHCDR1, VHCDR2 and VHCDR3, and a light chain variable domain (VL) with three light chain CDRs VLCDR1, VLCDR2, and VLCDR3, wherein the CDRs of the isolated antibody, or fragment, variant, or derivative thereof are identical to the CDRs of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

33. The method of claim 31 or claim 32, wherein one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a VH and a VL identical to the VH and VL of mouse monoclonal antibody m3B7.6 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870, or mouse monoclonal antibody m5B7.7 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871; and wherein one of the two or more anti-DPP-4 antibodies is an isolated antibody or antigen-binding fragment, variant, or derivative thereof comprising a VH and a VL identical to the VH and VL of rat monoclonal antibody R11A2.15 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or rat monoclonal antibody R11A9.11 produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873.

34. The method of any one of claims 31 to 33, wherein the assay is a sandwich immunoassay comprising a first anti-DPP-4 "capture" antibody or antigen-binding fragment thereof attached to a solid support, and a second anti-DPP-4 "detection" antibody or antigen-binding fragment thereof.

35. The immunoassay of claim 34, comprising:

- (a) attaching a capture antibody or antigen-binding fragment thereof to a solid support;
- (b) applying the test sample or a control sample under conditions sufficient to allow DPP-4, if present in the sample, to bind to the capture antibody or antigen-binding fragment thereof;

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- (c) applying the detection antibody or antigen-binding fragment thereof under conditions sufficient to allow binding to DPP-4 already bound to the capture antibody or antigen-binding fragment thereof; and
- (d) measuring the amount of detection antibody or antigen-binding fragment thereof bound to DPP-4.

36. The immunoassay of claim 35, wherein the detection antibody or antigen-binding fragment thereof further comprises a detectable label.

37. The immunoassay of claim 36, wherein the detectable label is biotin.

38. The immunoassay of claim 37, wherein the detectable label is ruthenium chelate.

39. The immunoassay of any one of claims 34 to 38, wherein:

- (a) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872, or an antigen-binding fragment, variant, or derivative thereof;
- (b) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (c) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;

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- (d) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (e) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof;
- (f) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof;
- (g) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof; or
- (h) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC

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under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

40. A method of measuring the amount of dipeptidyl peptidase-4 (DPP-4) in a sample obtained from a subject comprising assaying the sample using the immunoassay of any one of claims 34 to 39; the kit of any one of claims 23 to 30; and/or the antibody, or fragment, variant, or derivative thereof of any one of claims 1 to 4 or 7 to 12, or the antibody produced by the hybridoma of claim 5 or the cell culture of claim 6, or a fragment, variant, or derivative thereof.

41. The method of claim 40, wherein the sample obtained from a subject is one or more of whole blood, serum, plasma, saliva, urine, sputum, bronchoalveolar lavage fluid, lung epithelial cells, or nasal polyps, or skin.

42. The method of claim 40 or claim 41, wherein the subject has a disease or condition selected from the group consisting of: an IL-13-mediated disease or disorder, a pulmonary disease or disorder, and a chronic inflammatory skin disease or disorder.

43. The method of claim 42, wherein the disease or condition is selected from the group consisting of: asthma, atopic asthma, corticosteroid naive asthma, chronic asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, asthma due to smoking, asthma uncontrolled on corticosteroids, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), ulcerative colitis (UC), atopic dermatitis (AD), allergic rhinitis, chronic rhinosinusitis, skin fibrosis, allergic contact dermatitis, eczema and psoriasis.

44. The method of any one of claims 40 to 43, wherein a sample is obtained from the subject and is submitted for measurement of the DPP-4 level in the sample.

45. The method of any one of claims 40 to 44, wherein the immunoassay comprises a sandwich immunoassay comprising a first anti-DPP-4 "capture" antibody or antigen-binding fragment thereof attached to a solid support, and a second anti-DPP-4 "detection" antibody or antigen-binding fragment thereof.

46. The method of claim 45, wherein the immunoassay comprises:

(a) attaching a capture antibody or antigen-binding fragment thereof to a solid support;

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- (b) applying the patient sample or control sample under conditions sufficient to allow DPP-4, if present in the sample, to bind to the capture antibody or antigen-binding fragment thereof;
- (c) applying the detection antibody or antigen-binding fragment thereof under conditions sufficient to allow binding to DPP-4 already bound to the capture antibody or antigen-binding fragment thereof; and
- (d) measuring the amount of detection antibody or antigen-binding fragment thereof bound to DPP-4.

47. The method of claim 46, wherein the detection antibody, or fragment, variant, or derivative thereof further comprises a detectable label.

48. The method of claim 47, wherein the detectable label is biotin.

49. The method of claim 48, wherein the detectable label is ruthenium chelate.

50. The method of any one of claims 45 to 49, wherein:

- (a) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;
- (b) the capture antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (c) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-

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binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof;

- (d) the capture antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof;
- (e) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof;
- (f) the capture antibody is rat monoclonal antibody R11A2.15 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121872 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof;
- (g) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m3B7.6 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121870 or an antigen-binding fragment, variant, or derivative thereof; or

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- (h) the capture antibody is rat monoclonal antibody R11A9.11 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121873 or an antigen-binding fragment, variant, or derivative thereof and the detection antibody is mouse monoclonal antibody m5B7.7 as produced from a hybridoma deposited at the ATCC under Deposit No. PTA-121871 or an antigen-binding fragment, variant, or derivative thereof.

51. The method of any one of claims 40 to 50, wherein the subject is an asthma patient, and wherein the sample taken from the patient comprises serum.

Dorsal view

Ventral view

CFA/IFA

TiterMax Gold

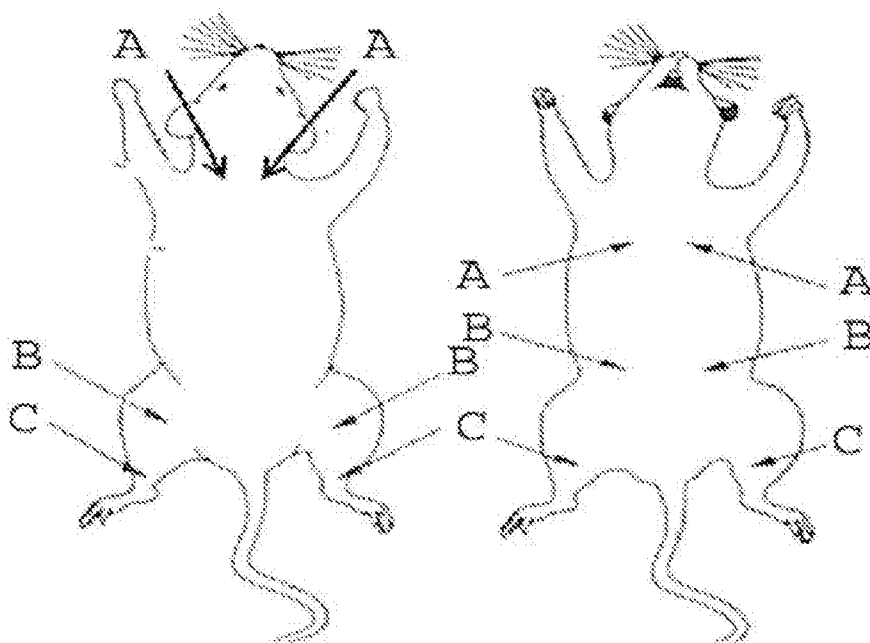


FIG. 1

SUBSTITUTE SHEET (RULE 26)

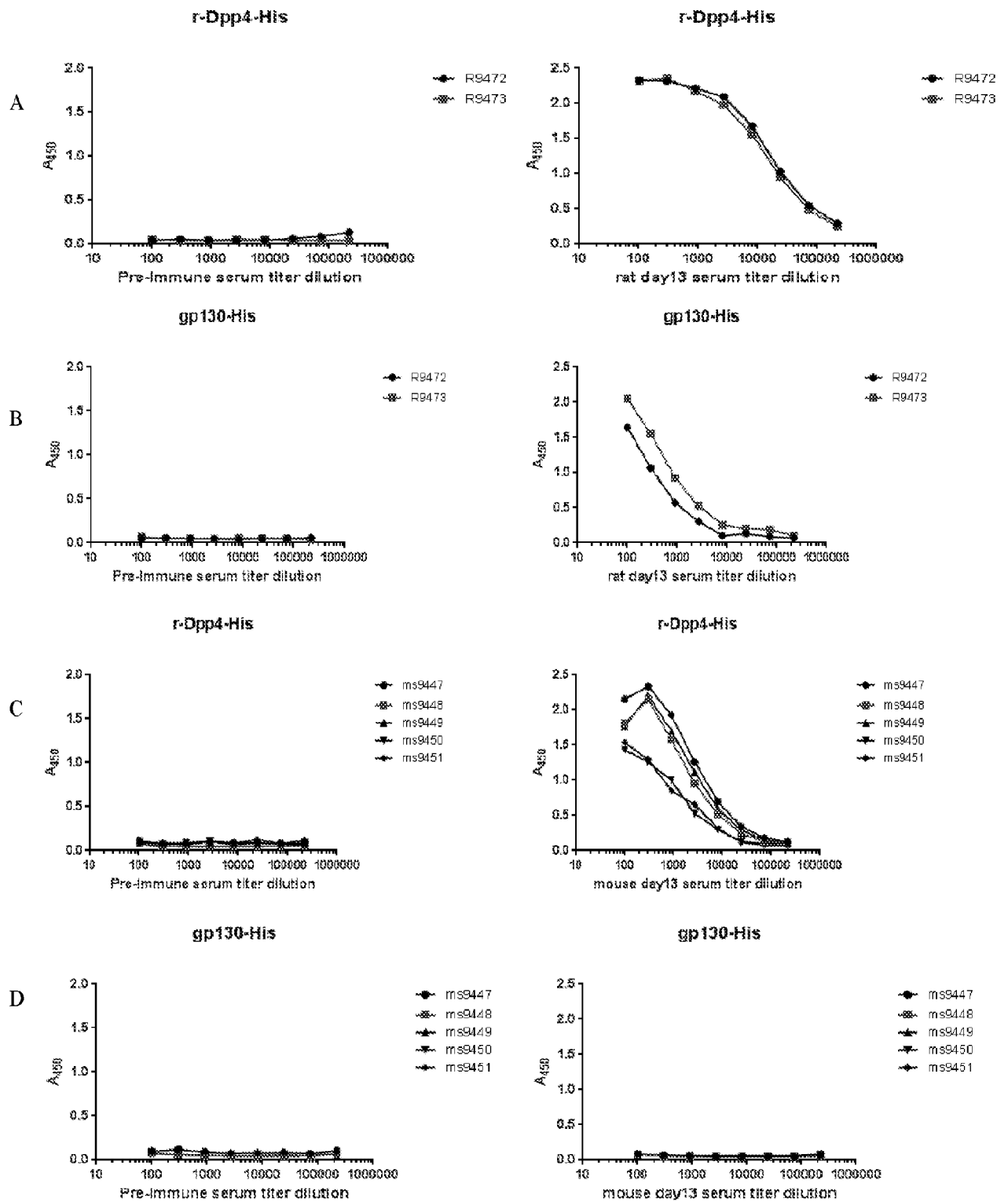
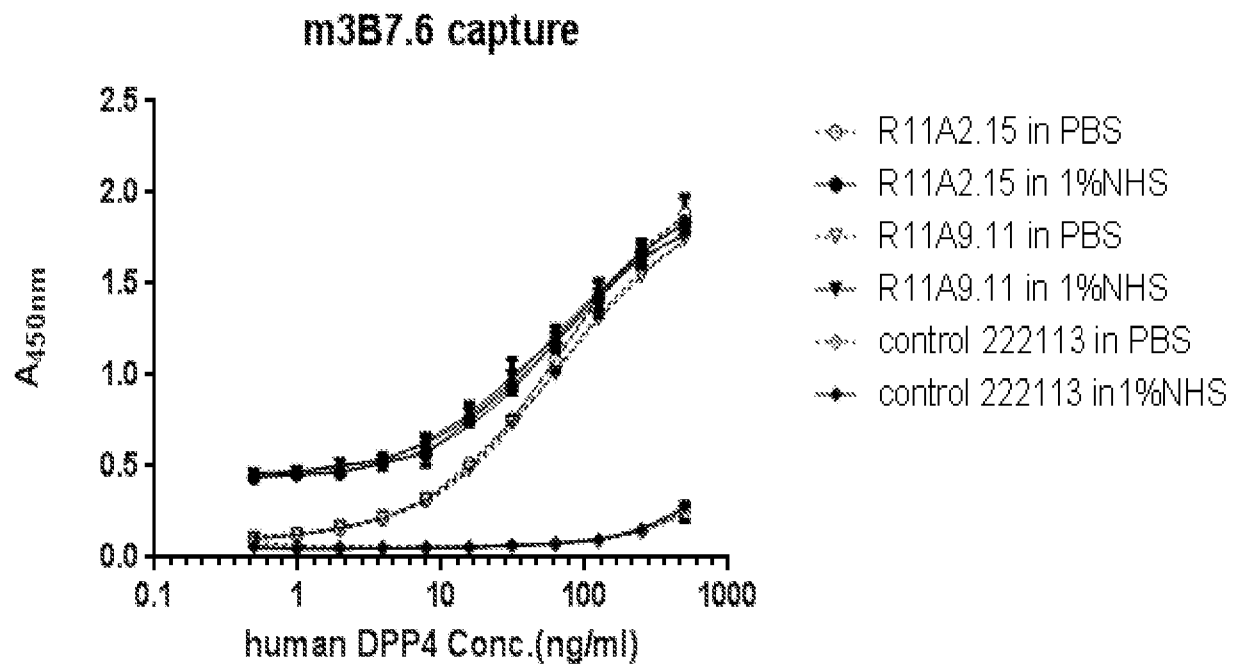
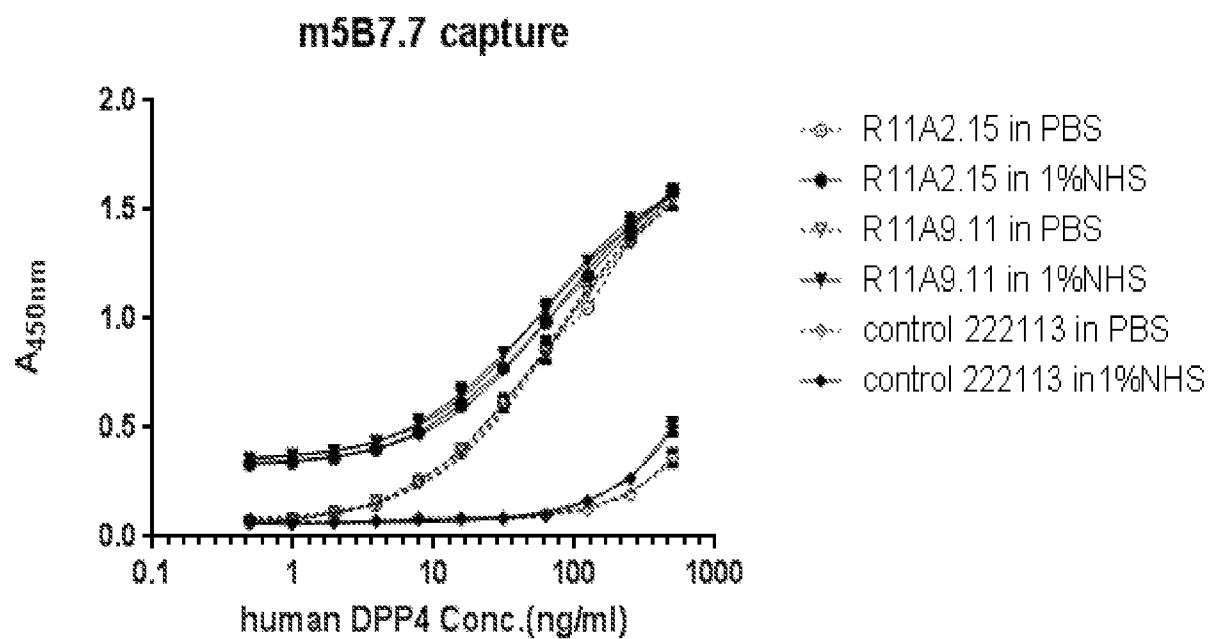


