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

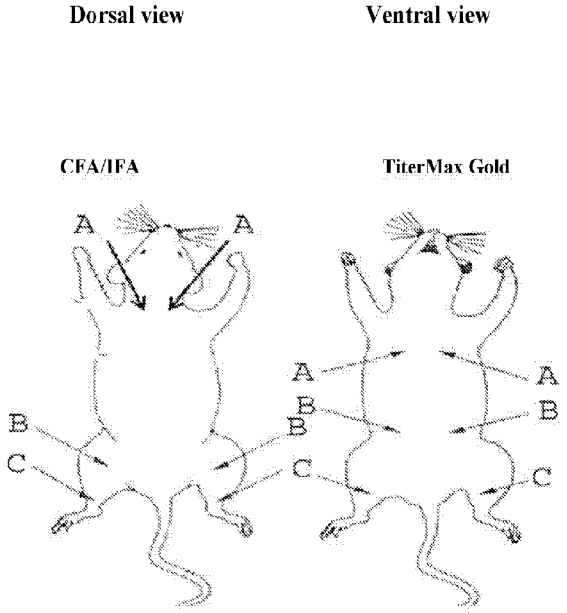

FIG. 1

(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 though 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

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

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

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

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

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,

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,

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

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

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

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

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 ± 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

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.

[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

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, pyodermas 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-13Rαl 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-4Rα antibody such as dupilumab/REGN668 (Regeneron); (f) a double-stranded oligonucleotide directed against IL-4Rα 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

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.

[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

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.

[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 dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose, or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONIC™, or polyethylene glycol (PEG). Compositions as provided

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

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

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

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

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

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

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,

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

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

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

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 µL of a 2 µg/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 µL/well wash buffer (PBS/0.1% TWEEN-20®). Following washing, 150 µL/well

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 µL 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 µl 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 µL/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 µL of stop solution (1M HCl) was added to each well. The plates were read on a spectrophotometer at $\lambda = 450$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 µm 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 $2.5x10^4$ 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

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 µg/mL in PBS; 50 µL/well), and were incubated overnight at 4°C. The plates were washed three times with 200 µL/well wash buffer (PBS/0.1% TWEEN-20®). Following washing, 150 µL/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 µL) 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 µL of detection mAb R11A2.15, R11A9.11 or 222113.11 (at 2 µg/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 µL 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 µL/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 µL of TMB stop solution (1M HCl) was added to each well. Finally the plates were read on a spectrophotometer at $\lambda = 450$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 DPPIV/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 µg/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 µg/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 µg/mL for 5 min and was washed with 200 µL of PBS buffer

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.

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[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.

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.

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.

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

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-

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

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;

(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;

(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

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;

(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-

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

(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.