FIG. 2

**FIG. 3A**

**FIG. 3B**

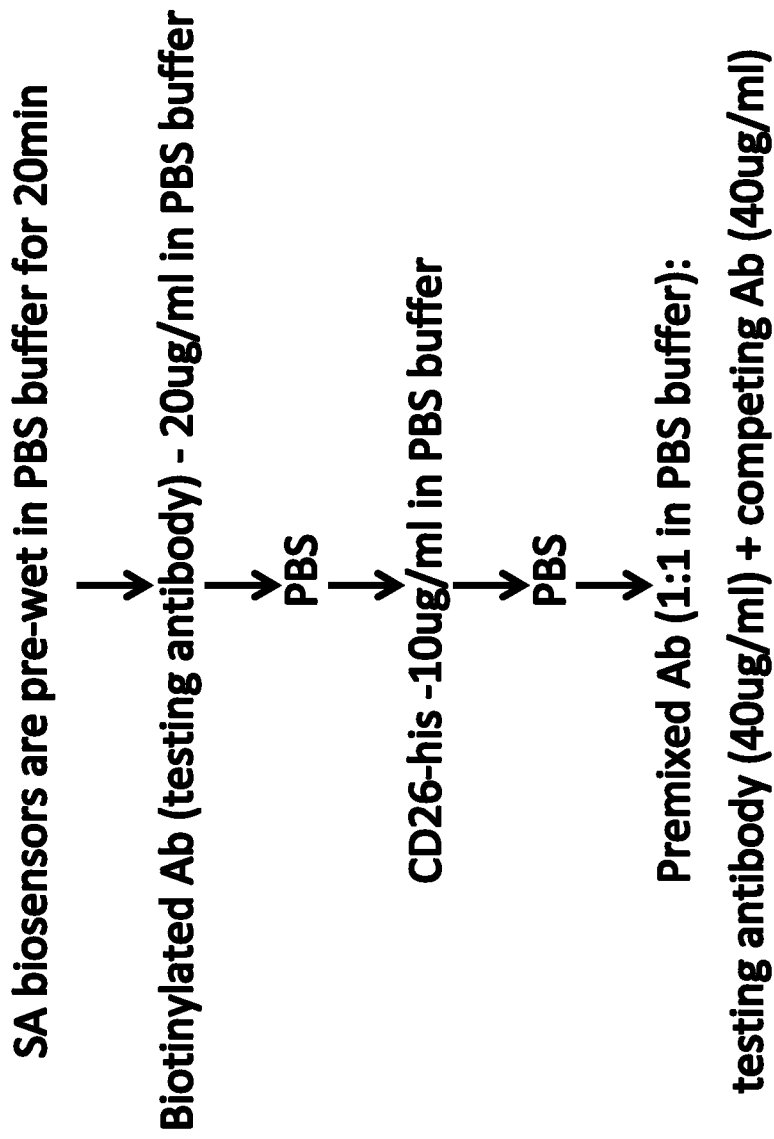


FIG. 4A

REPLACEMENT SHEET

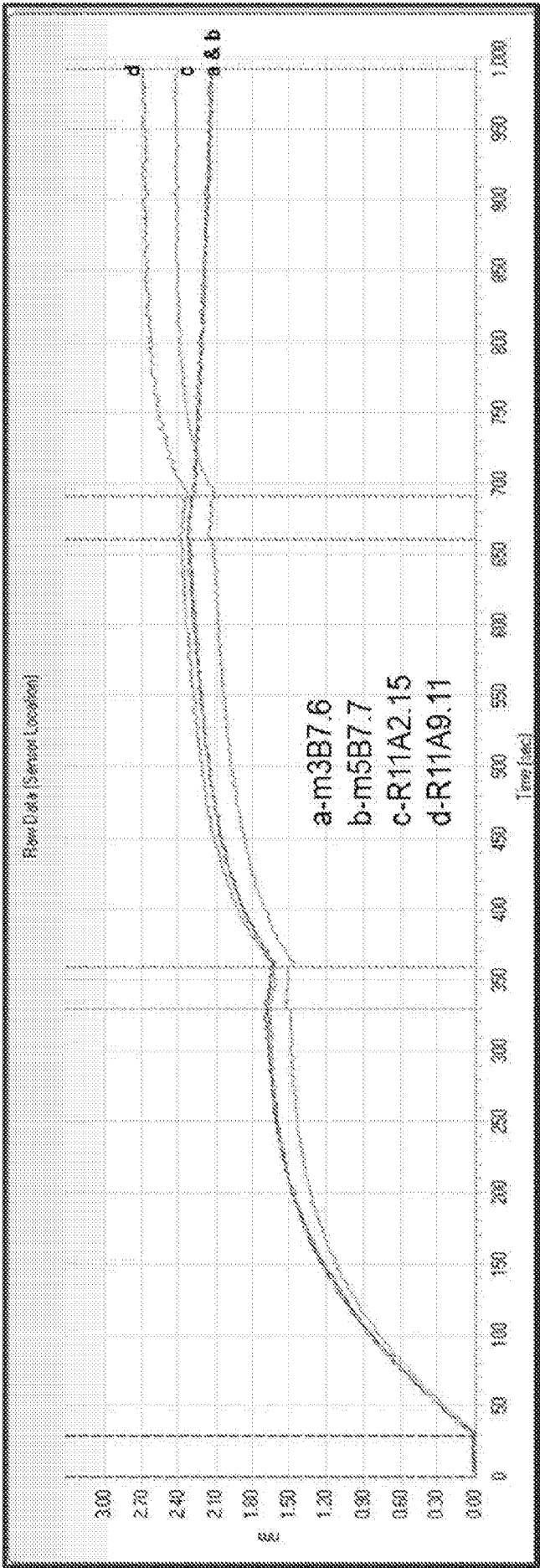


FIG. 4B

REPLACEMENT SHEET

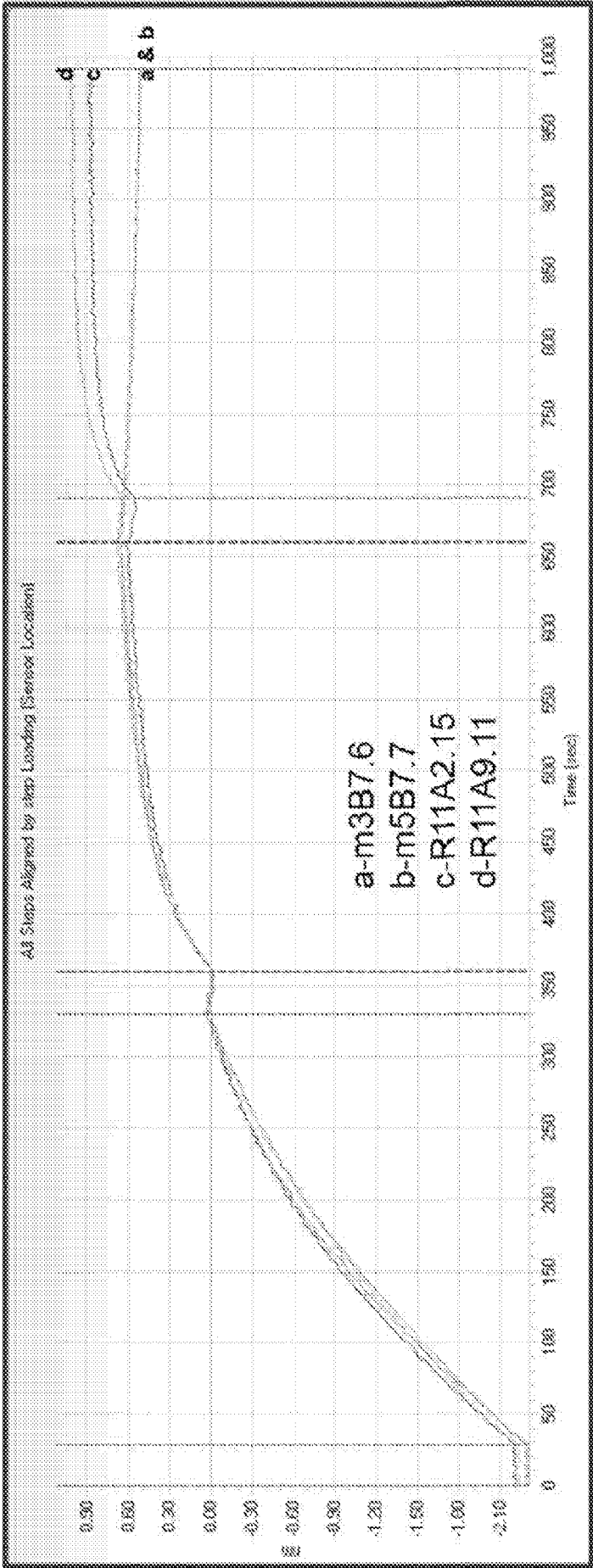


FIG. 4C

REPLACEMENT SHEET

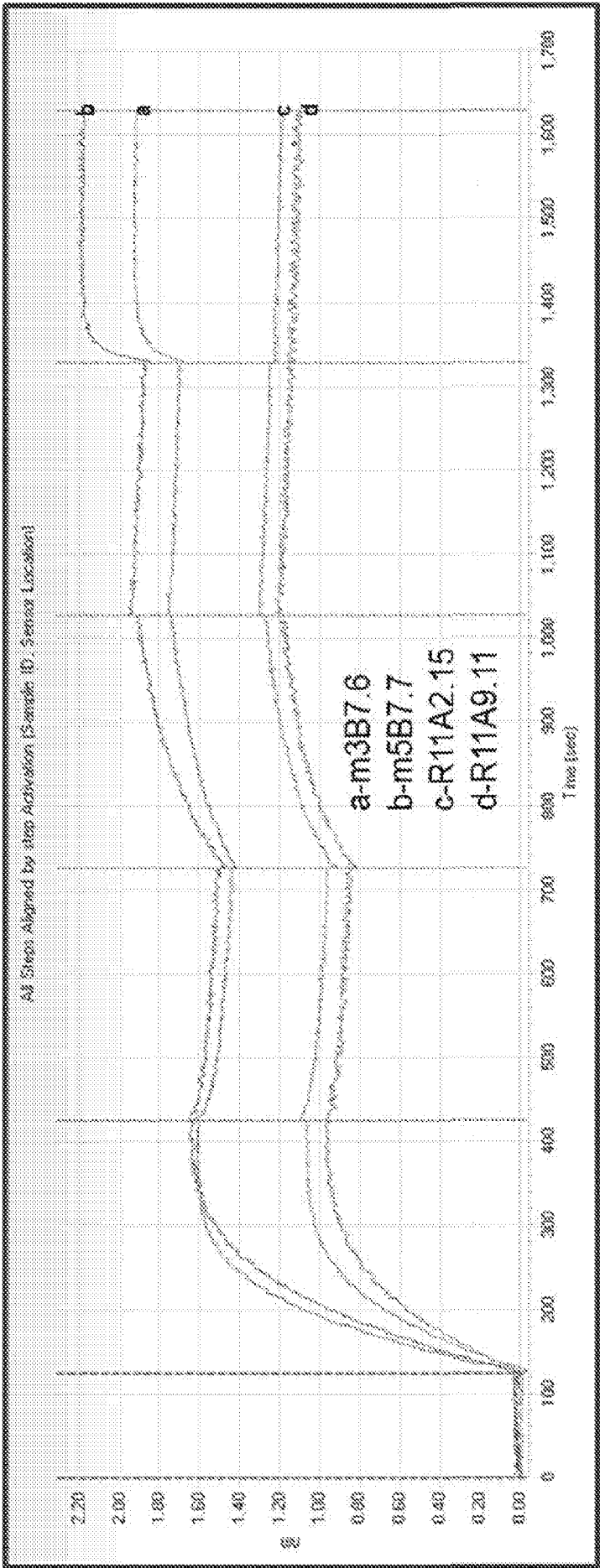


FIG. 4D

REPLACEMENT SHEET

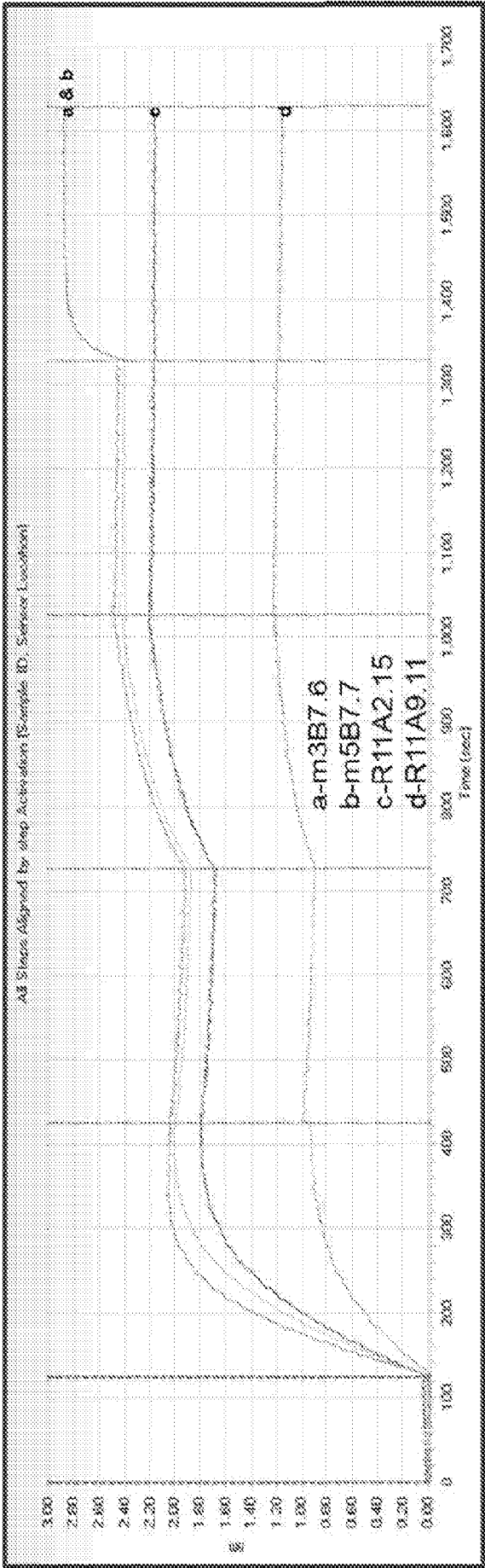


FIG. 4E



(51) International Patent Classification:

A61K 39/395 (2006.01) C12N 1/19 (2006.01)
A61P 35/00 (2006.01) C12N 1/21 (2006.01)
C07K 16/28 (2006.01) C12N 15/13 (2006.01)
C12N 1/15 (2006.01)

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(74) Agents: **SCOTT, Derek, B.** et al.; c/o Medimmune, LLC, One Medimmune Way, Gaithersburg, MD 20878 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ASSAY TO DETECT HUMAN DPP-4

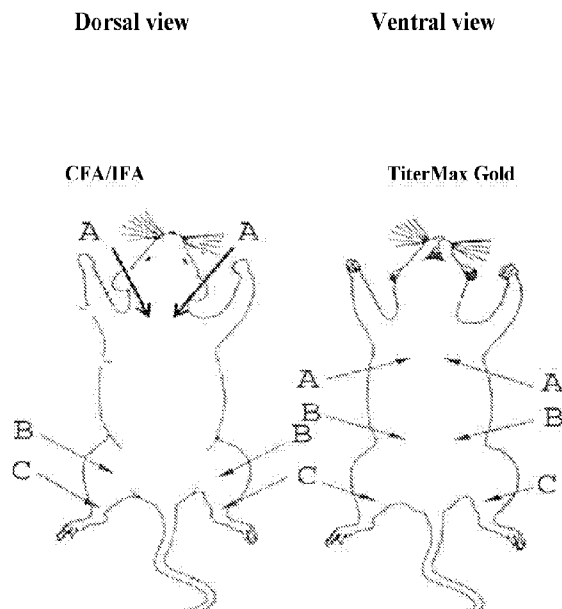


FIG. 1

(57) Abstract: This disclosure provides a robust, sensitive, and specific assay for the detection and measurement of DPP-4 levels in samples obtained from human patients. The disclosure further provides novel anti-DPP-4 monoclonal antibodies that recognize human DPP-4, and assay kits comprising one or more of these antibodies.

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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

(88) Date of publication of the international search report:

1 September 2016

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12603

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 39/395, A61P 35/00, C07K 16/28, C12N 1/15, C12N 1/19, C12N 1/21, C12N 15/13 (2016.01) CPC - A61K 47/48561, C07K 14/70596, C07K 16/2896, C12Y 304/14005, C12N 9/48, C12N 9/6424 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(8) - A61K 39/395, A61P 35/00, C07K 16/28, C12N 1/15, C12N 1/19, C12N 1/21, C12N 15/13 (2016.01) CPC - A61K 47/48561, C07K 14/70596, C07K 16/2896, C12Y 304/14005, C12N 9/48, C12N 9/6424 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC(8) - C12N 15/63, C12P 21/08 (2016.01); CPC- C12N 9/6491, C07K 16/40, A61K 2039/505 USPC- 530/387.3, 435/332, 530/388.2 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: human DPP4, ADABP, ADCP2, ADCP-2, Adenosine deaminase complexing protein 2, CD26, Dipeptidyl peptidase 4, Dipeptidyl peptidase IV, DPPIV, DPP IV, M3B7.6, m5B7.7, R11A2.15, R11A9.11, capture antibody, detection antibody, sandwich immunoassay....		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Hatano, et al. Establishment of monoclonal anti-human CD26 antibodies suitable for immunostaining of formalin-fixed tissue. Diagn Pathol. 2014, 9:30; pg 3	1-7, 31-33
A	US 7,250,492 B2 (Chen) 31 July 2007 (31.07.2007) col 9, ln 23-30; col 12, ln 63-67	1-7, 31-33
☐ Further documents are listed in the continuation of Box C. ☐		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 25 June 2016		Date of mailing of the international search report 29 JUL 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12603

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-30, 34-51
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

***** See Supplemental Sheet to continue *****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-7, 31-33, restricted to m3B7.6 and R11A2.15 monoclonal antibodies

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12603

***** Supplemental Sheet *****

In Continuation of Box III. Observations where unity of invention is lacking:

Group I+: claims 1-7, 31-33, directed to an anti-DDP-4 antibody, a hybridoma producing said antibody, and a method for detecting DDP-4 in a sample. The anti-DDP-4 antibody, hybridoma producing said antibody, and a method for detecting DDP-4 in a sample will be searched to the extent that they encompass m3B7.6 monoclonal antibody produced from a mouse hybridoma, ATCC Deposit No. PTA-121870 and R11A2.15 MAB produced from a rat hybridoma, ATCC Deposit No. PTA-121872. It is believed that claims 1-7, 31-33 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a mouse MAB m3B7.6 produced from a hybridoma, ATCC Deposit No. PTA-121870 and a rat MAB R11A2.15 produced from a hybridoma, ATCC Deposit No. PTA-121872, hybridomas producing said antibodies, and a method for detecting DDP-4 in a sample utilizing said antibodies. Additional mouse and/or rat MAB(s), hybridomas producing thereof, and a method utilizing thereof will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mouse and/or rat MAB(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a mouse MAB m5B7.7, rat MAB R11A9.11, hybridoma cells ATCC Deposit No. PTA-121871 and No. PTA-121873, and a method for detecting DDP-4 in a sample utilizing mouse MAB m5B7.7 and rat MAB R11A9.11, i.e., claims 1-7, 31-33.

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The special technical feature of each invention of Group I+ is a specific mouse anti-DDP-4 MAB and a specific rat anti-DDP-4 MAB.

Common Technical Features

Some inventions of Group I+ share the technical feature of a mouse anti-DDP-4 MAB and a hybridoma cells producing thereof.

However, this shared technical feature does not represent a contribution over prior art as being anticipated by a paper titled "Establishment of monoclonal anti-human CD26 antibodies suitable for immunostaining of formalin-fixed tissue" by Hatano, et al. (Diagn Pathol. 2014, 9:30) (hereinafter "Hatano").

Hatano discloses a mouse anti-DDP-4 MAB (pg 3, col 1, "mouse anti-human CD26 mAbs, murine anti-human CD26 mAbs (clone 4G8, 1F7, 14D10, 5F8, 16D4B or 9C11", NOTE: CD26 is synonymous with DDP-4 (see instant specification, para [0065], "The term "DPP-4" as used herein refers to the dipeptidyl peptidase IV protein... DPP-4 is also known as DPP-IV, adenosine deaminase complexing protein 2, or CD26 (cluster of differentiation 26)"; and Hatano, pg 1, para 1, "CD26 is a 110-kDa type II membrane-bound glycoprotein with dipeptidyl peptidase IV (DPPIV) activity in its extra cellular domain") and a hybridoma cells producing thereof (pg 3, col 2, "Development of hybridomas and monoclonal anti-human CD26 antibodies"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Group I+ share the technical feature of a rat anti-DDP-4 MAB and a hybridoma cells producing thereof. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 7,250,492 B2 (Chen) (31 July 2007).

Chen discloses an anti-DDP-4 MAB (col 9, In 23-30, "mono specific antibodies which specifically bind an epitope of a human DPPIV (dipeptidyl peptidase IV/CD26) ... the anti-DPP-4 antibodies E19 or E26"), and a hybridoma cells producing thereof (col 12, In 63-67, "The rat hybridoma that produces monoclonal antibody E26 was deposited... and assigned patent deposit accession number PTA-3377"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Group I+ share the technical feature of a method of detecting DPP-4 levels in a sample, comprising [contacting said one or more samples with] at least two anti-DPP-4 monoclonal antibodies. However, this shared technical feature does not represent a contribution over prior art as being obvious over a publication titled "Human Dipeptidyl Peptidase IV Elisa Kit (DPP4)" by Shanghai BlueGene Biotech CO.,LTD. (2011) [according to the properties of the posted document] [Retrieved from the Internet 28 April 2016: <http://www.hoelzel-biotech.com/media/import/pdf_manual/BG_Bluegene/E01D0039__Manual.pdf>] (hereinafter "BlueGene").

BlueGene discloses a method of detecting DPP-4 levels in a sample comprising [contacting said one or more samples with] at least two anti-DPP-4 antibodies (pg 1, 2nd para, "This DPP4 enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for DPP4. Standards or samples are then added to the microtiter plate wells and DPP4 if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of DPP4 present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for DPP4 are added to each well to "sandwich" the DPP4 immobilized on the plate... The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm"), wherein only the first, i.e., capture antibody is an anti-DPP-4 monoclonal antibody (pg 1, 2nd para, "The microtiter plate... has been pre-coated with a monoclonal antibody specific for DPP4"). BlueGene does not specifically disclose that the second/detection antibody is also a monoclonal anti-DPP-4 antibody. However, it would have been obvious to one of ordinary skill in the art to substitute, in the course of routine experimentation and with a reasonable expectation of success, the polyclonal anti-DPP-4 detection antibody disclosed by BlueGene (pg 1, 2nd para, "a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for DPP4 are added to each well to "sandwich" the DPP4 immobilized on the plate...") by a monoclonal anti-DPP-4 antibody that binds to an DPP-4 epitope other than the epitope to which the BlueGene monoclonal anti-DPP-4 antibody binds. As said technical feature would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

The inventions of Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

--- Continued on next sheet ---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12603

***** Supplemental Sheet *****

In Continuation of Box III. Observations where unity of invention is lacking:

NOTE re item 4: Claims 8-30, 34-51 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

NOTE re claim 31:

Claim 31 is objected to as unclear because a method cannot comprise an antibody. For the purpose of this Lack of Unity analysis, the ISA/USA has construed claim 31 as follows:

31. A method of detecting dipeptidyl peptidase-4 (DPP-4) levels in one or more samples, comprising [contacting said one or more samples with] at least two anti-DPP-4 antibodies or antigen-binding fragments thereof, wherein...



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权利要求书7页 说明书29页

序列表10页 附图8页

(54)发明名称

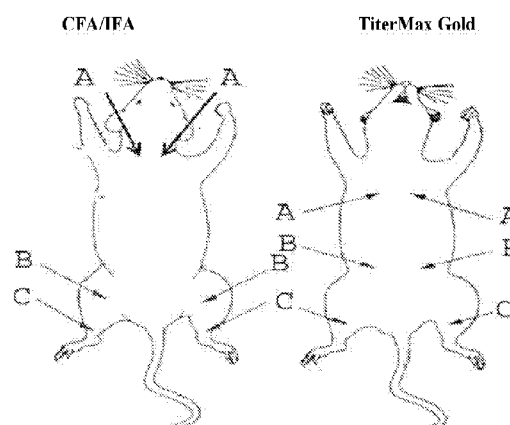
检测人DPP-4的测定法

(57)摘要

本公开内容提供稳健的、灵敏的和特异的用于检测和测量从人类患者获得的样品中的DPP-4水平的测定法。本公开内容进一步提供识别人DPP-4的新型抗DPP-4单克隆抗体,和包含一种或多种这些抗体的测定试剂盒。

背视图

腹视图



1. 一种分离抗体或其抗原结合片段、变体或衍生物,其竞争性抑制从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的结合和/或结合与其相同的二肽基肽酶-4(DPP-4)表位。

2. 一种分离抗体或其抗原结合片段、变体或衍生物,其结合二肽基肽酶-4(DPP-4),包含具有三个重链互补决定区(CDRs)VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRsVLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL),其中所述分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

3. 权利要求2的分离抗体或其片段、变体或衍生物,包含与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL。

4. 权利要求1-3中任一项的分离抗体或其片段、变体或衍生物,其中所述抗体片段为Fab片段、Fab'片段、F(ab')₂片段、Fv片段或单链抗体分子。

5. 一种杂交瘤,其选自在保藏号121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤和其组合。

6. 一种产抗体的细胞培养物,包含:在保藏号121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤或其组合。

7. 由权利要求5的杂交瘤或权利要求6的产抗体的细胞培养物产生的分离抗体或其抗原结合片段、变体或衍生物。

8. 权利要求1-4或7中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段、变体或衍生物,其中所述抗体或其片段、变体或衍生物进一步包含与其融合的异源多肽。

9. 权利要求8的抗体或其片段、变体或衍生物,其中所述异源多肽为稳定多肽、标签、标记或其组合。

10. 权利要求1-4或7中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段,或其片段、变体或衍生物,其中所述抗体或其片段、变体或衍生物与异源部分缀合。

11. 权利要求10的抗体或其片段、变体或衍生物,其中所述异源部分包含以下一种或多种:肽、蛋白、酶、脂质、异源抗体或其片段、可检测标记或聚乙二醇(PEG)。

12. 权利要求11的抗体或其片段、变体或衍生物,其中所述异源部分包括生物素或钕螯合物。

13. 一种组合物,包含权利要求1-4或7-12中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体,或其片段、变体或衍生物。

14. 一种组合物,包含至少两种权利要求1-4或7-12中任一项的抗体或其片段,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段,或其片段、变体或衍生物的组合。

15. 一种分离多核苷酸,包含编码权利要求1-4或7-12中任一项的抗体或其亚基、片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段,或其亚基、片段、变体或衍生物的核酸分子。

16. 一种组合物,包含两个或更多个编码权利要求1-4或7-12中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段,或其片段、变体或衍生物的核酸分子。

17. 权利要求16的组合物,其中所述两个或更多个核酸分子位于同一载体内。

18. 包含权利要求15的分离多核苷酸或权利要求16或17中任一项的两个或更多个核酸分子的载体。

19. 权利要求16的组合物,其中所述两个或更多个核酸分子位于至少两个单独的载体中。

20. 权利要求19的载体。

21. 包含权利要求18的载体或权利要求20的载体的分离的宿主细胞。

22. 制备权利要求1-4或7-12中任一项的抗DPP-4抗体或其亚基、片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段,或其亚基、片段、变体或衍生物的方法,包括(a) 培养权利要求21的宿主细胞,和(b) 回收抗体、其亚基、片段或衍生物。

23. 用于测量样品中的二肽基肽酶-4 (DPP-4) 水平的试剂盒,包含权利要求1-4或7-12中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体,或其片段、变体或衍生物。

24. 用于测量样品中的二肽基肽酶-4 (DPP-4) 水平的试剂盒,包含至少两种权利要求1-4或7-12中任一项的抗体或其片段、变体或衍生物,或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体,或其片段、变体或衍生物。

25. 权利要求23或权利要求24的试剂盒,进一步包含固体支持物和检测试剂。

26. 权利要求23-25中任一项的试剂盒,包含捕获抗体或其片段、变体或衍生物和检测抗体或其片段、变体或衍生物。

27. 权利要求26的试剂盒,其中:

(a) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

(b) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆

抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(c) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15 或其抗原结合片段、变体或衍生物;

(d) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(e) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;

(f) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物;

(g) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;或

(h) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

28. 权利要求23-27中任一项的试剂盒,其中所述检测抗体包含可检测标记。

29. 权利要求28的试剂盒,其中所述可检测标记为生物素并且检测试剂包括链霉亲和素-辣根过氧化物酶(HRP)缀合物和HRP的比色底物。

30. 权利要求29的试剂盒,其中所述可检测标记为钆螯合物。

31. 检测一个或多个样品中的二肽基肽酶-4 (DPP-4) 水平的方法,包括至少两种抗DPP-4抗体或其抗原结合片段,其中一种抗DPP-4抗体包括竞争性抑制从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的结合和/或结合与其相同DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物;并且其中一种抗DPP-4抗体包括竞争性抑制从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的结合和/或结合与其相同DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物。

物。

32. 权利要求31的方法,其中两种或更多种抗DPP-4抗体的一种为包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL)的分离抗体或其抗原结合片段、变体或衍生物,其中所述分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的CDRs相同;并且其中两种或更多种抗DPP-4抗体的一种为包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL)的分离抗体或其抗原结合片段、变体或衍生物,其中所述分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

33. 权利要求31或权利要求32的方法,其中两种或更多种抗DPP-4抗体的一种为包含与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物;并且其中两种或更多种抗DPP-4抗体的一种为包含与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物。

34. 权利要求31-33中任一项的方法,其中所述测定为夹心免疫测定,其包含附着在固体支持物上的第一个抗DPP-4“捕获”抗体或其抗原结合片段,和第二个抗DPP-4“检测”抗体或其抗原结合片段。

35. 权利要求34的免疫测定,包括:

- (a) 将捕获抗体或其抗原结合片段附着到固体支持物;
- (b) 在如果样品中存在DPP-4时足以允许DPP-4结合捕获抗体或其抗原结合片段的条件下施加检验样品或对照样品;
- (c) 在足以允许结合已经结合到捕获抗体或其抗原结合片段的DPP-4的条件下施加检测抗体或其抗原结合片段;和
- (d) 测量结合到DPP-4的检测抗体或其抗原结合片段的量。

36. 权利要求35的免疫测定,其中所述检测抗体或其抗原结合片段进一步包含可检测标记。

37. 权利要求36的免疫测定,其中所述可检测标记为生物素。

38. 权利要求37的免疫测定,其中所述可检测标记为钆螯合物。

39. 权利要求34-38中任一项的免疫测定,其中:

(a) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

(b) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆

抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(c) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

(d) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(e) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;

(f) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物;

(g) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;或

(h) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

40. 测量从受试者获得的样品中的二肽基肽酶-4 (DPP-4) 的量的方法,包括使用权利要求34-39中任一项的免疫测定,权利要求23-30中任一项的试剂盒和/或权利要求1-4或7-12中任一项的抗体或其片段、变体或衍生物或由权利要求5的杂交瘤或权利要求6的细胞培养物产生的抗体或其片段、变体或衍生物测定样品。

41. 权利要求40的方法,其中从受试者获得的样品为以下的一种或多种:全血、血清、血浆、唾液、尿液、痰、支气管肺泡灌洗液、肺上皮细胞或鼻息肉或皮肤。

42. 权利要求40或权利要求41的方法,其中所述受试者具有选自IL-13-介导的疾病或病症、肺疾病或病症和慢性炎症性皮肤病或病症的疾病或病况。

43. 权利要求42的方法,其中所述疾病或病况选自:哮喘、特应性哮喘、未经皮质类固醇治疗的哮喘、慢性哮喘、皮质类固醇抵抗性哮喘、皮质类固醇难治性哮喘、抽烟引起的哮喘、不受皮质类固醇控制的哮喘、特发性肺纤维化(IPF)、慢性阻塞性肺病(COPD)、溃疡性结肠炎(UC)、特应性皮炎(AD)、过敏性鼻炎、慢性鼻窦炎、皮肤纤维化、过敏性接触性皮炎、湿疹

和银屑病。

44. 权利要求40-43中任一项的方法,其中从受试者获得样品并提交用于测量样品中的DPP-4水平。

45. 权利要求40-44中任一项的方法,其中所述免疫测定包括夹心免疫测定,其包括附着到固体支持物的第一个抗DPP-4“捕获”抗体或其抗原结合片段,和第二个抗DPP-4“检测”抗体或其抗原结合片段。

46. 权利要求45的方法,其中所述免疫测定包括:

(a) 将捕获抗体或其抗原结合片段附着到固体支持物;

(b) 在如果样品中存在DPP-4时足以允许DPP-4结合捕获抗体或其抗原结合片段的条件下施加患者样品或对照样品;

(c) 在足以允许结合已经结合到捕获抗体或其抗原结合片段的DPP-4的条件下施加检测抗体或其抗原结合片段;和

(d) 测量结合到DPP-4的检测抗体或其抗原结合片段的量。

47. 权利要求46的方法,其中所述检测抗体或其片段、变体或衍生物进一步包含可检测标记。

48. 权利要求47的方法,其中所述可检测标记为生物素。

49. 权利要求48的方法,其中所述可检测标记为钆螯合物。

50. 权利要求45-49中任一项的方法,其中:

(a) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

(b) 捕获抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(c) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

(d) 捕获抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

(e) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;

(f) 捕获抗体为从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆

抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物;

(g) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;或

(h) 捕获抗体为从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

51. 权利要求40-50中任一项的方法,其中所述受试者为哮喘患者,并且其中从患者获取的样品包括血清。

检测人DPP-4的测定法

[0001] 对电子提交的序列表的引用

与本申请一起提交的以ASCII文本文件(名称DPP4-100P1_sequence_listing.txt;大小:26,741字节;和创建日期:2014年12月15日)电子提交的序列表的内容通过引用以其整体结合到本文中。

[0002] 发明背景

二肽基肽酶IV (DPP-4) (也称为CD26或腺苷脱氨酶结合蛋白)为脯氨酰寡肽酶家族中的II型跨膜丝氨酸蛋白酶,其催化来自在第2位具有脯氨酸或丙氨酸的多肽的N端的N端二肽的水解(酶学委员会(EC)号3.4.14.5 (BRENDA | IUBMB))。许多趋化因子和肽激素,包括GLP-1、GLP-2、胃抑制性多肽(GIP)、垂体腺苷酸环化酶激活多肽(PACAP)和神经肽Y被DPP-4切割和灭活。参见,例如,Yaron A., Naider F. Crit. Rev. Biochem. Mol. Biol. 28, 31-81 (1993); Mentlein R. Regul Pept. 85(1):9-24 (1999)。因此,DPP-4通过其抑制趋化因子和肽激素的能力调节葡萄糖代谢、食欲和疼痛调节。

[0003] 除了作为调节性蛋白酶的作用,DPP-4还结合若干分子并诱导胞内信号转导。具体地,DPP-4通过结合至包括腺苷脱氨酶(ADA)在内的若干配体诱导T细胞共刺激/增殖和淋巴细胞-上皮细胞粘附。参见,例如,Gines等人, Biochem. J. 361:203-209 (2002)。还已经报道DPP-4增强T-细胞成熟和迁移、细胞因子分泌、抗体产生、B细胞的免疫球蛋白同种型转换,和细胞毒性T细胞活化。Ohnuma等人Front Biosci. 13:2299-310 (2008)。

[0004] DPP-4为110 kDa的糖蛋白,由位于染色体2 (2q24.3)上的基因编码(Abbott等人Immunogenetics. 40(5):331-8 (1994)),作为由776个氨基酸组成的同型二聚体起作用。每一单体由两个结构域组成: α/β 水解酶结构域和8-片 β -螺旋桨结构域。DPP-4在几种组织中广泛表达,包括肝、肺、肾、上皮细胞和淋巴细胞。Heike等人Clin Exp Immunol. 74:431-434 (1988); Gorrell等人Scand J Immunol. 54:249-264 (2001)。当T细胞活化时,DPP-4表达在静止T细胞上调。还已经观察到DPP-4的可溶、活性形式,包含大部分的胞外结构域(残基39-766),其包括关键催化结构域。Ikushima H等人 Cell Immunol. 215(1):106-10 (2002)。

[0005] DPP-4基因敲除小鼠显示改善的对口服葡萄糖负荷的葡萄糖耐受性、增加的胰岛素和GLP-1活性、对饮食诱导的肥胖的抗性和高脂饮食之后增加的胰岛素敏感性。Marguet等人, Proc Natl Acad Sci U S A. 97(12):6874-9 (2000); Conarello等人, Proc Natl Acad Sci U S A. 100(11):6825-30 (2003)。除了其在代谢病症和血糖控制中的作用,DPP-4还牵涉控制免疫功能、细胞迁移、病毒进入细胞、癌症转移和炎症。参见,例如,Aytac等人, Curr Drug Targets Immune Endocr Metabol Disord 4(1):11-8 (2004)。

[0006] 最近,DPP-4表达经报道高度受白细胞介素-13 (IL-13)诱导。参见,例如,Zhang等人, Am J Respir Crit Care Med 189:A4875 (2014); Shiobara等人, Am J Respir Crit Care Med 189:A4239 (2014); Brightling等人, Am J Respir Crit Care Med 189:A6670 (2014); 2014年1月27日提交的美国临时申请号61/931,878,和2014年5月9日提交的美国临时申请号61/990,932,对于所有目的其各自通过引用以其整体结合到本文

中。IL-13为114个氨基酸的细胞因子,未修饰的分子量大约12 kDa。McKenzie, A. N.等人 J Immunol, 1993. 150:5436-44; Minty, A.等人 Nature, 1993. 362:248-50。IL-13水平已经显示在许多疾病或病症中与疾病严重性相关联,包括,但不限于,哮喘、慢性阻塞性肺病、特发性肺纤维化和特应性皮炎。例如,在过敏性炎症的哮喘和啮齿动物模型中,提高的IL-13水平已经报道与疾病严重性相关联(参见美国专利申请公开号2012-0052060,2012年3月1日出版,并通过引用以其整体结合到本文中)。

[0007] 慢性阻塞性肺病(COPD)包括具有不同程度慢性支气管炎、小气道疾病和肺气肿的患者群体,特征在于进行性不可逆肺功能下降,其对当前基于哮喘的疗法响应差。Zheng等人(J Clin Invest, 2000. 106:1081-93)证明在小鼠肺中IL-13的过表达引起肺气肿、提高的粘液产生和炎症,反映人COPD的方面。因此迹象为IL-13在COPD的发病机制中发挥重要作用,特别是在具有哮喘样特征的患者中。

[0008] IL-13也可在炎症性肠疾病的发病机制中发挥作用,并且已经与纤维化病况例如特发性肺纤维化(IPF)联系在一起。参见,例如,Jovani, M.,等人 Curr Drug Targets. 2013.12:1444-52; 和Rafii, R.,等人 J Thorac Dis. 2013. 1:48-73。

[0009] 特应性皮炎是一种常见的慢性炎症性皮肤病,其常常与其它特应性病况例如过敏性鼻炎和哮喘相关(Bieber, New England Journal of Medicine, 2008, 358: 1483-1494)。IL-13 mRNA的上调已经在亚急性和慢性特应性皮炎损伤中观察到(Tazawa等人, Arch. Dermatol. Res., 2004, 295:459-464; Purwar等人, J. Invest. Derm., 2006, 126, 1043-1051; Oh等人, J Immunol., 2011, 186:7232-42)。

[0010] 提高的DPP-4水平已经在哮喘、COPD和AD患者中观察到(参见,例如,美国临时申请号61/931,878,2014年1月27日提交;和美国临时申请号61/990,932,2014年5月9日提交,各自通过引用以其整体结合到本文中)。另外,在涉及哮喘患者的2B期临床研究中,在用IL-13抗体拮抗剂(tralokinumab)治疗的患者中高血清DPP-4水平预测提高的响应率,将DPP-4鉴定为IL-13介导的疾病或病症,包括IL-13介导的肺疾病或病症(例如,哮喘、IPF或COPD)或IL-13介导的慢性炎症性皮肤病疾病或病症(例如,特应性皮炎)的预测性生物标志。参见Brightling等人, Am J Respir Crit Care Med 189:A6670 (2014);和美国临时申请号61/931,878,2014年1月27日提交;和美国临时申请号61/990,932,2014年5月9日提交,其各自通过引用以其整体结合到本文中。因此,尽管已知增加的DPP-4水平与某些IL-13介导的疾病或病症相关联并且已知DPP-4血清水平预测患者对抗IL-13疗法的响应,但仍存在对测量患者(包括,但不限于,患有IL-13介导的疾病或病症的患者)中DPP-4的量和/或测定DPP-4水平变化的特异和灵敏的测定的需求。尽管存在市售可得的可用于测量患者血清DPP-4水平的试剂,但这些市售可得的试剂或试剂盒依赖于多克隆抗体,其不仅由于批次间差异引入测定变异,并且/或者不是非常灵敏。因此,仍存在对特异和灵敏的抗体、试剂和/或免疫测定的需求,以测量患者中DPP-4的量和/或测定DPP-4水平变化。

[0011] 发明概述

本公开内容提供抗二肽基肽酶-4 (DPP-4) 抗体,其可用在,例如,诊断测定中以测定受试者的DPP-4水平。本公开内容的示例性的抗DPP-4抗体包括:(1) 小鼠单克隆抗体m3B7.6,由在用于专利程序目的的关于微生物保藏的国际识别的布达佩斯条约的规定下于2015年1月8日保藏在美国典型培养物保藏中心(American Type Culture Collection) (ATCC),

10801 University Boulevard, Manassas, Va. 20110-2209并分配ATCC登录号PTA-121870的杂交瘤产生;(2) 小鼠单克隆抗体m5B7.7,由在用于专利程序目的的关于微生物保藏的国际识别的布达佩斯条约的规定下于2015年1月8日保藏在美国典型培养物保藏中心(American Type Culture Collection) (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209并分配ATCC登录号PTA-121871的杂交瘤产生;(3) 大鼠单克隆抗体R11A2.15,由在用于专利程序目的的关于微生物保藏的国际识别的布达佩斯条约的规定下于2015年1月8日保藏在美国典型培养物保藏中心(American Type Culture Collection) (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209并分配ATCC登录号PTA-121872的杂交瘤产生;和(4) 大鼠单克隆抗体R11A9.11,由在用于专利程序目的的关于微生物保藏的国际识别的布达佩斯条约的规定下于2015年1月8日保藏在美国典型培养物保藏中心(American Type Culture Collection) (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209并分配ATCC登录号PTA-121873的杂交瘤产生。

[0012] 在某些方面,本公开内容提供分离抗体或其抗原结合片段、变体或衍生物,或两种或更多种这样的抗体,其中所述一种或多种抗体竞争性抑制(1) 从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6,(2) 从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7,(3) 从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15,或(4) 从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11,或其任何组合的结合和/或结合与其相同的DPP-4表位。

[0013] 在某些方面,本公开内容提供结合DPP-4的分离抗体或其抗原结合片段、变体或衍生物,其包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH),和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL),其中分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

[0014] 在某些方面,本公开内容提供的抗体或其抗原结合片段、变体或衍生物可包含与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL。

[0015] 在某些方面,本公开内容提供如上所述的抗原结合抗体片段。在某些方面,所述抗体片段可为Fab片段、Fab'片段、F(ab')₂片段、Fv片段或单链抗体分子。

[0016] 本公开内容进一步提供在保藏号121870下保藏在ATCC的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤和/或其组合。在某些方面,本公开内容提供产抗体的细胞培养物,其包括在保藏号121870下保藏在ATCC的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏

号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤和/或其组合。在另一个方面,本公开内容提供由如本文所提供的杂交瘤或如本文所提供的产抗体的细胞培养物产生的分离抗体或其抗原结合片段、变体或衍生物。

[0017] 在某些方面,本公开内容所提供的抗体或其片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体,或其片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11),进一步包含与其融合的异源多肽。例如,在某些方面,所述异源多肽为稳定多肽、标签、标记或其组合。

[0018] 在某些方面,本公开内容提供的抗体或其片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体,或其片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11),缀合至异源部分。在某些方面,所述异源部分包括以下一种或多种:肽、蛋白、酶、脂质、异源抗体或其片段、可检测标记或聚乙二醇(PEG)。在某些方面,异源部分为,例如,生物素或钕螯合物。

[0019] 本公开内容进一步提供包含如本公开内容所提供的抗体或其片段、变体或衍生物,和/或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体,或其片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11)的组合物。在某些方面,所述组合物包含至少两种这样的抗体的组合。

[0020] 在另一个方面,本公开内容提供分离的多核苷酸,其包含编码如本文所提供的抗体或其亚基、片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物所产生的抗体或其片段,或其亚基、片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11)的核酸分子。本公开内容进一步提供包含如所提供的多核苷酸的载体。

[0021] 在某些方面,本公开内容提供包含两种或更多种编码如本公开内容所提供的抗体或其片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体或其片段,或其片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11)的核酸分子的组合物。在某些方面,所述两种或更多种核酸分子位于同一载体中。本公开内容进一步提供包含位于同一载体中的如所提供的两种或更多种核酸分子的载体。在某些方面,两种或更多种核酸分子位于至少两个单独的载体中。本公开内容进一步提供所述两个单独的载体。