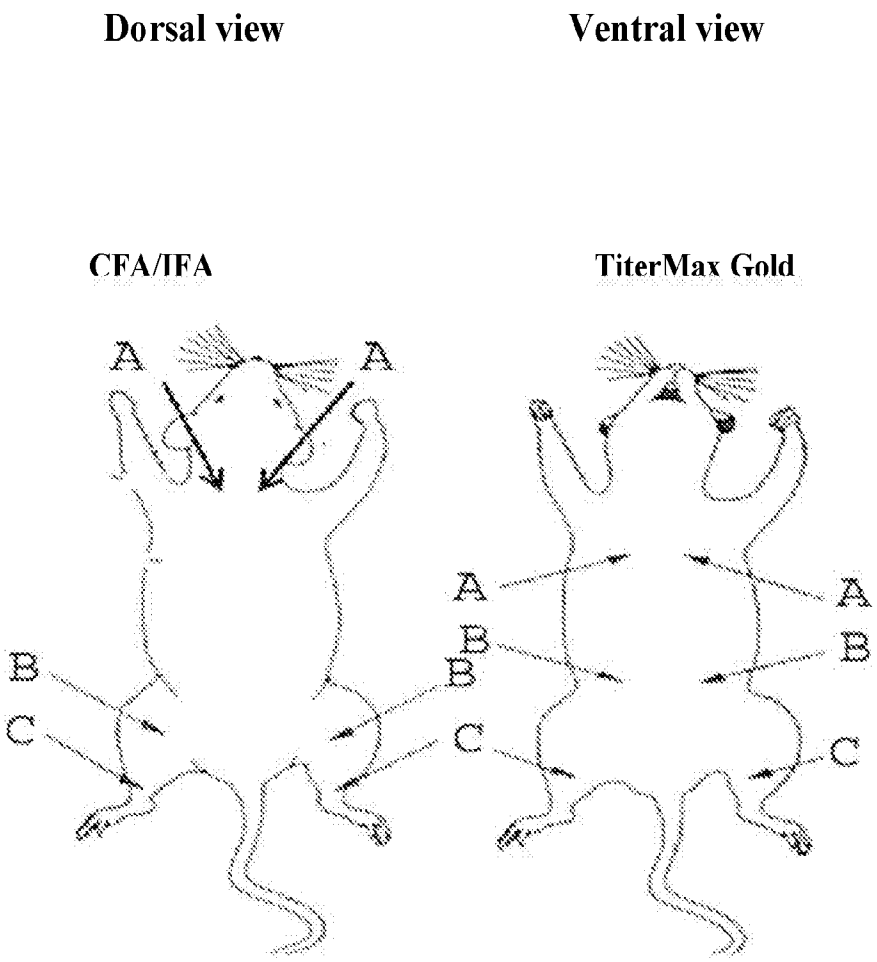
Dorsal view
Ventral view
CFA/IFA
TiterMax Gold
A
A
B
C
A
B
B
C
A
B
C

FIG. 1

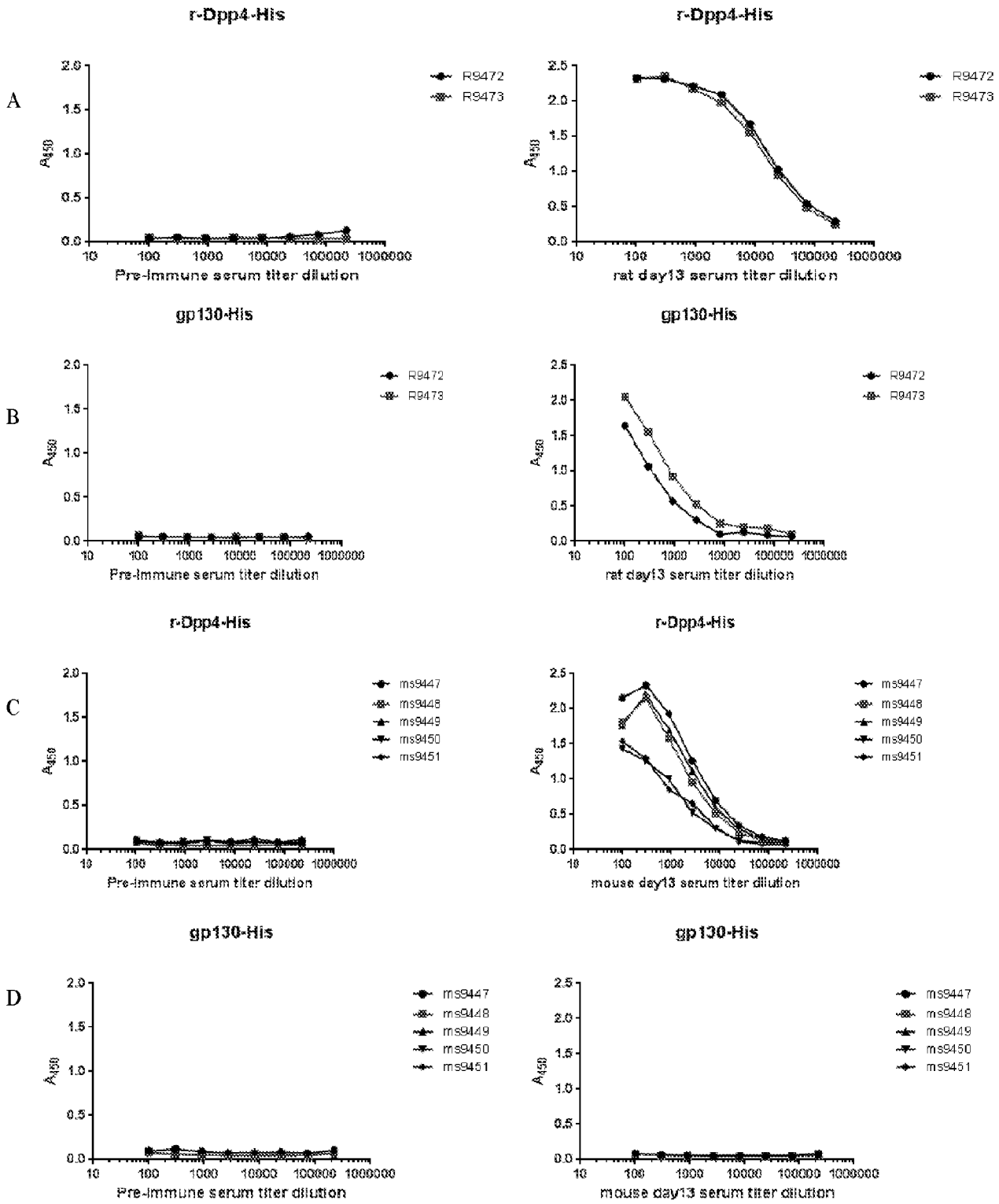
A
r-Dpp4-His
R9472
R9473
A450
Pre-Immune serum titer dilution
r-Dpp4-His
R9472
R9473
A450
rat day13 serum titer dilution
B
gp130-His
R9472
R9473
A450
Pre-Immune serum titer dilution
gp130-His
R9472
R9473
A450
rat day13 serum titer dilution
C
r-Dpp4-His
ms9447
ms9448
ms9449
ms9450
ms9451
A450
Pre-Immune serum titer dilution
r-Dpp4-His
ms9447
ms9448
ms9449
ms9450
ms9451
A450
mouse day13 serum titer dilution
D
gp130-His
ms9447
ms9448
ms9449
ms9450
ms9451
A450
Pre-Immune serum titer dilution
gp130-His
ms9447
ms9448
ms9449
ms9450
ms9451
A450
mouse day13 serum titer dilution