[0022] 本公开内容进一步提供分离的宿主细胞,其包含提供的载体,或两个或更多个提供的载体。本公开内容进一步提供制备如本公开内容所提供的抗DPP-4抗体或其亚基、片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体,或其亚基、片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15和/或R11A9.11)的方法,其中所述方法包括(a) 培养所提供的宿主细胞,和(b) 回收抗体、其亚基、片段或衍生物。

[0023] 在另一个方面,本公开内容提供用于测量样品中的DPP-4水平的试剂盒,其中所述试剂盒包含如本公开内容所提供的抗体或其片段、变体或衍生物,或由本公开内容所提供的杂交瘤或细胞培养物产生的抗体,或其片段、变体或衍生物(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15或R11A9.11)。在某些方面,所述试剂盒包含至少两种这样的抗体或其片段、变体或衍生物。试剂盒可进一步包含固体支持物和/或检测试剂。在某些方面,至少两种抗体或其片段、变体或衍生物的一个可为捕获抗体或其片段、变体或衍生物,并且至少两种抗体或其片段、变体或衍生物的一个可为检测抗体或其片段、变体或衍生物。在某些方面,

检测抗体可包含可检测标记,例如,可检测标记可为生物素,并且试剂盒可包含检测试剂例如链霉亲和素-辣根过氧化物酶(HRP)缀合物和HRP的比色底物,或可检测标记可为钆螯合物。在某些方面:

捕获抗体可为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物;

捕获抗体可为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;和/或

捕获抗体可为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体可为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0024] 本公开内容进一步提供用于检测一个或多个样品中的DPP-4水平的免疫测定,其中所述免疫测定包括使用至少两种抗DPP-4抗体或其抗原结合片段、变体或衍生物,其中一种抗DPP-4抗体可为竞争性抑制从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗

体m5B7.7的结合和/或结合与其相同的DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物；并且其中一种抗DPP-4抗体包括竞争性抑制从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的结合和/或结合与其相同的DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物。

[0025] 在某些方面，所述免疫测定包括使用至少两种抗DPP-4抗体或其抗原结合片段、变体或衍生物，其中一种抗DPP-4抗体可为包含具有三个重链CDRs VHCDR1、VHCDR2和VHCDR3的VH和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的VL的分离抗体或其抗原结合片段、变体或衍生物，其中所述分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的CDRs相同；并且其中两种或更多种抗DPP-4抗体的一种可为包含具有三个重链CDRs VHCDR1、VHCDR2和VHCDR3的VH和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的VL的分离抗体或其抗原结合片段、变体或衍生物，其中所述分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

[0026] 在某些方面，免疫测定包括使用至少两种抗DPP-4抗体或其抗原结合片段、变体或衍生物，其中两种或更多种抗DPP-4抗体的一种可为包含与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物；并且其中两种或更多种抗DPP-4抗体的一种可为包含与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物。

[0027] 在某些方面，免疫测定可为夹心免疫测定，其包括使用附着在固体支持物上的第一个抗DPP-4“捕获”抗体或其抗原结合片段，和第二个抗DPP-4“检测”抗体或其抗原结合片段（包括，例如，抗体m3B7.6、m5B7.7、R11A2.15和/或R11A9.11）。例如，免疫测定可包括将捕获抗体或其抗原结合片段附着到固体支持物；在足以允许DPP-4（如果样品中存在的话）结合捕获抗体或其抗原结合片段的条件下施加检验样品或对照样品；在足以允许结合已经结合到捕获抗体或其抗原结合片段的DPP-4的条件下施加检测抗体或其抗原结合片段；和测量结合到DPP-4的检测抗体或其抗原结合片段的量。在某些方面，检测抗体可包含可检测标记，例如，可检测标记可为生物素，并且试剂盒可包含检测试剂例如链霉亲和素-辣根过氧化物酶（HRP）缀合物和HRP的比色底物，或可检测标记可为钆螯合物。

[0028] 在某些方面，免疫测定可包括使用捕获抗体和检测抗体，其中：

捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物，并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物；

捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗

体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物;

捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物;

捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;

捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物;

捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物;和/或

捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0029] 在某些方面,本公开内容提供测量从受试者获得的样品中的DPP-4的量的方法,其中所述方法包括使用提供的免疫测定或试剂盒,和/或所提供的抗体或其片段、变体或衍生物,或两种或更多种这样的抗体(包括,例如,抗体m3B7.6、m5B7.7、R11A2.15和/或R11A9.11)的组合测定样品。在某些方面,样品可为全血、血清、血浆、唾液、尿液、痰、支气管肺泡灌洗液、肺上皮细胞或鼻息肉或皮肤的一种或多种。在某些方面,受试者具有选自以下的疾病或病况:IL-13-介导的疾病或病症、肺疾病或病症和慢性炎症性皮肤病或病症。例如,疾病或病况可为哮喘、特应性哮喘、未经皮质类固醇治疗的哮喘(corticosteroid naive asthma)、慢性哮喘、皮质类固醇抵抗性哮喘、皮质类固醇难治性哮喘、抽烟引起的哮喘、不受皮质类固醇控制的哮喘、特发性肺纤维化(IPF)、慢性阻塞性肺病(COPD)、溃疡性结肠炎(UC)、特应性皮炎(AD)、过敏性鼻炎、慢性鼻窦炎、皮肤纤维化、过敏性接触性皮炎、湿疹和/或银屑病。在某些方面,样品可从受试者获得并且可提交用于测量样品中的DPP-4水平。在某些方面,受试者可为哮喘患者,并且从患者获取的样品可为血清。

[0030] 附图简述

图1:用于RIMMS方案的引流淋巴结注射位点。对人DPP-4特异的小鼠和大鼠单克隆抗体使用多个位点重复免疫(RIMMS)方案(Kilpatrick, K., 等人, Hybridoma 16:381-389 (2009))产生。简言之,两只Wistar大鼠分别在第0、2、5、7、9和13天用30、15、7、3、3和3 μ g CD26免疫,至如图1中所示的引流淋巴结位点A、B和C。小鼠免疫按照相同的时间表,但在每一时间点用一半的抗原量。

[0031] 图2A-D:抗DPP-4小鼠和大鼠抗体对人DPP-4特异,如使用直接结合ELISA所显示的。针对人DPP-4 (r-DPP-4-his) (A) 或人gp130-his (B) 的直接结合ELISA使用来自用人DPP-4免疫的两只大鼠(R9472和R9473)的免疫前(左侧)或第13天检验-出血(右侧)血清。第13天的大鼠血清特异性结合人DPP-4,而使用免疫前的血清未观察到特异性结合。针对人DPP-4 (r-DPP-4-his) (C) 或人gp130-his (D) 的直接结合ELISA使用来自用人DPP-4免疫的5只小鼠(ms9447、ms9448、ms9449、ms9450和ms9451)的免疫前(左侧)或第13天检验-出血(右侧)血清。第13天的小鼠血清特异性结合人DPP-4,而使用免疫前的血清未观察到特异性结合。

[0032] 图3A-B:在免疫测定中使用m3B7.6抗体(A)或m5B7.7 (B) 作为捕获抗体检测人DPP-4。A. 在夹心ELISA测定中,将DPP-4加入PBS或1%正常人血清(NHS)中,使用m3B7.6作为捕获抗体,R11A2.15、R11A9.11或对照抗体R222113 (R&D SystemsCatalog No. MAB1180) 作为检测抗体。在夹心ELISA测定中,m3B7.6 + R11A2.15或m3B7.6 + R11A9.11在测量人DPP-4中提供最好的灵敏度。B. 在夹心ELISA测定中,将DPP-4加入PBS或1%正常人血清(NHS)中,使用m5B7.7作为捕获抗体,R11A2.15、R11A9.11或对照抗体R222113 (R&D SystemsCatalog No. MAB1180) 作为检测抗体。在夹心ELISA测定中,m5B7.7 + R11A2.15或m5B7.7 + R11A9.11在测量人DPP-4中提供最好的灵敏度。这些结果,结合图2中报告的结果,证明抗体m3B7.6、m5B7.7、R11A2.15和R11A9.11检测内源和重组人DPP-4二者。另外,与检测DPP-4的抗体m3B7.6、m5B7.7、R11A2.15和R11A9.11相比,市售可得的抗体(R222113)在这些免疫测定中未能检测到DPP-4。

[0033] 图4A-E:小鼠单克隆抗体m3B7.6和m5B7.7互相竞争,而大鼠单克隆抗体R11A2.15和R11A9.11互相竞争。A. 用于测定如图4B-E中所示的m3B7.6、m5B7.7、R11A2.15和R11A9.11的相对结合特异性和竞争概况的OCTET测定的示意图。简言之,首先将检验抗体生物素化,然后在链霉亲和素生物传感器上在200 μ L中以20 μ g/ml的浓度捕获5 min。生物传感器然后用200 μ L PBS缓冲液洗涤1 min,然后用10 μ g/mL重组人DPP-4孵育5 min,并用200 μ L PBS缓冲液洗涤1 min。将竞争抗体与检验抗体以1:1比率和各自20 μ g/ml的终浓度混合并以200 μ L装载到生物传感器上5 min。显示额外结合检验抗体的竞争抗体被认为具有不同表位。否则其被认为是共享相同表位。B. 使用m3B7.6 (a) 作为检验抗体的OCTET测定结果。m5B7.7 (b) 显示与m3B7.6 (a) 相同的结合概况,而R11A2.15 (c) 和R11A9.11 (d) 显示与m3B7.6 (a) 不同的结合概况。基于这些结果,抗体m3B7.6和m5B7.7共享相同或重叠的表位。C. 使用m5B7.7 (b) 作为检验抗体的OCTET测定结果。m3B7.6 (a) 显示与m5B7.7 (b) 相同的结合概况,而R11A2.15 (c) 和R11A9.11 (d) 显示与m5B7.7 (b) 不同的结合概况。基于这些结果,抗体m5B7.7和m3B7.6共享相同或重叠的表位。D. 使用R11A2.15 (c) 作为检验抗体的OCTET测定结果。R11A9.11 (d) 显示与R11A2.15 (c) 相同的结合概况,而m3B7.6

(a) 和 m5B7.7 (b) 显示与 R11A2.15 (c) 不同的结合概况。基于这些结果, 抗体 R11A2.15 和 R11A9.11 共享相同或重叠的表位。E. 使用 R11A9.11 (d) 作为检验抗体的 OCTET 测定结果。R11A2.15 (c) 显示与 R11A9.11 (d) 相同的结合概况, 而 m3B7.6 (a) 和 m5B7.7 (b) 显示与 R11A9.11 (d) 不同的结合概况。基于这些结果, 抗体 R11A9.11 和 R11A2.15 共享相同或重叠的表位。

[0034] 发明详述

本公开内容提供抗 DPP-4 单克隆抗体、包含一种或多种这些抗体的测定试剂盒, 和使用一种或多种这些抗体用于检测和测量获自人类患者的样品中的 DPP-4 水平的稳健、灵敏和特异的免疫测定。

[0035] 在本说明书和所附的权利要求中, 单数形式“a”、“an”和“the”包括复数, 除非上下文另有清楚指示。术语“a”(或“an”), 以及术语“一个或多个”和“至少一个”在本文中可互换使用。

[0036] 此外, 本文中所使用的“和/或”理解为两个指定特征或组分各自的具体公开内容, 具有或不具有另一特征或组分。因此, 如在本文中在短语例如“A和/或B”中使用的, 术语“和/或”意在包括“A和B”、“A或B”、“A”(单独的)和“B”(单独的)。同样, 如在短语例如“A、B和/或C”中使用的, 术语“和/或”意在涵盖每一下列方面: A、B和C; A、B或C; A或C; A或B; B或C; A和C; A和B; B和C; A(单独的); B(单独的)和C(单独的)。

[0037] 无论哪一方面在本文中用语言“包括”描述, 也提供以“由……组成”和/或“基本由……组成”描述的其它类似方面。

[0038] 如贯穿说明书和权利要求与数值相连使用的, 术语“约”表示本领域技术人员熟悉和可接受的准确度区间。一般而言, 这样的准确度区间为 $\pm 10\%$ 。

[0039] 除非另有定义, 本文使用的所有技术和科学术语具有与本公开内容相关领域普通技术人员所通常理解的相同的含义。例如, Concise Dictionary of Biomedicine and Molecular Biology (生物医学和分子生物学简明词典), Juo, Pei-Show, 第2版, 2002, CRC Press; The Dictionary of Cell and Molecular Biology (细胞和分子生物学词典), 第3版, 1999, Academic Press; 和 Oxford Dictionary Of Biochemistry And Molecular Biology (生物化学和分子生物学牛津词典), 2000年修订, Oxford University Press, 为技术人员提供本公开内容中使用的许多术语的通用词典。

[0040] 单位、前缀和符号以其 Système International de Unites (SI) 接受的形式表示。数值范围包括定义范围的数字。除非另有说明, 氨基酸序列从左至右以氨基至羧基定向书写。本文提供的标题并非对公开内容各方面的限制, 其可作为整体参考说明书。相应地, 紧接下面定义的术语通过以其整体参考说明书更完整定义。

[0041] “分离”的物质、组合物、实体和/或物质、组合物或实体的任何组合, 或其任何语法变体, 例如, 分离的生物学材料, 为不在其天然环境中的物质。不需要特定的纯化水平。例如, 分离抗体为不在其天然或自然环境中产生或不位于其天然或自然环境中的抗体。重组产生的生物学材料被认为是如本文所公开的分离的, 在非天然细胞例如杂交瘤中产生的材料同样如此。物质, 例如, 生物学材料, 如果已经通过任何合适的技术分离、分级或部分或基本上纯化, 也认为是“分离的”。在某些方面, 分离的物质, 例如, 分离的生物学材料, 可为“非天然存在的”。

[0042] 如本文所使用的,术语“非天然存在的”物质、组合物、实体和/或物质、组合物或实体的任何组合,或其任何语法变体是一种条件术语,其明确排除,但仅排除,本领域普通技术人员很好地理解为“天然存在的”或由法官或行政机构例如美国专利商标局(United States Patent and Trademark Office)或司法机构确定或解释为在任何时间或可能在任何时间“天然存在”的物质、组合物、实体和/或物质、组合物或实体的任何组合的那些形式。例如,术语“非天然存在的抗体”明确排除天然存在的那些抗体,例如,将会天然存在于暴露于正常抗原刺激环境的小鼠的免疫系统中的抗体,或由行政机构,例如美国专利商标局,或司法机构,例如联邦法院,最终确定为“天然存在的”抗体。

[0043] “多核苷酸”或“核酸”,如本文中可互换使用的,指任何长度的核苷酸聚合物,并且包括DNA和RNA。核苷酸可为脱氧核糖核苷酸、核糖核苷酸、修饰的核苷酸或碱基,和/或其类似物,或可由DNA或RNA聚合酶引入聚合物中的任何底物。多核苷酸可包含修饰的核苷酸,例如,甲基化的核苷酸和其类似物。前述描述适用于本文提及的所有多核苷酸,包括RNA和DNA。

[0044] 如本文所使用的术语“表达”指基因产生生化物质,例如本文所提供的转运体分子的过程。该过程包括基因在细胞中功能存在的任何表现,包括,但不限于,基因敲减以及瞬时表达和稳定表达二者。其包括但不限于基因转录为一个或多个mRNAs,和这样的mRNAs翻译为一个或多个多肽。如果终产物为生化物质,表达包括产生该生化物质或任何前体。

[0045] “表达产物”可为核酸,例如基因转录产生的信使RNA,或多肽。本文所描述的表达产物进一步包括具有转录后修饰例如多腺苷酸化的核酸,或具有翻译后修饰例如甲基化、糖基化、添加脂质、与其它蛋白亚基缔合、蛋白水解切割等的多肽。

[0046] 术语“载体”或“表达载体”在本文中用于指用作媒介以引入宿主细胞和在宿主细胞中表达目的表达产物的载体。如本领域技术人员所已知的,这样的载体可容易地选自质粒、噬菌体、病毒和反转录病毒。一般而言,载体可包含选择标志、适当的限制位点以帮助克隆特定核酸和进入真核或原核细胞和/或在真核或原核细胞中复制的能力。载体的实例包括,但不限于,病毒载体、裸露DNA或RNA表达载体、质粒、粘粒或噬菌体载体、与阳离子缩合剂缔合的DNA或RNA表达载体、包裹在脂质体中的DNA或RNA表达载体,和某些真核细胞,例如生产细胞。

[0047] 术语“宿主细胞”指内含使用重组DNA技术构建的并且编码至少一个表达产物的载体的细胞。在描述从重组宿主分离表达产物的过程中,除非另有清楚说明,术语“细胞”和“细胞培养物”可互换使用,指表达产物的源,即从“细胞”回收表达产物意指从离心下来的全细胞回收,或从包含培养基和悬浮细胞二者的细胞培养物回收。

[0048] 术语“多肽”、“肽”和“蛋白质”在本文中可互换使用,指任何长度的氨基酸聚合物。聚合物可为线性或分支的,其可包含修饰的氨基酸,并且非氨基酸可使其中断。该术语还涵盖已经天然或干预修饰的氨基酸聚合物;例如,二硫键形成、糖基化、脂质化、乙酰化、磷酸化或任何其它操作或修饰,例如与标记元件缀合。定义中还包括,例如,包含一个或多个氨基酸类似物(包括,例如,非天然氨基酸等)以及本领域已知的其它修饰的多肽。应理解,由于本公开内容的多肽基于抗体,在某些实施方案中,多肽可作为单链或缔合链存在。

[0049] 如本文所使用的术语“DPP-4”指由DPP-4基因(cDNA: SEQ ID NO: 7)编码的二肽基肽酶IV蛋白(EC 3.4.14.5; Uniprot: P27487 (膜结合形式;SEQ ID NO: 5;可溶形式:

SEQ ID NO: 6))。DPP-4也称为DPP-IV、腺苷脱氨酶复合蛋白2或CD26 (分化簇26)。DPP-4与吸引素、FAP、DPP8和DPP9有关。DPP-4为高度保守的多功能II型跨膜糖蛋白,其存在于循环中(血浆)和几种细胞类型表面上,包括上皮、内皮和淋巴细胞。DPP-4为丝氨酸蛋白酶家族的部分,牵涉T细胞共刺激、趋化因子生物学、II型糖尿病和肿瘤生物学(Zhong等人, *Atherosclerosis* 2013;226:305-314)。DPP-4的内源底物包括广泛不同的含脯氨酸肽,例如生长因子、趋化因子、神经肽和血管活性肽(Gorrell, M., *Clin. Sci.* 108, 277-292, 2005; McIntosh, C. H. S., 等人 *Int. J. Biochem. Cell Biol.* 38, 860-872, 2006)。Giovannini-Chami提示了DPP-4在炎症性呼吸疾病如哮喘中的作用(Giovannini-Chami等人, *European Respiratory Journal*. 2012 May;39(5):1197-205),其发现具有尘螨过敏性鼻炎的儿童的上皮中提高的DPP-4转录(和其它Th2标记基因)与不受控的哮喘相关。术语DPP-4也包括其片段、变体(例如,本领域已知的K1R、V7I、S437I、T557I、D663E变体)和衍生物(例如,DPP-4蛋白的糖基化或无糖基化蛋白形式,或该蛋白的其它化学修饰形式)。

[0050] 术语“水平”或“量”,例如,如在“DPP-4水平”或“DPP-4的量”中,指使用用于检测生物样品中DPP-4 (蛋白表达)的存在或表达的分析方法做出的测量,其指示生物样品中DPP-4的或DPP-4对应的存在、不存在、绝对量或浓度、相对量或浓度、滴度、表达水平、测量水平的比率等。“值”或“水平”的准确性质取决于用以检测DPP-4的具体分析方法(例如,如本文中所提供的免疫测定)的具体设计和元件。参见,例如,U.S. 2010/00221752。

[0051] 如本文关于DPP-4所使用的,术语“提高的DPP-4”、“高DPP-4”、“提高的DPP-4水平”或“高DPP-4水平”指比正常水平或范围高的生物样品(例如,血清)中的水平。DPP-4的正常水平或范围根据标准实践定义。因此,可将在特定生物样品中测量的水平与相似正常样品中测定的水平或水平范围比较。当DPP-4在检验样品中以比正常样品中更高的水平或范围存在时,DPP-4的水平称为提高的。

[0052] 如本文所使用的,术语“抗体”(或其片段、变体或衍生物)指在B细胞产生的典型抗体的背景下,至少能够结合抗原的最小抗体部分,例如,至少重链的可变结构域(VH)和轻链的可变结构域(VL)。脊椎动物系统中基本的抗体结构已相对较好地理解。参见,例如,Harlow等人, *Antibodies: A Laboratory Manual* (抗体:实验室手册), (Cold Spring Harbor Laboratory Press, 第2版. 1988)。除非另外指出,抗体“片段”、“变体”或“衍生物”指抗原结合“片段”、“变体”或“衍生物”。

[0053] 当关于如本文所公开的抗体时,术语“片段”、“变体”、“衍生物”和“类似物”可包括保留参考抗体的至少一些活性,例如抗原结合活性,但结构上不同的任何抗体。抗体的片段包括,例如,Fab、Fab'和F(ab')₂、Fd、Fvs、单链Fvs (scFv)、单链抗体、二硫键连接的Fvs (sdFv)片段。变体包括如上所述的片段,并且还包括由于氨基酸置换、缺失或插入引起的具有改变的氨基酸序列(例如,在可变结构域中)的抗体。变体可自发发生或有意构建。有意构建的变体可使用本领域已知的突变技术产生。变体抗体可包括保守或非保守氨基酸置换、缺失或插入。变化仅受抗体保留参考抗体功能的约束限制,例如,与参考抗体结合相同表位,或竞争性抑制参考抗体。衍生物为已经改变以便呈现天然抗体上不存在的额外特征的抗体。实例包括包含抗体的抗原结合结构域的融合蛋白或缀合的抗体。“衍生的”抗体可还包含通过功能侧基反应化学衍生的一个或多个氨基酸。“衍生物”还包括包含一个或多个标准或合成的20种标准氨基酸的氨基酸衍生物的那些抗体。例如,4-羟脯氨酸可置换脯氨酸;

5-羟赖氨酸可置换赖氨酸,3-甲基组氨酸可置换组氨酸;高丝氨酸可置换丝氨酸;和鸟氨酸可置换赖氨酸。

[0054] 轻链和重链二者均分为结构和功能同源区。术语“恒定”和“可变”功能上使用。在这方面,将会理解轻链(VL)和重链(VH)部分二者的可变结构域决定抗原识别和特异性。相反,轻链(CL)和重链(CH1、CH2或CH3)的恒定结构域赋予重要的生物学性质,例如分泌、经胎盘迁移、Fc受体结合、互补结合等。

[0055] 如上文所指示的,可变区允许结合分子选择性识别和特异性结合抗原上的表位。即,抗体的VL结构域和VH结构域,或互补决定区(CDRs)子集,联合形成定义三维抗原结合位点的可变区。该四元结合分子结构形成在Y的每一臂的末端存在的抗原结合位点。更具体地,抗原结合位点由每一VH和VL链上的三个CDRs定义。

[0056] 在抗体中,每一抗原结合结构域中存在的6个“互补决定区”或“CDRs”为短的非连续的氨基酸序列,随着抗体在水性环境中呈现其三维构象,其特别定位以形成抗原结合结构域。抗原结合结构域中的剩余氨基酸,称为“框架”区,显示更少的分子间可变性。框架区主要采用 β 折叠构象并且CDRs形成环,其连接 β 折叠结构,并且在一些情况下形成 β 折叠结构的一部分。因此,框架区作用于形成提供通过链间非共价相互作用以正确定向放置CDRs的支架。通过放置的CDRs形成的抗原结合结构域定义与免疫反应性抗原上的表位互补的表面。该互补表面促进抗体与其同源表位的非共价结合。对于任何给定的重链或轻链可变区,本领域普通技术人员可容易地鉴定分别包含CDRs和框架区的氨基酸,因为其已被精确定义(参见,“Sequences of Proteins of Immunological Interest (免疫相关蛋白序列),”Kabat, E.,等人, U.S. Department of Health and Human Services, (1983); 以及 Chothia和Lesk, J. Mol. Biol., 196:901-917 (1987),通过引用以其整体结合到本文中)。

[0057] 在其中术语的两个或更多个定义在本领域中使用和/或接受的情况下,如本文所使用的术语的定义意在包括所有这样的含义,除非明确说明相反。一个具体实例为使用术语“互补决定区”(“CDR”)描述重链和轻链多肽二者的可变区中存在的非连续抗原结合位点。该特定区域已经由Kabat等人, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest (免疫相关蛋白序列)” (1983)和由Chothia等人, J. Mol. Biol. 196:901-917 (1987)描述,其通过引用结合到本文中,其中该定义包括当相互比较时氨基酸残基的重叠或子集。虽然如此,任一定义指抗体或其变体的CDR的应用意在在如本文所定义和使用的术语的范围中。

[0058] 抗体或其抗原结合片段、变体或衍生物包括,但不限于,多克隆、单克隆、人、人源化或嵌合抗体,单链抗体,表位结合片段,例如Fab、Fab'和F(ab')₂,Fd、Fvs、单链Fvs(scFv)、单链抗体、二硫键连接的Fvs(sdFv)、包含VL或VH结构域的片段、Fab表达文库产生的片段。ScFv分子为本领域所已知并且在,例如,美国专利5,892,019中描述。本公开内容涵盖的免疫球蛋白或抗体分子可为任何类型(例如,IgG、IgE、IgM、IgD、IgA和IgY)、种类(例如,IgG1、IgG2、IgG3、IgG4、IgA1和IgA2)或亚类的免疫球蛋白分子。

[0059] “特异性结合”一般指抗体或其片段、变体或衍生物经由其抗原结合结构域结合表位,并且结合需要抗原结合结构域和表位之间的一些互补性。根据该定义,当经由其抗原结合结构域结合表位比结合随机不相关的表位更容易时,抗体被称为“特异性结合”表位。

[0060] 如果抗体或其片段、变体或衍生物优先结合表位至其在某种程度上阻断参考抗体或抗原结合片段与表位结合的程度,那么称其为竞争性抑制参考抗体或抗原结合片段与给定表位结合。竞争性抑制可通过本领域已知的任何方法测定,例如,竞争ELISA测定。结合分子可称为竞争性抑制参考抗体或抗原结合片段与给定表位结合至少90%、至少80%、至少70%、至少60%或至少50%。

[0061] 本文所公开的抗体或其抗原结合片段、变体或衍生物可依据其识别或特异性结合的抗原的表位或部分,例如靶多糖描述或定义。例如,与本公开内容中提供的抗体的抗原结合结构域特异性相互作用的人DPP-4的部分为“表位”。

[0062] 如本文所使用的,术语“IL-13介导的疾病或病症”指在具有病症的受试者中异常IL-13水平(单独或与其它介质一起)引起、恶化、相关或延长的任何病理学。IL-13介导的疾病或病症的非限制性实例包括哮喘、特发性肺纤维化(IPF)、慢性阻塞性肺病(COPD)、溃疡性结肠炎(UC)、特应性皮炎(AD)、过敏性鼻炎或慢性鼻窦炎。

[0063] 如本文所使用的,术语“肺疾病或病症”指至少部分影响肺或呼吸系统的任何病理学。非限制性实例包括哮喘、特应性哮喘、未经皮质类固醇治疗的哮喘、慢性哮喘、皮质类固醇抵抗性哮喘、皮质类固醇难治性哮喘、抽烟引起的哮喘、不受皮质类固醇控制的哮喘、IPF、COPD、过敏性鼻炎或慢性鼻窦炎。在某些方面,肺疾病或病症为IL-13介导的。

[0064] 如本文所使用的,术语“慢性炎症性皮肤病疾病或病症”指至少部分影响皮肤的任何病理学。非限制性实例包括特应性皮炎、皮肤纤维化、过敏性接触性皮炎、湿疹或银屑病。在某些方面,慢性炎症性皮肤病疾病或病症为IL-13介导的。

[0065] 术语“哮喘”指作为可逆的气道阻塞和/或支气管高反应性存在的疾病,在一些情况下与潜在的炎症有关。哮喘的实例包括过敏性哮喘、特应性哮喘、未经皮质类固醇治疗的哮喘、慢性哮喘、皮质类固醇抵抗性哮喘、皮质类固醇难治性哮喘、抽烟引起的哮喘、不受皮质类固醇控制的哮喘和如,例如,Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma, National Asthma Education and Prevention Program (2007) (“NAEPP Guidelines”) (通过引用以其整体结合到本文中)中所提及的其它哮喘。

[0066] 如本文所使用的术语“COPD”指慢性阻塞性肺病。术语“COPD”包括两种主要病况:肺气肿和慢性阻塞性支气管炎。

[0067] 术语“特发性肺纤维化”(IPF)指以肺的进展性瘢痕或纤维化为特征的疾病。其为特定类型的间质性肺病,其中肺泡逐渐被纤维组织替代。伴随IPF,进展性瘢痕形成导致正常薄和柔韧的组织增厚和变得坚硬,使得肺更难扩张,防止氧容易地进入血流。参见,例如,Am. J. Respir. Crit. Care Med. 2000. 161:646-664。

[0068] 术语“溃疡性结肠炎”(UC)指影响结肠直肠的胃肠(GI)道的炎症性病症,包括特征性溃疡或开放性溃疡。UC为间歇性疾病,具有恶化的症状周期和相对无症状的周期。活动疾病的症状包括长期(数周)持续的混有血液的恒定腹泻、体重减轻、从GI道慢性失血、贫血、腹痛和轻微不适至痛苦的肠运动或伴随肠运动的痛苦的腹部绞痛。参见,例如,Danese,等人 N Engl J Med. 2011 365(18):1713-25。

[0069] 如本文所使用的,术语“特应性皮炎”指慢性炎症性、复发性、非接触传染的和发痒的皮肤病症,其常常与其它特应性疾病例如过敏性鼻炎和哮喘相关(Bieber, New England

Journal of Medicine, 2008, 358: 1483-1494)。术语“特应性皮炎”等于“神经性皮炎”、“特应性湿疹”或“内源性湿疹”。特异性皮炎的具体形式,从其发生的位置或从其出现或从引起其的应激因素得名,根据本公开内容也被术语“特应性皮炎”包括。这些包括,但不限于,屈侧性湿疹(eczema flexurarum)、eczema mulluscatum、eczema verrucatum、种痘后湿疹、eczema dyskoides、dyshydrotic eczema、微生物性湿疹、钱币状湿疹、seborrhoeic eczema和其它形式的湿疹;口周皮炎和眶周皮炎。如本文所使用的,术语特应性皮炎还包括频繁发生的细菌二次感染(例如由于金黄色葡萄球菌感染引起的那些)、脓皮病例如接触性脓疱病及其衍生病,以及毛囊性须疮或病毒二次感染。IL-13牵涉疾病的发病机制并且是重要的体内诱导物。参见,例如,Oh等人, J. Immunol. 186:7232-42 (2011); Tazawa等人, Arch. Dermatol. Res. 295:459-464 (2004); Metwally等人 Egypt J. Immunol. 11:171-7 (2004)。

[0070] 如本文所使用的术语“受试者”或“患者”指任何受试者,特别是哺乳动物受试者,包括任何人类或非人动物。术语“非人动物”包括所有脊椎动物,例如,哺乳动物和非哺乳动物,例如,非人灵长类、绵羊、狗、猫、马、牛、熊、鸡、两栖类和爬行类等。

[0071] 在本公开内容的一些方面,受试者为幼稚受试者。幼稚受试者为尚未给予治疗,例如治疗剂的受试者。在一些方面,幼稚受试者在诊断为具有IL-13-介导的疾病或病症,例如哮喘、IFP、COPD、AD或UC之前尚未用治疗剂治疗。在另一个方面,受试者在诊断为具有IL-13-介导的疾病或病症之前已经接受治疗和/或一个或多个剂量的治疗剂(例如,能够调整与IL-13-介导的疾病或病症、肺疾病或病症、慢性炎症性皮肤病疾病或病症或炎症性肠疾病或病症有关的炎性应答的治疗剂)。

[0072] 如本文所使用的,术语“IL-13拮抗剂”指可影响IL-13在体外或体内的表达、活性或半衰期,或具有IL-13-介导的疾病或病症的受试者中IL-13引起或恶化的症状、病理学或后遗症的任何药剂。IL-13拮抗剂可为如下定义的任何“治疗剂”,其可直接或间接抑制、减少或中和IL-13活性,抑制或减少IL-13表达、减少IL-13半衰期,或可防止由于IL-13引起的症状的恶化。在某些方面,IL-13拮抗剂为抗IL-13单克隆抗体,例如,tralokinumab (SEQ ID NOs 3和4),或2012年3月1日出版的美国专利申请公开号2012-0052060 (通过引用以其整体结合到本文中)中描述的其它抗IL-13单克隆抗体。在其它方面,IL-13拮抗剂包括,但不限于:(a) 抗人IL-13抗体,例如,来金珠单抗(Lebrikizumab) (SEQ ID NOs 1和2) (MILR1444A / RG3637, Roche/Genentech)、ABT-308 (Abbott)、GSK679586 (GlaxoSmithKline)或QAX576 (Novartis);(b) 抗人IL-13R α 1抗体,例如,Merck MK6105;(c) IL-13-毒素缀合物,例如IL-13-PE38QQR (NeoPharm, Inc.);(d) IL-4突变蛋白 Aerovant[™] (Aerovance, Inc.);(e) 抗IL-4R α 抗体例如dupilumab/REGN668 (Regeneron);(f) 定向于IL-4R α 的双链寡核苷酸例如AIR645 (Isis);或(g) IL-4 / IL-13双特异性抗体例如GSK2434735 (Glaxo SmithKline)。

[0073] 如本文所使用的术语“治疗剂”指给予受试者以产生期需的,通常有益的效果的任何治疗活性物质。术语治疗剂包括,例如,典型的低分子量治疗剂,通常称为小分子药物,和生物制品,包括但不限于:抗体或其活性片段、肽、脂质、蛋白药物、蛋白缀合物药物、酶、寡核苷酸、核酶、遗传物质、朊病毒、病毒、细菌和真核细胞。治疗剂也可作为前药,当给予受试者时其代谢为所需的治疗活性物质。在一些方面,治疗剂为预防剂。另外,治疗剂可为药学上

配制的。治疗剂也可作为放射性同位素或通过一些其它形式的能量例如光或超声能量,或通过可全身给予的其它循环分子活化的药剂。

[0074] 如本文所使用的术语“样品”包括从受试者获得的任何生物学流体或组织,例如全血、血清、肌肉、唾液。样品包括任何生物学流体或组织,例如全血、血清、肌肉、唾液、尿液、滑液、骨髓、脑脊液、鼻分泌物、痰、羊水、支气管肺泡灌洗液、肺组织、外周血单核细胞、总白血细胞、淋巴结细胞、脾细胞、扁桃体细胞或皮肤。在一些特定方面,样品为血液或其级分、肌肉、皮肤或其组合。样品可通过本领域已知的任何手段获得。

[0075] 为了应用本公开内容的方法和系统,来自患者的样品可在任何时间获得。在一些情况下,连续的样品可在治疗开始后或治疗停止后从患者获得。样品可,例如,由医疗提供者(例如,医生)或医疗福利提供者要求,通过相同或不同的医疗提供者(例如,护士、医院)或临床实验室获得和/或处理,并且处理后,结果可转发到又一个医疗提供者、医疗福利提供者或患者。相似地,测量/测定一个或多个分数、分数之间比较、评估分数和治疗决定可由一个或多个医疗提供者、医疗福利提供者和/或临床实验室进行。

[0076] 如本文所使用的,术语“医疗提供者”指直接交互和管理活受试者,例如人类患者的个体或机构。医疗提供者的非限制性实例包括医生、护士、技术人员、治疗者、药剂师、咨询者、替代医学实践者、医疗设施、医生办公室、医院、急诊室、诊所、急诊中心、替代医学门诊/设施和提供与全部或任何部分患者健康状态有关的一般和/或专门治疗、评估、维护、疗法、药物和/或劝告(包括但不限于一般药物、专门药物、手术和/或任何其它类型的治疗、评估、维护、疗法、药物和/或劝告)的任何其它实体。

[0077] 如本文所使用的,术语“临床实验室”指用于检查或处理从活受试者例如人类来源的材料的设施。处理的非限制性实例包括为了提供信息,例如为诊断、预防或治疗活受试者例如人类任何疾病或损伤,或评估活受试者例如人类的健康的目的,从人体来源的材料的生物学、生物化学、血清学、化学、免疫血液学、血液学、生物物理学、细胞学、病理学、遗传学或其它检查。这些检查还可包括收集或另外获得样品、准备、测定、测量或另外描述活受试者例如人类体内或从活受试者例如人类的身体获得的样品中各种物质的存在或不存在的程序。

[0078] 如本文所使用的,术语“医疗福利提供者”包括提供、呈现、提供、全部或部分支付或另外关于给予患者一种或多种医疗福利、福利计划、健康保险和/或医疗费用账户程序的个体当事人、机构或组。

[0079] 在一些方面,医疗提供者可管理或指示另一个医疗提供者给予或使用任何本文公开的免疫测定或试剂盒以测量DPP-4。医疗提供者可实现或指示另一个医疗提供者或患者进行下列行动:获得样品、处理样品、提交样品、接收样品、转移样品、分析或测量样品、定量样品、提供分析/测量/定量样品后获得的结果、接收分析/测量/定量样品后获得的结果、比较/评分分析/测量/定量一个或多个样品后获得的结果、从一个或多个样品提供比较/分数、从一个或多个样品获得比较/分数、给予治疗(例如治疗IL-13-介导的疾病或病症例如哮喘、IPF、COPD、AD或UC的治疗剂)、开始给予治疗、停止给予治疗、继续给予治疗、临时中断给予治疗、增加给予的治疗剂的量、减少给予的治疗剂的量、继续给予一定量的治疗剂、增加给予治疗剂的频率、减少给予治疗剂的频率、在一种治疗剂上维持相同的给剂频率、用至少另一种治疗或治疗剂替代治疗或治疗剂、组合治疗或治疗剂与至少另一种治疗或额外的

治疗剂。

[0080] 在一些方面,医疗福利提供者可授权或拒绝,例如,获得样品、处理样品、提交样品、接收样品、转移样品、分析或测量样品、定量样品、提供分析/测量/定量样品后获得的结果、转移分析/测量/定量样品后获得的结果、比较/评分分析/测量/定量一个或多个样品后获得的结果、转移来自一个或多个样品的比较/分数、给予治疗或治疗剂、开始给予治疗或治疗剂、停止给予治疗或治疗剂、继续给予治疗或治疗剂、临时中断给予治疗或治疗剂、增加给予的治疗剂的量、减少给予的治疗剂的量、继续给予一定量的治疗剂、增加给予治疗剂的频率、减少给予治疗剂的频率、在一种治疗剂上维持相同的给剂频率、用至少另一种治疗或治疗剂替代治疗或治疗剂,或组合治疗或治疗剂与至少另一种治疗或额外的治疗剂。

[0081] 另外,医疗福利提供者可,例如,授权或拒绝治疗处方、授权或拒绝治疗覆盖、授权或拒绝偿付治疗费用、决定或拒绝治疗资格等。

[0082] 在一些方面,临床实验室可,例如,收集或获得样品、处理样品、提交样品、接收样品、转移样品、分析或测量样品、定量样品、提供分析/测量/定量样品后获得的结果、接收分析/测量/定量样品后获得的结果、比较/评分分析/测量/定量一个或多个样品后获得的结果、从一个或多个样品提供比较/分数、从一个或多个样品获得比较/分数,或其它相关活动。

[0083] DPP-4作为生物标志

提高的DPP-4水平已经在哮喘、COPD和AD患者中观察到(参见,例如,美国临时申请号61/931,878,2014年1月27日提交;和美国临时申请号61/990,932,2014年5月9日提交,各自通过引用以其整体结合到本文中)。另外,在哮喘患者的2B期临床研究中,在用IL-13抗体拮抗剂(tralokinumab)治疗的患者中高血清DPP-4水平预测提高的响应率,将DPP-4鉴定为IL-13介导的疾病或病症包括IL-13介导的肺疾病或病症(例如,哮喘、IPF或COPD)或IL-13介导的慢性炎症性皮肤病疾病或病症(例如,特应性皮炎)的预测性生物标志。参见Brightling等人, Am J Respir Crit Care Med 189:A6670 (2014); 和美国临时申请号61/931,878,2014年1月27日提交;和美国临时申请号61/990,932,2014年5月9日提交,其各自通过引用以其整体结合到本文中。

[0084] 因此,在具有哮喘、IPF、COPD、AD和UC,以及其它炎症性疾病、肺部疾病或病症或慢性炎症性皮肤病疾病或病症的患者中,提高的DPP-4水平可用于鉴定可获益于特定治疗,包括但不限于中和IL-13活性的治疗的那些患者。提供了可用于测量患者的DPP-4水平的抗DPP-4抗体和使用本文公开的抗DPP-4抗体的免疫测定和试剂盒。