FIG. 2

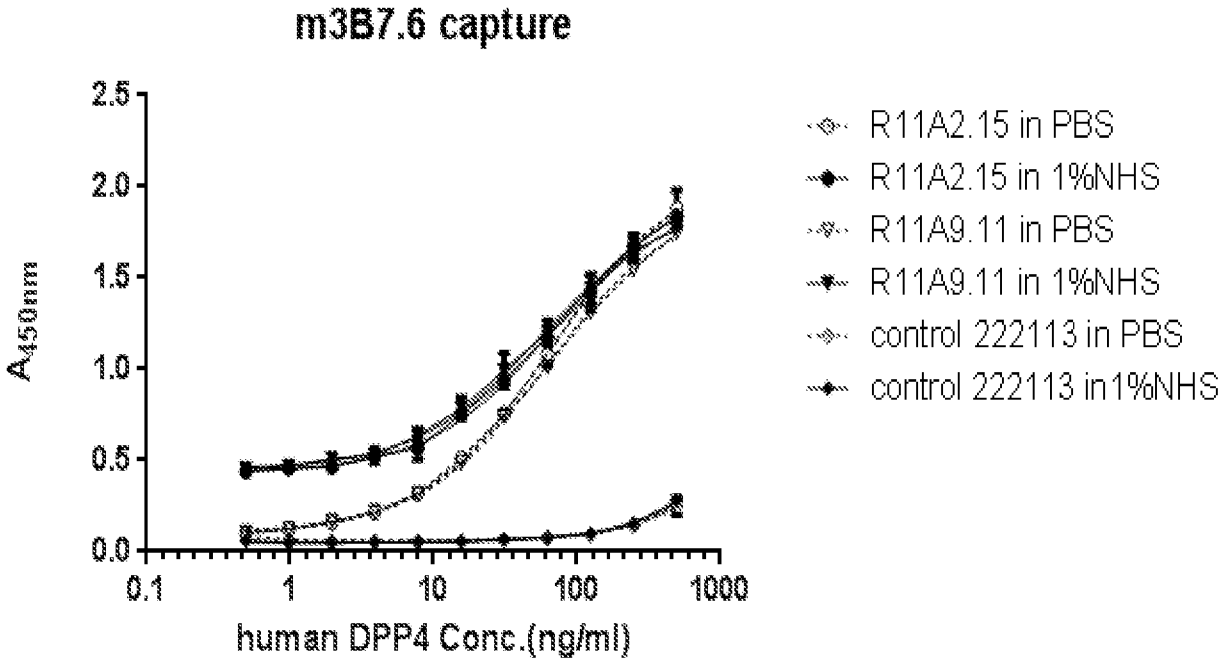
m3B7.6 capture
2.5
2.0
1.5
1.0
0.5
0.0
A450nm
0.1
1
10
100
1000
human DPP4 Conc.(ng/ml)
R11A2.15 in PBS
R11A2.15 in 1%NHS
R11A9.11 in PBS
R11A9.11 in 1%NHS
control 222113 in PBS
control 222113 in1%NHS

FIG. 3A

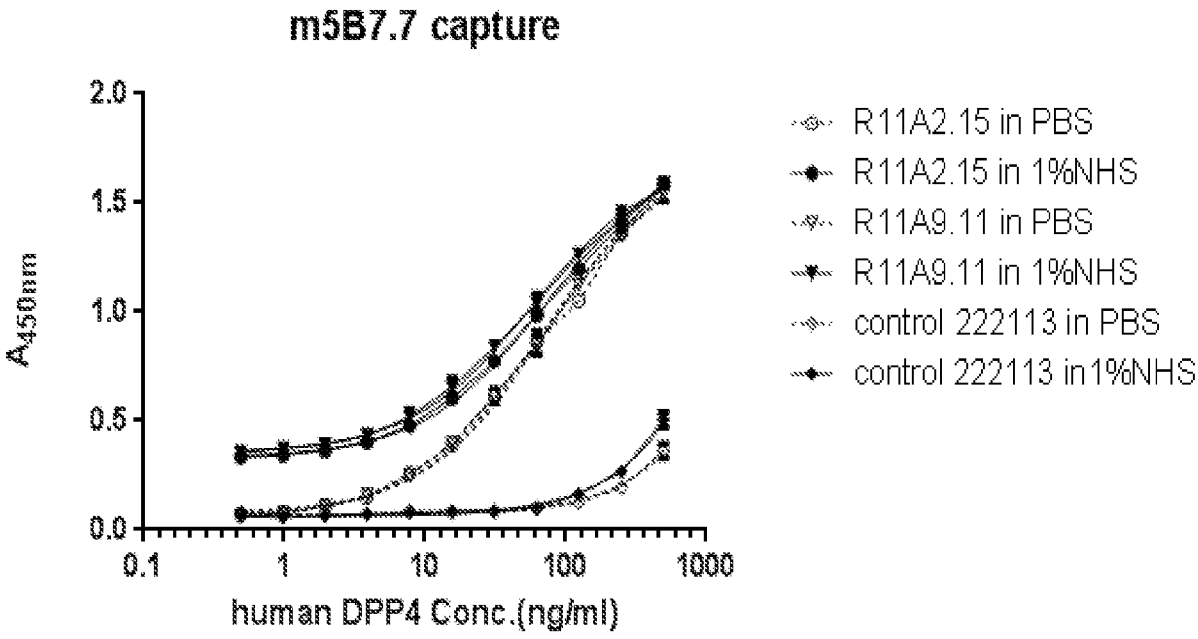
m5B7.7 capture
2.0
1.5
1.0
0.5
0.0
A_{450nm}
0.1
1
10
100
1000
human DPP4 Conc.(ng/ml)
R11A2.15 in PBS
R11A2.15 in 1%NHS
R11A9.11 in PBS
R11A9.11 in 1%NHS
control 222113 in PBS
control 222113 in1%NHS