[0085] 抗DPP-4抗体

本公开内容提供分离的抗DPP-4抗体和其抗原结合片段。在某些方面,本文提供的抗DPP-4抗体和抗原结合片段可结合人DPP-4。

[0086] 具体地,本公开内容提供结合人DPP-4的两种小鼠单克隆抗体和两种大鼠单克隆抗体。这些抗体通过标准杂交瘤技术产生,并且产生这些抗体的杂交瘤已经在2015年1月8日在布达佩斯条约下保藏于美国典型培养物保藏中心, Manassas, VA。小鼠抗DPP-4抗体在本文中称为m3B7.6和m5B7.7,大鼠抗DPP-4抗体在本文中称为R11A2.15和R11A9.11。还提供这些抗体的抗原结合片段、变体和/或衍生物。还提供在其结合相同表位或其能够竞争性抑制m3B7.6、m5B7.7、R11A2.15和R11A9.11中的一个或多个方面与这些抗体相关的抗体。小

鼠单克隆抗体m3B7.6从在保藏号PTA-121870下保藏在美国典型培养物保藏中心, Manassas, VA (ATCC)的杂交瘤产生,小鼠单克隆抗体m5B7.7从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生,大鼠单克隆抗体R11A2.15从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生,和大鼠单克隆抗体R11A9.11从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生。

[0087] 在某些方面,提供分离抗体或其抗原结合片段、变体或衍生物,其中抗体与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11结合相同DPP-4表位。

[0088] 在某些方面,提供分离抗体或其抗原结合片段、变体或衍生物,其中抗体竞争性抑制从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11结合DPP-4,例如,人DPP-4。例如,单克隆抗体m3B7.6和m5B7.7能够相互竞争性抑制结合人DPP-4,单克隆抗体R11A9.11和R11A2.15能够相互竞争性抑制结合人DPP-4。

[0089] 在某些方面,提供分离的抗DPP-4抗体或其片段、变体或衍生物,其中抗体包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL),其中分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

[0090] 在某些方面,提供分离的抗DPP-4抗体或其片段、变体或衍生物,其中抗体包含与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL。

[0091] 当从一个或多个保藏的杂交瘤获得一种或多种抗体时,本领域普通技术人员可分离、克隆和测序表达的抗体,以确定VH、VL和CDR区域,而无过度实验。

[0092] 在某些方面,提供杂交瘤,其中杂交瘤包括在保藏号121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤或其组合。

[0093] 在某些方面,提供产抗体的细胞培养物,其中所述细胞培养物可用于表达如本文所提供的抗DPP-4抗体或其片段、变体或衍生物。在某些方面,细胞培养物包括在保藏号

121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤、在保藏号121871下保藏在ATCC的杂交瘤、在保藏号121872下保藏在ATCC的杂交瘤、在保藏号121873下保藏在ATCC的杂交瘤,或其组合。

[0094] 本公开内容所提供的任何抗DPP-4抗体或其片段、变体或衍生物可进一步包含额外的多肽,例如,信号肽以定向分泌。另外,本公开内容提供的抗DPP-4抗体或其片段、变体或衍生物可为,例如,融合多肽、Fab片段、scFvs或其它衍生物,如本文所描述。

[0095] 在某些方面,本公开内容所提供的抗DPP-4抗体或其片段、变体或衍生物可为融合蛋白的部分,即,抗体或其抗原结合片段可与异源多肽融合。术语“异源多肽”在本文中用于指从与抗DPP-4抗体或其片段、变体或衍生物不同的实体衍生的多肽。在非限制性实例中,与抗体或其抗原结合片段、变体或衍生物融合的“异源多肽”可衍生自相同物种的非免疫球蛋白多肽,或免疫球蛋白或非免疫球蛋白异源多肽。在一些方面,异源多肽可为,例如,稳定多肽、标签、标记或其组合。

[0096] 在某些方面,本公开内容提供的抗DPP-4抗体或其片段、变体或衍生物可包含异源氨基酸序列或与抗体天然不关联的一个或多个其它部分(例如,肽、蛋白、酶、脂质、异源抗体或其片段、变体或衍生物、可检测标记、聚乙二醇(PEG)或两个或更多个任何所述试剂的组合)。在进一步的方面,本公开内容提供的抗DPP-4抗体或其片段、变体或衍生物可包含可检测标记,其选自酶、荧光标记、化学发光标记、生物发光标记、放射性标记或两种或更多种任何所述可检测标记的组合。在某些方面,可检测标记为生物素,其可与缀合至,例如,酶,例如,辣根过氧化物酶(HRP)的链霉亲和素相互作用。在某些方面,可检测标记为钆螯合物,其可在暴露于电流时发光。其它可检测的标记为本领域普通技术人员所熟知。

[0097] 本文还提供包含一种或多种如上文所指出的抗DPP-4抗体或其片段的组合物。在某些方面,组合物包含“捕获”抗体和“检测”抗体,如本文它处所述。如本文所提供的组合物包括但不限于缓冲液、载体和防腐剂。防腐剂、稳定剂、缓冲液、抗氧化剂和/或其它添加剂可包括缓冲液例如磷酸盐、柠檬酸盐和其它有机酸;抗氧化剂,例如抗坏血酸和甲硫氨酸;防腐剂例如十八烷基二甲基苄基氯化铵;氯化六甲双铵;苯扎氯铵;苄索氯铵;苯酚、丁醇或苯甲醇;烷基对羟基苯甲酸酯类,例如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯;邻苯二酚;间苯二酚;环己醇;3'-戊醇和间甲酚;低分子量多肽;蛋白质,例如血清白蛋白、明胶或免疫球蛋白;亲水聚合物,例如聚乙烯吡咯烷酮;氨基酸,例如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其它糖类包括葡萄糖、甘露糖或糊精;螯合剂,例如EDTA;糖类,例如蔗糖、甘露醇、海藻糖或山梨醇;成盐反离子,例如钠;金属络合物(例如Zn-蛋白质络合物)和/或非离子表面活性剂,例如TWEEN™、PLURONIC™或聚乙二醇(PEG)。本文所提供的组合物可在单一的小瓶或容器中混合,或可在两个或更多个小瓶或容器中或作为试剂盒的部分提供,如本文它处所述。

[0098] 多核苷酸

本公开提供编码本文所提供的任何抗DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的多核苷酸。在某些方面提供分离的多核苷酸或包含两种或更多种多核苷酸的多核苷酸组合物,其单独或共同编码本文所提供的抗DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物。

[0099] 在某些方面,本公开提供分离的多核苷酸,其包含编码抗DPP-4抗体或其亚基(例

如,重链或轻链)、片段、变体或衍生物的核酸,其中抗体包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL),其中分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在美国典藏培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

[0100] 本公开还提供包含编码抗-DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的核酸的分离的多核苷酸,其中抗体包含与从在保藏号PTA-121870下保藏在美国典藏培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL。

[0101] 本公开进一步提供包含两种或更多种单独或共同编码本文所提供的抗-DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的多核苷酸的组合物。

[0102] 在某些方面,提供一种载体或两种或更多种载体,以帮助本文所提供的抗-DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的展示、筛选、分离、克隆和/或表达。在某些方面,一种或多种载体为表达载体。

[0103] 在某些方面,多核苷酸组合物的两个或更多个核酸分子可位于相同的载体中。在某些方面,多核苷酸组合物的两个或更多个核酸分子可位于至少两个单独的载体中。

[0104] 表达载体用于表达编码本文所提供的抗DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的分离的多核苷酸。重组表达载体为可复制的DNA构建体,其具有编码转运体分子的多肽链的合成的或cDNA衍生的DNA片段,与从哺乳动物、微生物、病毒或昆虫基因衍生的合适的转录或翻译调节元件操作性连接。转录单元一般包括以下序列的装配:(1)在基因表达中具有调节作用的一个或多个遗传元件,例如,转录启动子或增强子,(2)结构或编码序列,其转录为mRNA并翻译为蛋白,和(3)适当的转录和翻译起始和终止序列,如下文详细描述。这样的调节元件可包括操纵子序列以控制转录。可额外地并入通常由复制起点赋予的在宿主中复制的能力,和帮助识别转化子的选择基因。

[0105] 在某些方面提供分离的多核苷酸或包含两个或更多个分离的多核苷酸的组合物,包括与启动子操作性连接的核酸分子,或与两个或更多个启动子操作性连接的两个或更多个核酸分子,其中启动子可为相同的或不同的。

[0106] 当核酸区域彼此功能相关时,其为“操作性连接的”。例如,如果其作为参与多肽分泌的前体表达,信号肽的DNA(分泌前导序列)与多肽的DNA操作性连接;如果其控制序列的转录,启动子与编码序列操作性连接;或如果其放置为允许翻译,核糖体结合位点与编码序列操作性连接。

[0107] 表达控制序列和表达载体的选择取决于宿主的选择。广泛不同的表达宿主/载体组合可使用。用于真核宿主的有用的表达载体包括,例如,包含来自SV40、牛乳头瘤病毒、腺病毒和巨细胞病毒的表达控制序列的载体。用于细菌宿主的有用的表达载体包括已知的细

菌质粒,例如来自大肠杆菌的质粒,包括pCR 1、pBR322、pMB9和其衍生物,较宽宿主范围质粒,例如M13和丝状单链DNA噬菌体。

[0108] 在某些方面提供包含如本文所提供的多核苷酸的分离的宿主细胞。在某些方面提供包含本文提供的多核苷酸组合物的两个或更多个多核苷酸的一种或多种分离的宿主细胞。

[0109] 合适的用于表达本文所提供的转运体分子的宿主细胞包括在适当的启动子控制下的原核生物、酵母、昆虫或更高等的真核细胞。原核生物包括革兰氏阴性或革兰氏阳性生物体,例如大肠杆菌或芽孢杆菌。更高等的真核细胞包括如下所述的已建立的哺乳动物来源的细胞系。也可采用无细胞翻译系统。关于蛋白质生产,包括抗体生产的方法的额外信息可见于例如美国专利出版号2008/0187954、美国专利号6,413,746和6,660,501以及国际专利出版号W0 04009823,其各自通过引用以其整体结合到本文中。

[0110] 各种哺乳动物或昆虫细胞培养系统也可用于表达本文提供的抗DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物。合适的哺乳动物宿主细胞系的实例包括HEK-293和HEK-293T、Gluzman (Cell 23:175, 1981)描述的猴肾细胞COS-7系,或其它细胞系,包括,例如,L细胞、C127、3T3、中国仓鼠卵巢(CHO)、NS0、HeLa和BHK细胞系。哺乳动物表达载体可包含非转录元件例如复制起点、与待表达基因操作性连接的合适的启动子和增强子和其它5'或3'侧翼非转录序列,和5'或3'非翻译序列,例如核糖体结合位点、多腺苷酸化位点、剪接供体和受体位点和转录终止序列。用于在昆虫细胞中产生异源蛋白的杆状病毒系统由Luckow和Summers, BioTechnology 6:47 (1988)综述。

[0111] 本文提供的宿主细胞可在制造本文所提供的抗DPP-4抗体或其亚基(例如,重链或轻链)、片段、变体或衍生物的方法中使用,其中所述方法包括(a) 培养宿主细胞和(b) 从宿主细胞分离表达的抗体、片段或亚基。

[0112] 用于检测DPP-4水平的测定

本公开内容提供测量从受试者获得的样品中的DPP-4水平的方法,包括使用利用至少一种,例如,至少两种识别人DPP-4上的不同表位的抗DPP-4抗体或其抗原结合片段的免疫测定测定样品。用于该方法的示例性抗体包括以下一种或多种:从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11,和/或其抗原结合片段、变体或衍生物,如本文所描述的。

[0113] 所述方法涉及使用特异和灵敏的用于检测从受试者获得的样品中的DPP-4的免疫测定。样品在利用至少一种,例如,至少两种本文提供的抗DPP-4抗体的免疫测定中测定,所述抗体例如,从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11,和/或其抗原结合片段、变体或衍生物,如本文所描述的。

[0114] 例如,本公开内容提供测量从受试者获得的样品中的DPP-4水平的方法,包括在利用至少一种,例如,至少两种识别人DPP-4上的不同表位的抗DPP-4抗体或其抗原结合片段、

变体或衍生物的免疫测定中测定样品。在一个方面,至少两种抗DPP-4抗体的一种包括与从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7结合相同DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物,并且至少两种抗DPP-4抗体的一种包括与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11结合相同DPP-4表位的分离抗体或其抗原结合片段、变体或衍生物。

[0115] 在另一个方面,至少两种抗DPP-4抗体的一种包括竞争性抑制从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6和/或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7结合DPP-4的分离抗体或其抗原结合片段、变体或衍生物;并且至少两种抗DPP-4抗体的一种包括竞争性抑制从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11结合DPP-4的分离抗体或其抗原结合片段、变体或衍生物。

[0116] 在另一个方面,两种或更多种抗DPP-4抗体的一种为包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL)的分离抗体或其抗原结合片段、变体或衍生物,其中分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6和/或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的CDRs相同;并且两种或更多种抗DPP-4抗体的一种为包含具有三个重链互补决定区(CDRs) VHCDR1、VHCDR2和VHCDR3的重链可变结构域(VH)和具有三个轻链CDRs VLCDR1、VLCDR2和VLCDR3的轻链可变结构域(VL)的分离抗体或其抗原结合片段、变体或衍生物,其中分离抗体或其片段、变体或衍生物的CDRs与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的CDRs相同。

[0117] 在另一个方面,两种或更多种抗DPP-4抗体的一种为包含与从在保藏号PTA-121870下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m3B7.6和/或从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物;并且两种或更多种抗DPP-4抗体的一种为包含与从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11的VH和VL相同的VH和VL的分离抗体或其抗原结合片段、变体或衍生物。

[0118] 任何这些抗体或其片段可与一个或多个异源多肽融合,例如,稳定多肽、标签、标记或其组合,或可缀合到异源部分,例如,肽、蛋白、酶、脂质、异源抗体或其片段、可检测标记、聚乙二醇(PEG)或两种或更多种任何所述试剂的组合。在某些方面,抗体包含可检测标记例如生物素或钆螯合物。其它可检测标记为本领域普通技术人员所熟知,并且包含在本公开内容中。

[0119] 在某些方面,免疫测定包括夹心免疫测定,例如,酶联免疫吸附测定(ELISA)或夹心电化学发光(ECL)测定,其中将第一种抗DPP-4“捕获”抗体或其抗原结合片段附着到固

体支持物,让来自样品的抗原或标准品与捕获抗体结合,然后加入第二种抗DPP-4“检测”抗体或其抗原结合片段,并通过酶促反应、电化学发光反应、放射性或其它检测方法检测。

[0120] 在某些方面,免疫测定包括以下:首先,让捕获抗体或其片段、变体或衍生物结合到固体支持物,例如,多孔板或本领域普通技术人员已知的其它测定装置。让捕获抗体附着一段时间,例如,过夜,然后去除未结合的抗体。然后可将板洗涤以去除任何未结合的捕获抗体。然后可将板用封闭溶液处理以让非特异性蛋白结合固体支持物的任何未结合区域。典型的封闭溶液包括不相关的蛋白,例如,脱脂奶粉或血清白蛋白。然后可将板再次洗涤以去除任何未结合的封闭溶液。接着,将疑似包含DPP-4的样品加入板。样品通常连续稀释并一式两份或一式三份铺板。包含标准量的DPP-4或其合适片段的对照和不同阴性对照也包括在内。让抗原结合捕获抗体一段时间,例如,室温1小时。孵育之后,然后可将板洗涤以去除任何未结合的抗原。

[0121] 接着,加入检测抗体。检测抗体通常为结合与捕获抗体不同的DPP-4表位的抗DPP-4抗体。检测抗体可为标记的或未标记的。当检测抗体未标记时,标记的二抗可用于检测,如本领域普通技术人员所熟知的。检测抗体可用酶,例如,辣根过氧化物酶或碱性磷酸酶直接标记,或可用将会允许酶结合的标签标记。例如,检测抗体可缀合生物素,并且酶在后续步骤中通过允许酶缀合的链霉亲和素结合生物素标签连接。或者检测抗体可缀合化学发光、荧光或电化学发光标签。后者的实例为钆螯合物。孵育之后,然后可将板洗涤以去除任何未结合的检测抗体。

[0122] 检测抗体的检测通过基于使用的检测抗体类型而不同的方法完成。如果检测抗体用生物素标记,则加入酶缀合的链霉亲和素,洗去未结合的链霉亲和素,并加入底物,底物提供可,例如,在分光光度计上阅读的比色反应。如果检测抗体缀合钆螯合物,板经受电流,并测量光发射。

[0123] 在某些方面,所述方法直接测量患者样品中的DPP-4水平,其中绝对水平通过在使用,例如,纯化的全长DPP-4或DPP-4片段的标准曲线上标绘免疫测定结果计算。检测到的来自检测抗体的信号然后可基于板上包括的各个标准品和对照定量。通过在标准曲线上标绘结果,检验样品中DPP-4的绝对水平或量可,例如,以ng/mL或pg DPP-4/mL蛋白计算。

[0124] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0125] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121871下保藏在ATCC的杂

交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0126] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0127] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0128] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0129] 在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物。在本文所提供的免疫测定和方法的某些方面,捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0130] 多种不同的受试者样品可用在本文所呈现的方法中。样品的示例性的非限制实例包括以下一种或多种:全血、血清、血浆、唾液、痰、鼻息肉、鼻粘液、支气管肺泡灌洗液、皮肤细胞或肺组织,例如,肺上皮细胞。在特定方面,样品为血清样品、皮肤细胞或肺组织。

[0131] 在具体的方面,本文所公开的方法包括告知受试者DPP-4测定和/或至少部分基于DPP-4水平的诊断的结果。患者可口头、书面和/或电子告知。诊断也可记录在患者的医疗记录上。

[0132] 术语“医疗记录”或“患者医疗记录”指患者的检查和/或治疗报告,其通常包括下列一个或多个:患者的医疗历史和投诉、医生的身体发现、诊断检验和程序的结果,和以及患者用药和治疗程序。医疗记录通常由一个或多个医生和/或医生助理做出,并且其为书写的、抄写的或以其它方式记录的记录和/或需要医疗护理的各种疾病或损伤和/或接种和/或过敏和/或治疗和/或预后的历史,和/或关于患者、兄弟姐妹和/或职业的常见健康信息。在一些情况下,记录可由医生在诊断病况中回顾。

[0133] 医疗记录可为纸质形式和/或可维持在计算机可读介质上。医疗记录可由实验室、医生办公室、医院、健康护理维护组织、保险公司和/或个人医疗记录网站维持。在一些方面,至少部分基于DPP-4水平的诊断记录在医疗警报物品上,例如卡、佩戴品和/或射频识别(RFID)标签。如本文所使用的,术语“佩戴品”指可佩戴在受试者身体上的任何物品,包括,但不限于,标签、腕带、项圈、臂带和头带。

[0134] 在某些方面,所述方法可需要命令和/或进行一个或多个额外的测定。例如,如果DPP-4水平经测定在正常范围内(即,未提高),DPP-4测定可重复以排除假阴性结果,和/或一个或多个额外的DPP-4测定可进行以监测受试者的状态。如果DPP-4水平经测定提高,重复DPP-4测定以排除假阳性结果可为期需的。

[0135] DPP-4检测方法、测定和试剂盒

本公开内容提供方法、测定和试剂盒帮助测定或分析样品中的DPP-4水平或DPP-4的量。在一些方面,本文所公开的方法、测定和试剂盒由医疗提供者、医疗福利提供者或临床实验室进行或使用,以测定来自受试者的样品中的DPP-4水平或DPP-4的量。

[0136] 在某些方面,免疫测定在从受试者获得的样品上由治疗患者的医护专业人员进行,例如,使用如本文所述的免疫测定,其配制为“护理点(point of care)”诊断试剂盒。在一些方面,样品从患者获得并且提交至,例如,临床实验室,按照医护专业人员的指令,例如,使用如本文所述的免疫测定测量样品中的DPP-4水平。

[0137] 在某些方面,具有或疑似具有IL-13介导的疾病或病症的患者经诊断患有肺疾病或病症、炎症性肠疾病或病症或慢性炎症性皮肤病况。在某些方面,具有或疑似具有IL-13介导的病理学的疾病或病症为哮喘、特发性肺纤维化(IPF)、慢性阻塞性肺病(COPD)、溃疡性结肠炎(UC)、特应性皮炎、过敏性鼻炎或慢性鼻窦炎。

[0138] 本公开内容还提供在本文所公开的免疫测定的实践中使用的试剂盒。这样的试剂盒可包括容器,各自具有方法中利用的一种或多种不同试剂(例如,以浓缩的形式),包括,例如,一种或多种抗DPP-4抗体。一种或多种抗DPP-4抗体,例如,捕获抗体可已经附着在固体支持物上提供,并且一种或多种抗体,例如,检测抗体可已经缀合到可检测标记例如生物素或钆螯合物提供。试剂盒可还提供用于偶联可检测标记与抗体的试剂(以及标记本身)、缓冲液和/或支持本文所提供测定的实践的试剂和设备。在某些方面,提供结合检测抗体的标记的二抗。本公开内容提供的试剂盒可进一步包含合适的容器、板和实践本文所提供的测定必需的任何其它试剂或材料。

[0139] 用于测量样品中的DPP-4水平的试剂盒可包含一种或多种本文提供的抗DPP-4抗体或其片段,例如,从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆

抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11,和/或其抗原结合片段,变体或衍生物,或相关抗体或其抗原结合片段,同样如本文所描述的。

[0140] 在某些方面,如本文所提供的试剂盒包含两种分离抗体或其抗原结合片段,捕获抗体和检测抗体。在某些方面捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0141] 在某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,或如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0142] 在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物。在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0143] 在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物。在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物。

[0144] 在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物。在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121872下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A2.15或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0145] 在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并

且检测抗体为如从在保藏号PTA-121870下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m3B7.6或其抗原结合片段、变体或衍生物。在本文所提供的试剂盒的某些方面,捕获抗体为如从在保藏号PTA-121873下保藏在ATCC的杂交瘤所产生的大鼠单克隆抗体R11A9.11或其抗原结合片段、变体或衍生物,并且检测抗体为如从在保藏号PTA-121871下保藏在ATCC的杂交瘤所产生的小鼠单克隆抗体m5B7.7或其抗原结合片段、变体或衍生物。

[0146] 在某些方面,检测抗体被可检测地标记。在某些方面,可检测标记为生物素并且检测试剂包括链霉亲和素-辣根过氧化物酶(HRP)缀合物和HRP的比色底物。在某些方面可检测标记为钆螯合物。如本文别处所描述的其它抗体、标记和试剂也可用在如本文所提供的试剂盒中。

[0147] 在某些方面,本公开内容提供用于检测一个或多个样品中的DPP-4水平的免疫测定,包括使用至少一种,例如,至少两种抗DPP-4抗体或其抗原结合片段、变体或衍生物,例如,如本文所描述的从在保藏号PTA-121870下保藏在美国典型培养物保藏中心(ATCC)的杂交瘤产生的小鼠单克隆抗体m3B7.6、从在保藏号PTA-121871下保藏在ATCC的杂交瘤产生的小鼠单克隆抗体m5B7.7、从在保藏号PTA-121872下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A2.15和/或从在保藏号PTA-121873下保藏在ATCC的杂交瘤产生的大鼠单克隆抗体R11A9.11,或其抗原结合片段、变体或衍生物,或相关抗体或其抗原结合片段,同样如本文所描述的。

[0148] 在某些方面,本文所提供的免疫测定为夹心免疫测定,例如,ELISA测定或ECL测定,包括附着在固体支持物上的第一个抗DPP-4“捕获”抗体或其抗原结合片段,和第二个抗DPP-4“检测”抗体或其抗原结合片段。免疫测定通过本文提供的方法或本领域普通技术人员所熟知和理解的方法进行。在一个方面,免疫测定包括将捕获抗体或其片段、变体或衍生物附着到固体支持物;施加检验样品或对照样品,允许DPP-4(如果存在于样品中的话)结合捕获抗体或其片段、变体或衍生物;施加检测抗体或其片段、变体或衍生物,其可结合已经结合至捕获抗体或其片段、变体或衍生物的DPP-4;和测量结合至DPP-4的检测抗体或其片段、变体或衍生物的量。在某些方面,测定可进一步包括洗涤步骤、封闭步骤和孵育步骤。

[0149] 在某些方面,检测抗体或其片段、变体或衍生物进一步包含可检测标记,例如生物素或钆螯合物。

[0150] 本公开内容的方面可通过参考下列非限制性实施例进一步定义,其详细描述本公开内容某些抗体的制备和使用本公开内容的抗体的方法。对本领域技术人员将会显而易见的是,对材料和方法二者的许多修饰可实践而不脱离本公开内容的范围。

实施例

[0151] 实施例1:对人HPP-4特异的大鼠和小鼠单克隆抗体的产生和表征

对人DPP-4特异的小鼠和大鼠单克隆抗体通过下列方法产生。将动物使用多个位点重复免疫(RIMMS)方案(Kilpatrick, K.,等人, Hybridoma 16:381-389 (2009))免疫。简言之,5只Balb/c小鼠和2只Wistar大鼠(所有均为4-6周龄)在6个背侧位点皮下注射混合完全弗氏佐剂(Sigma)的r-人DPP4-His (R&D Systems Catalog No. 1180)(第一轮,接着不完全弗氏佐剂用于后续免疫),并在每一给剂日期在6个腹部位点皮下注射混合TiterMax

(Sigma) 佐剂的抗原。总共,每一动物在12个位点接受注射,如图1中所示。检验出血在第13天收集,并且出血前和免疫的血清二者通过直接结合ELISA使用结合至ELISA板的DPP-4 (r-DPP-4-His, R&D Systems目录号1180) 和阴性对照糖蛋白130 (r-gp130-His) 检验。将高结合ELISA板的孔在4℃用50 μ L 2 μ g/mL r-人DPP-4-His或gp130-His/PBS包被过夜。然后将板用200 μ L/孔洗涤缓冲液(PBS/0.1% TWEEN-20[®]) 洗涤3次。洗涤之后,将150 μ L/孔封闭缓冲液(PBS/3%脱脂奶粉/0.1% TWEEN-20[®]) 加入每一孔,并将板室温孵育1 h。然后如上面提到的,将板洗涤3次。

[0152] 将前-和检验-血清在PBS, pH7.2缓冲液中连续稀释,从最初的1:100稀释度至最终的稀释度1:218,600。将50 μ L每一稀释的样品加入板并室温孵育1 h。将板如上面提到的洗涤3次。洗涤之后,将50 μ L检测抗体加入每一孔。对于小鼠组,使用在PBS中1:8000稀释的驴抗小鼠(H+L):HRP (Jackson ImmunoResearch, Catalog No. 715-035-151)。对于大鼠组,使用在PBS中1:8000的驴抗大鼠(H+L):HRP (Jackson ImmunoResearch, Catalog No. 715-035-153)。将板室温孵育1 h。然后将板洗涤3次,并加入预热至室温的50 μ L/孔的TMB底物(KPL, Catalog No. 52-00-04)。将板在黑暗中室温孵育10 min,将50 μ L终止溶液(1M HCl)加入每一孔中。将板在分光光度计上在 $\lambda = 450$ nm处读数。

[0153] 对DPP-4的出血前和免疫血清抗体滴度在图2中显示。在第16和19天,从显示稳健血清滴度的大鼠和小鼠收集淋巴结,杂交瘤如下产生。在第19天,从动物收集淋巴结,从淋巴组织提取淋巴细胞并通过70 μ m细胞过滤器过滤。抗原特异性B细胞通过使用MACS、链霉亲和素微珠(Miltenyi Biotec Catalog # 130-048-101) 使用制造商建议的方案分离。然后将DPP-4特异性B细胞与骨髓瘤P3x/63Ag8.653细胞以1:1比率按照PEG (Roche) 融合方法融合。将融合的细胞以 2.5×10^4 B细胞/孔的密度接种在添加1xHAT的杂交瘤生长培养基(Ex-Cell 610 + 10% Hi-FBS + 1% 青霉素-链霉素 + 1X BM-condimed H1杂交瘤克隆补充物)中。融合后7天,将包含HAT的培养基替换为添加1xHT的生长培养基。

[0154] 将杂交瘤上清液筛查结合人DPP-4的抗体,并选择四个产生杂交瘤的抗体用于进一步研究:小鼠单克隆抗体m3B7.6 (IgG1/ κ)、小鼠单克隆抗体m5B7.7 (IgG1/ κ)、大鼠单克隆抗体R11A2.15 (IgG2a/ κ) 和大鼠单克隆抗体R11A9.11 (IgG2a/ κ)。表达这4个单克隆抗体的杂交瘤细胞系在2015年1月8日在保藏号PTA-121870 (m3B7.6)、保藏号PTA-121871 (m5B7.7)、保藏号PTA-121872 (R11A2.15) 和保藏号PTA-121873 (R11A9.11) 下在布达佩斯条约下保藏在美国典型培养物保藏中心(ATCC)。

[0155] 实施例2:夹心ELISA测定以检测DPP-4

材料和方法

A. 具有HRP检测系统的DPP-4夹心ELISA方案

基于HRP的夹心ELISA测定如下进行。具体细节例如抗DPP-4捕获抗体、待检验样品和抗DPP-4检测抗体在各个实施例中具体实验指出。对标准ELISA方案的变更为本领域普通技术人员所熟知,并且可根据本公开内容使用。

[0156] 将高结合ELISA板用抗DPP-4捕获mAb,例如,m3B7.6或m5B7.7 (在PBS中5 μ g/mL; 50 μ L/孔)包被,并在4℃孵育过夜。将板用200 μ L/孔洗涤缓冲液(PBS/0.1% TWEEN-20[®]) 洗涤3次。洗涤之后,将150 μ L/孔封闭缓冲液(PBS/3%脱脂奶粉/0.1% TWEEN-20[®]) 加入每一孔,并将板室温孵育1 h。然后如上面所提到的将板洗涤3次。

[0157] 对于标准曲线,将DPP-4标准品(例如,从R & D Systems Catalog # 1180-SE可得的标准品)在PBS, pH7.2缓冲液或1%正常人血清中连续稀释,例如,从500 ng/mL 2倍稀释至0.49 ng/mL。将50微升(50 μ L)每一标准品或稀释的样品加入板,并将板室温孵育1 h。再次,如上面所提到的将板洗涤3次。洗涤之后,将50 μ L检测mAb R11A2.15、R11A9.11或222113.11 (2 μ g/mL)加入每一孔,并将板室温孵育1 h。再次,如上面提到的将板洗涤3次。洗涤之后,将在PBS中1:8,000稀释的50 μ L驴抗大鼠(H+L)-HRP缀合物(Jackson Immunoresearch catalog # 712-035-153)加入每一孔,并将板室温孵育1h。再次,如上面提到的将板洗涤3次。洗涤之后,加入预热至室温的50 μ L/孔 TMB底物(KPL, Catalog No. 52-00-04),将板在黑暗中室温孵育10 min,并将50 μ L TMB终止溶液(1M HCl)加入每一孔。最后将板在分光光度计上在 $\lambda = 450\text{nm}$ 处读数。

[0158] 结果

使用小鼠m3B7.6和m5B7.7作为捕获抗体的结果分别在图3A和图3B中显示。两种小鼠和两种大鼠mAbs (mAb R11A2.15或R11A9.11)形成4对不同的用于人CD26的检测剂,其具有相似的检测下限(LLOD)并在夹心ELISA测定测量人DPP-4中提供高灵敏度。相反,市售可得的大鼠mAb (R222113) (来自R&D Systems的人DPPIV/CD26 MAbs (克隆222113)、大鼠IgG2A目录号MAB1180)检测到较少的人CD26并且当与两种小鼠mAbs m3B7.6和m5B7.7配对时在夹心ELISA测定测量人DPP-4中明显不太灵敏。参见图3A-B。这些结果证明与市售可得的大鼠抗体R222113相比,本文提供的抗体(m3B7.6、m5B7.7、R11A2.15和R11A9.11)在免疫测定检测人CD26方面优越。另外,本文提供的抗体(m3B7.6、m5B7.7、R11A2.15和R11A9.11)检测内源和重组人CD26二者,使其可用作诊断试剂。

[0159] 如图3中所示,m3B7.6 + R11A2.15、m3B7.6 + R11A9.11、m5B7.7 + R11A2.15和/或m5B7.7 + R11A9.11在夹心ELISA测定中有效测量人DPP-4。另外,无论选择哪一对,研究中DPP-4的血清浓度在1-2 μ g/mL之间。

[0160] 实施例3:使用OCTET的抗DPP-4 mAb表位框并(binining)

四种抗DPP-4抗体(m3B7.6、m5B7.7、R11A2.15和R11A9.11)的表位框并通过OCTET进行。将检验抗体生物素化并以20 μ g/mL的浓度和以200 μ L/孔在链霉亲和素生物传感器上捕获5 min,然后用200 μ L PBS缓冲液洗涤1 min。然后将生物传感器用10 μ g/mL重组人DPP-4孵育5 min并用200 μ L PBS缓冲液洗涤1 min。将竞争抗体以1:1比率与检验抗体以终浓度20 μ g/mL混合,并在200 μ L中与生物传感器一起孵育5 min。

[0161] 测定的示意图在图4A中示出,以四种不同抗体(m3B7.6、m5B7.7、R11A2.15和R11A9.11)作为检验抗体的结果分别在图4B-E中示出。结果证明小鼠单克隆抗体m3B7.6和m5B7.7互相竞争,并且大鼠单克隆抗体R11A2.15和R11A9.11互相竞争。小鼠抗体不与大鼠抗体竞争,因此不共享表位。基于该数据,我们得出结论两种小鼠抗体(m3B7.6和m5B7.7)共享相同或重叠的表位,而两种大鼠抗体(R11A2.15和R11A9.11)共享表位或重叠表位,并且表位与小鼠抗体共享的表位不同。

[0162] 前文对具体方面的描述将会完全揭示本公开内容的一般特征,以致他人在无过度实验下可通过应用本领域技术内的知识容易地为不同应用修饰和/或改编这样的具体方面,而不脱离本公开内容的一般概念。因此,基于本文呈现的教导和指南,这样的改编和修饰意在落入所公开方面的相等物的含义和范围内。应理解本文中的短语和术语是为了说明

的目的,而非限制,以致本说明书的术语或短语应由技术人员按照教导和指南解释。

[0163] 本公开内容的宽度和范围不应受任何上述示例性方面限制,而是应仅由下列权利要求及其相等物定义。

[0164] 本申请中引用的所有出版物、专利、专利申请书和/或其它文献对于所有目的通过引用以其整体结合,至如同每个单独的出版物、专利、专利申请书和/或其它文献单独指明对于所有目的通过引用结合相同的程度。

序列表

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〈223〉 来金珠单抗重链

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Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

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Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu

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Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser

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Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly

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Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn

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Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln

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Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser

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Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser

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[0002]

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 Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
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 245 250 255
 Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
 260 265 270
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
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 Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
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 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
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 20 25 30
 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro

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35 40 45
Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
50 55 60

[0005]

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Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp			
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Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln			
	100	105	110
Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr			
	115	120	125
Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr			
	130	135	140
Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val			
145	150	155	160
Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile			
	165	170	175
Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp			
	180	185	190
Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe			
	195	200	205
Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala			
210	215	220	
Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe			
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Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr			
	245	250	255
Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn			
	260	265	270
Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr			
	275	280	285
Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr			
290	295	300	
Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln			
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Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg			
	325	330	335
Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly			
	340	345	350
Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly			
	355	360	365
Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile			
370	375	380	
Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly			
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Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr			
	405	410	415

[0006]

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Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu		
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Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu		
450	455	460
Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr		
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Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp		
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Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys		
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Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met		
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Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu		
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Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg		
545	550	555
Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala		
565	570	575
Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His		
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Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp Gln Ile Glu		
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Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn Lys Arg Ile		
610	615	620
Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser Met Val Leu		
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Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val Ala Pro Val		
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Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg Tyr Met Gly		
660	665	670
Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn Ser Thr Val		
675	680	685
Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu Leu Ile His		
690	695	700
Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala Gln Ile Ser		
705	710	715
Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met Trp Tyr Thr		
725	730	735
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Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu Pro		
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 Ser Val Phe Leu Glu Asn Ser Thr Phe Asp Glu Phe Gly His Ser Ile
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 Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln Phe Ile Leu Leu Glu Tyr
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 Asn Tyr Val Lys Gln Trp Arg His Ser Tyr Thr Ala Ser Tyr Asp Ile
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 Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp Ile Ile Tyr Asn Gly Ile
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 Thr Asp Trp Val Tyr Glu Glu Glu Val Phe Ser Ala Tyr Ser Ala Leu
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 Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp
 180 185 190
 Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu
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 Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val
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 Asn Pro Thr Val Lys Phe Phe Val Val Asn Thr Asp Ser Leu Ser Ser
 225 230 235 240
 Val Thr Asn Ala Thr Ser Ile Gln Ile Thr Ala Pro Ala Ser Met Leu
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 Ile Gly Asp His Tyr Leu Cys Asp Val Thr Trp Ala Thr Gln Glu Arg
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	Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg Trp Asn Cys Leu Val Ala		
	290	295	300
	Arg Gln His Ile Glu Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg		
	305	310	315
	Pro Ser Glu Pro His Phe Thr Leu Asp Gly Asn Ser Phe Tyr Lys Ile		
	325	330	335
	Ile Ser Asn Glu Glu Gly Tyr Arg His Ile Cys Tyr Phe Gln Ile Asp		
	340	345	350
	Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly Thr Trp Glu Val Ile Gly		
	355	360	365
	Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr		
	370	375	380
	Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr Lys Ile Gln Leu Ser Asp		
	385	390	395
	Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu Leu Asn Pro Glu Arg Cys		
	405	410	415
	Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu		
	420	425	430
	Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr Thr Leu His Ser Ser Val		
	435	440	445
[0008]	Asn Asp Lys Gly Leu Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Lys		
	450	455	460
	Met Leu Gln Asn Val Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Ile		
	465	470	475
	Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe		
	485	490	495
	Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro		
	500	505	510
	Cys Ser Gln Lys Ala Asp Thr Val Phe Arg Leu Asn Trp Ala Thr Tyr		
	515	520	525
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Phe Lys Gln Val Glu Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn			
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Val His Phe Gln Gln Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val			
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背视图

腹视图

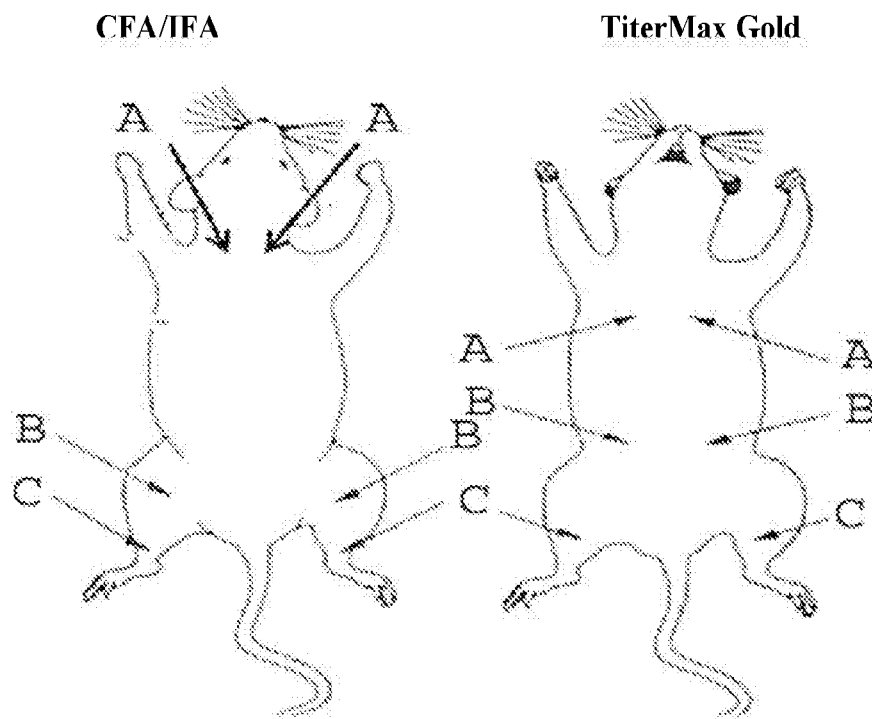


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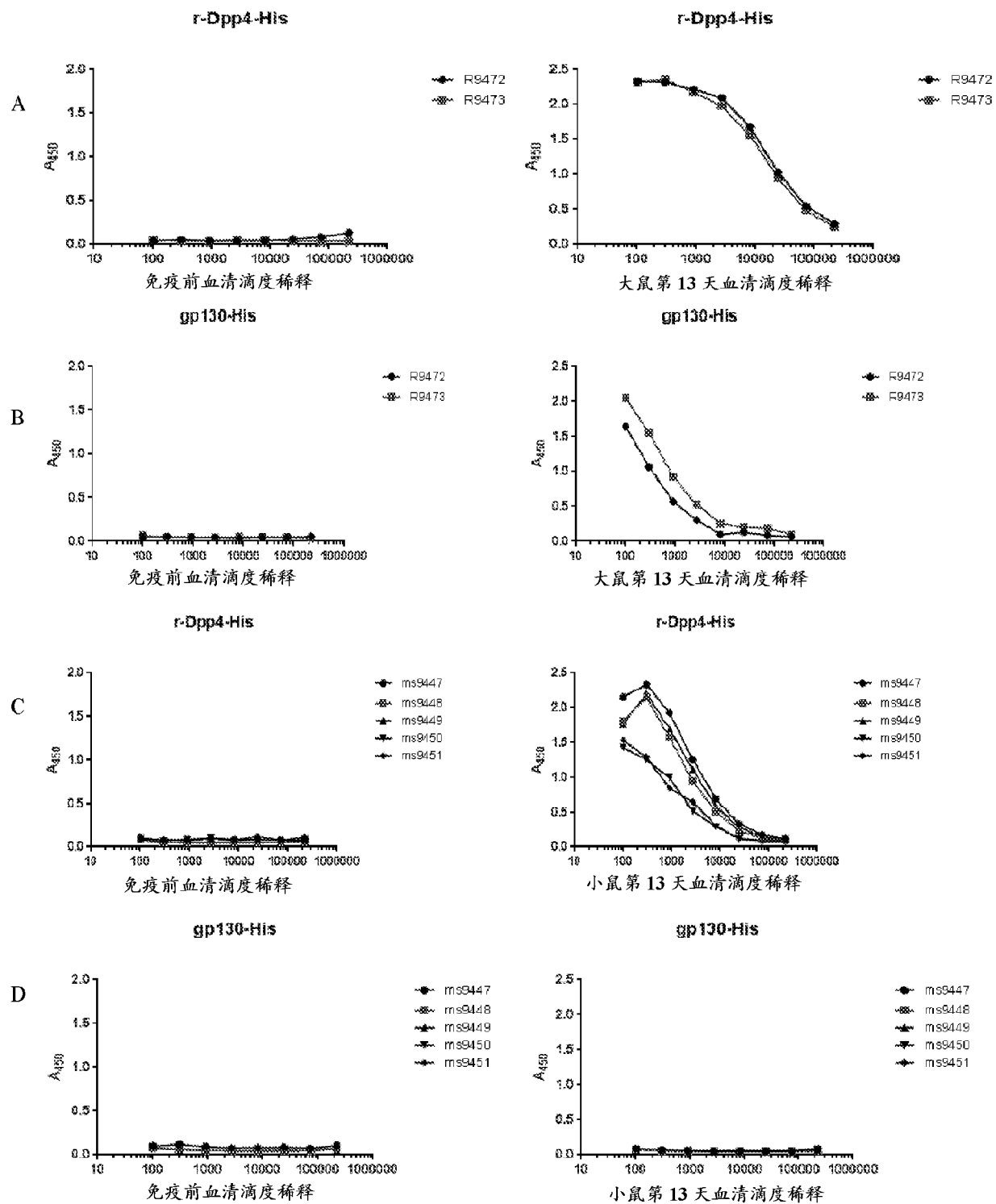


图 2

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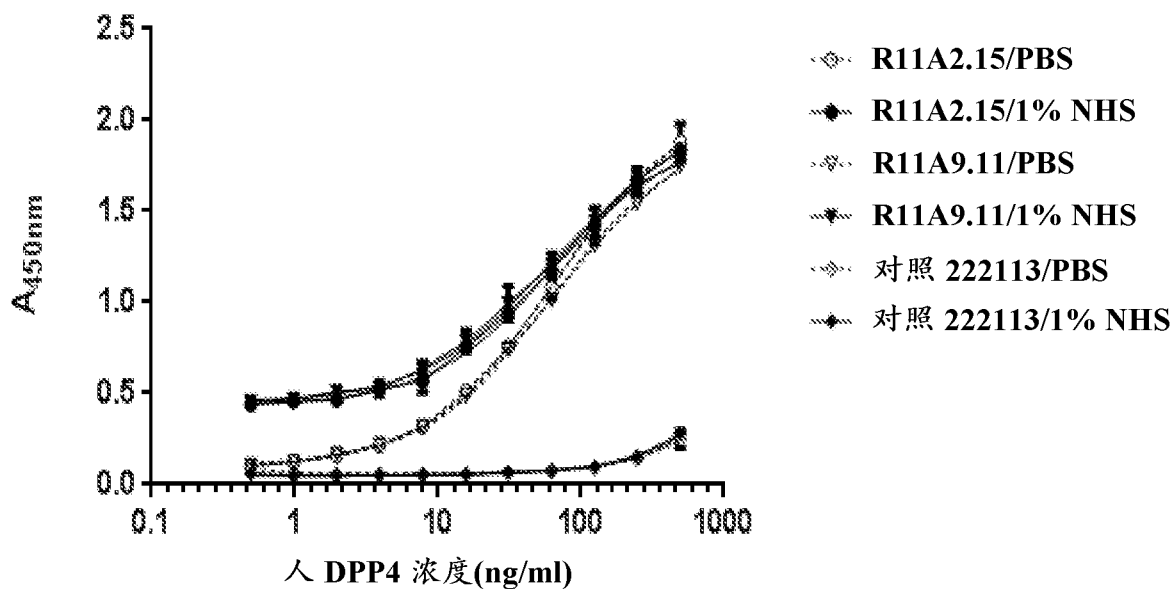


图 3A

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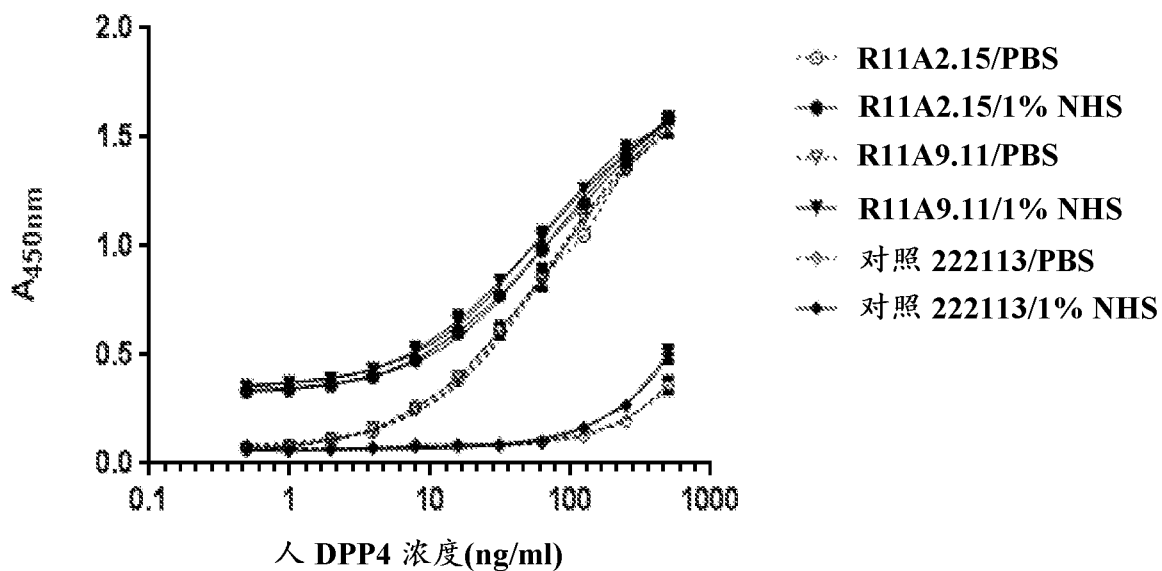


图 3B

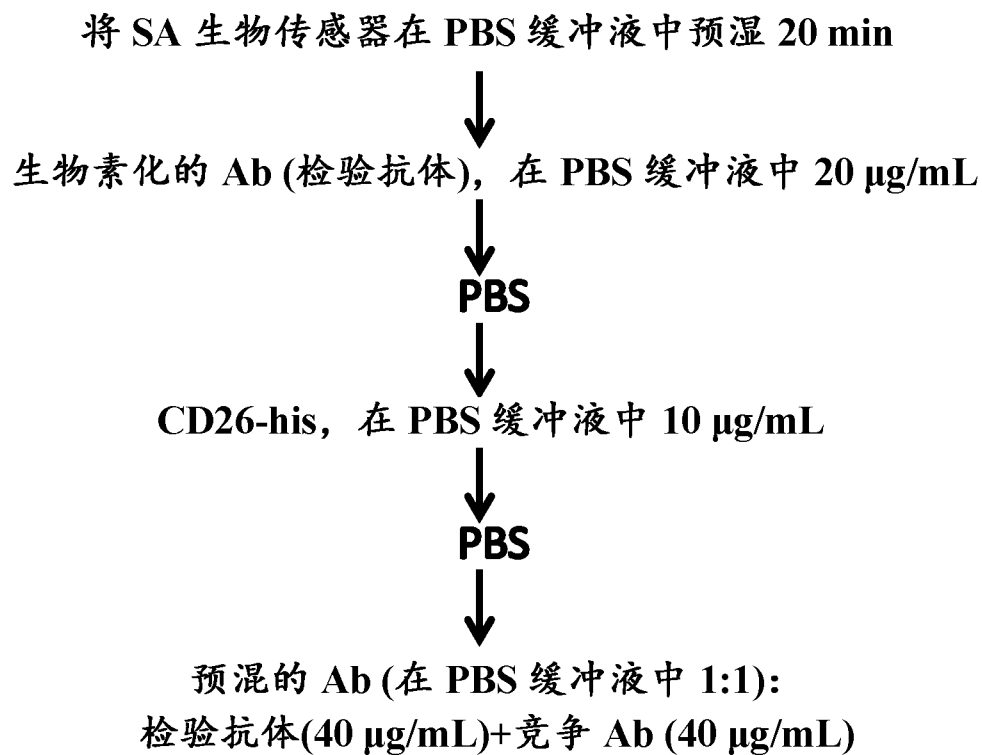


图 4A

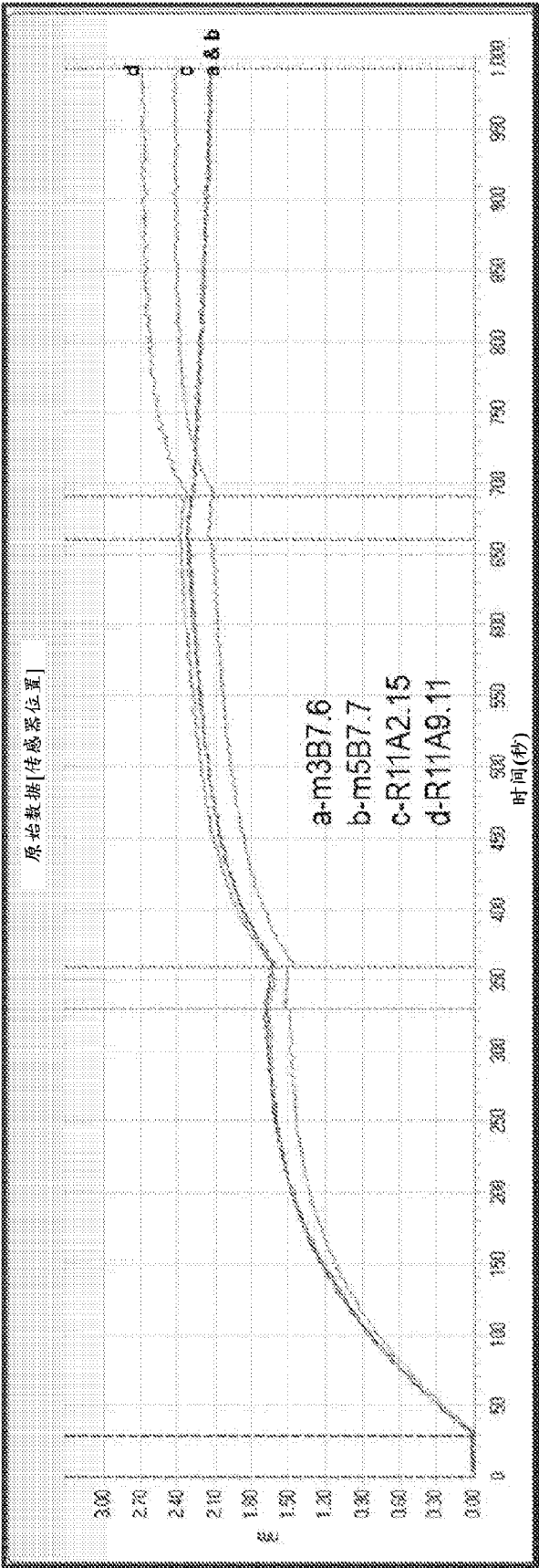


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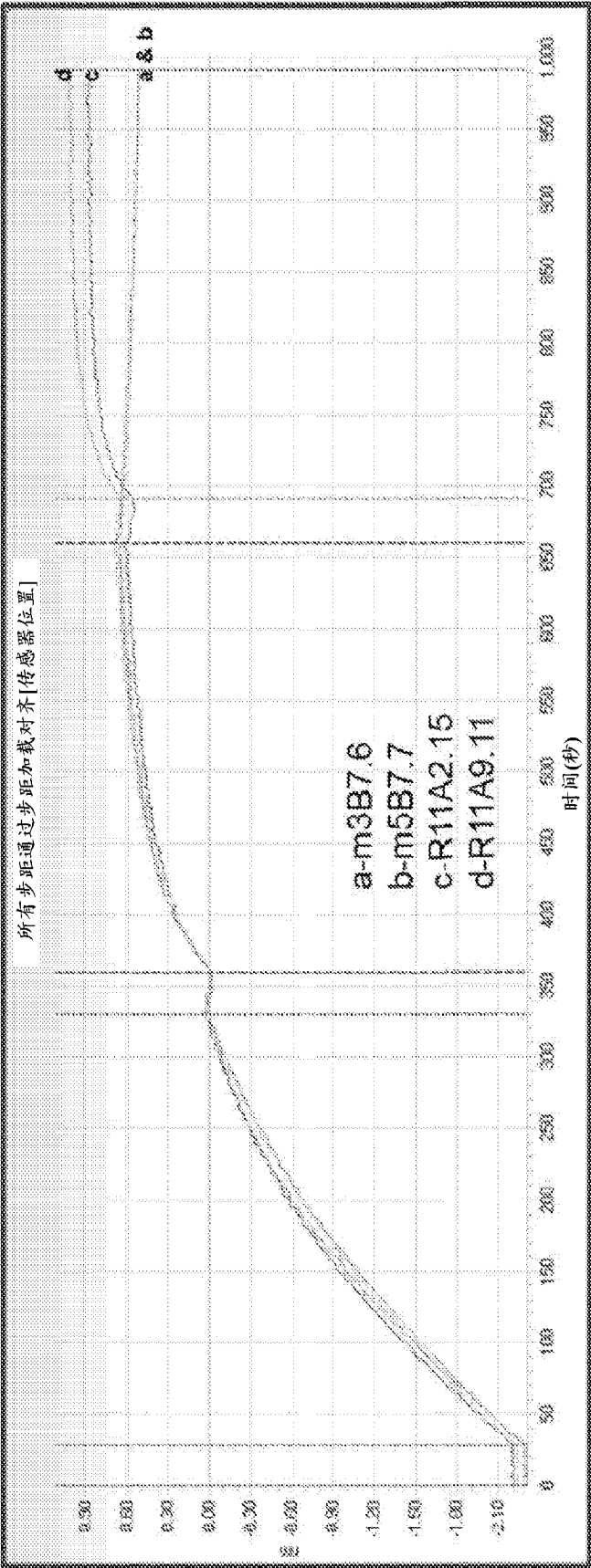


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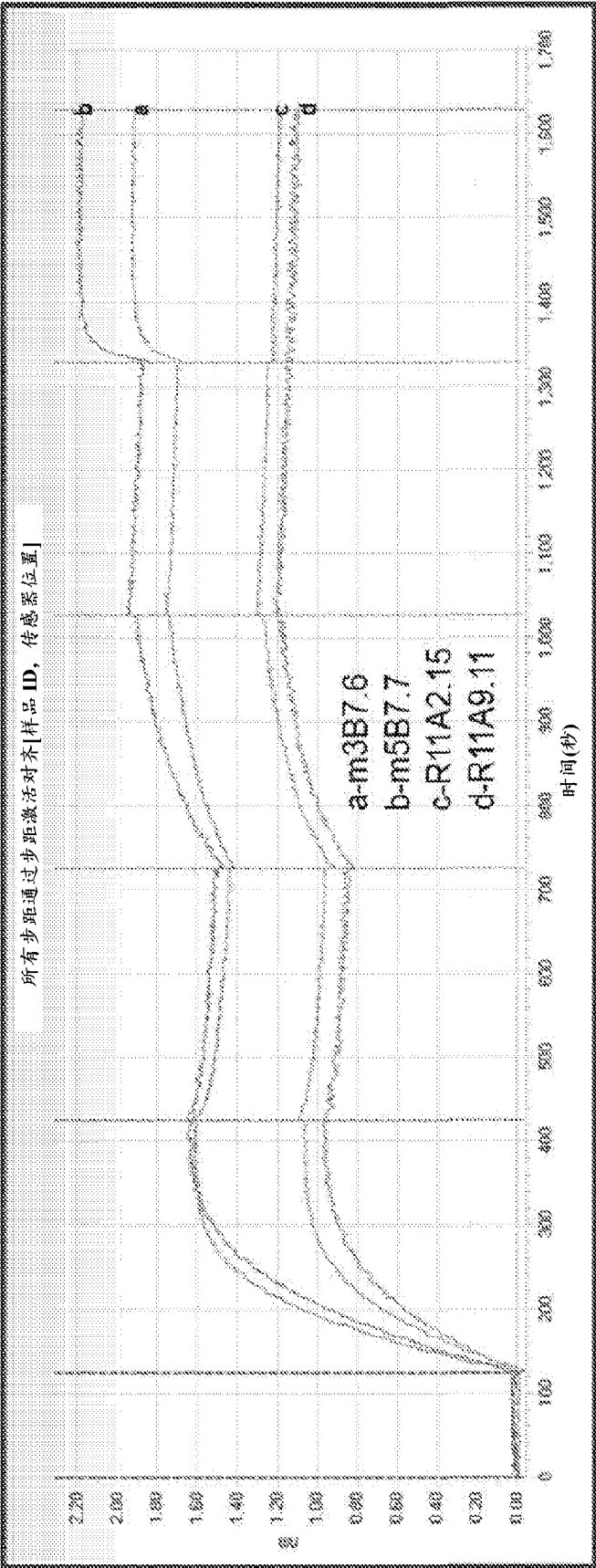


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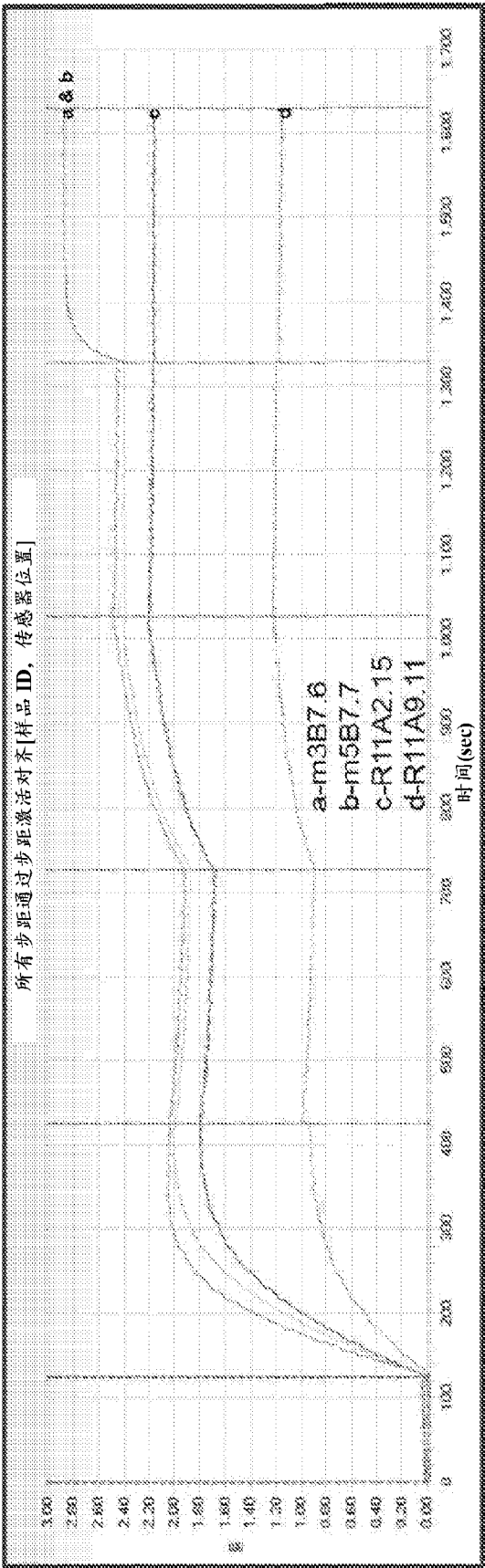


图 4E