FIG. 3B

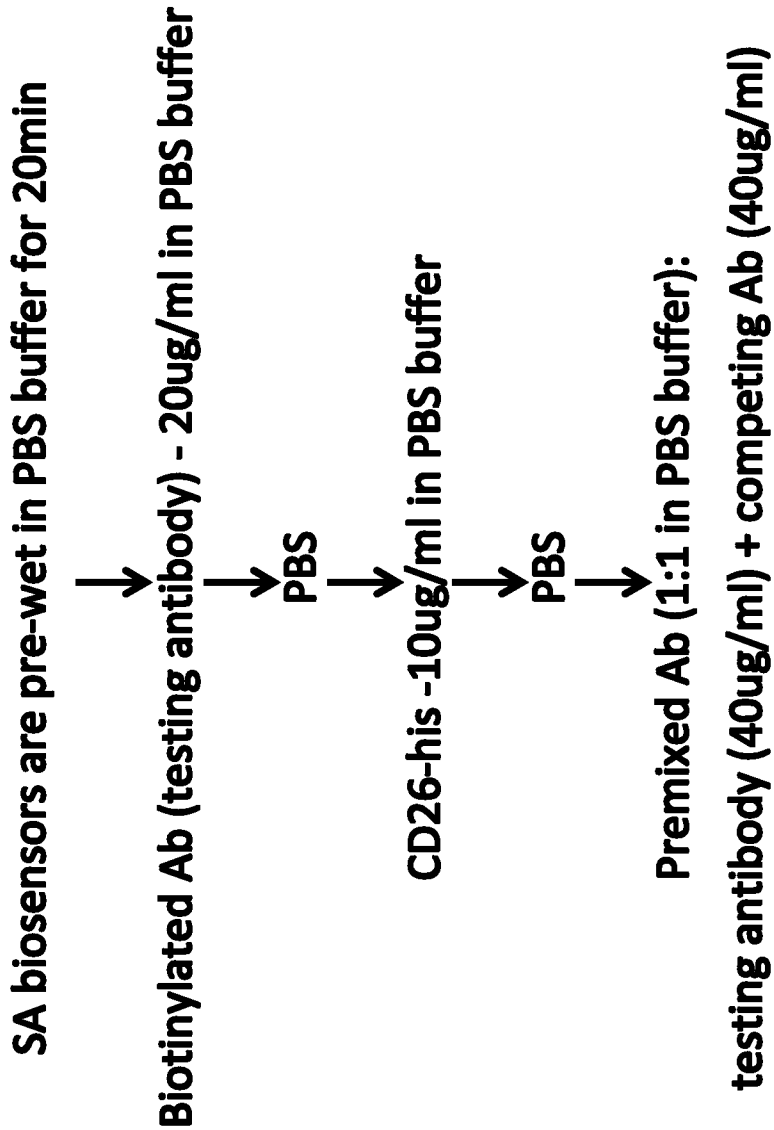
SA biosensors are pre-wet in PBS buffer for 20min
Biotinylated Ab (testing antibody) - 20ug/ml in PBS buffer
PBS
CD26-his -10ug/ml in PBS buffer
PBS
Premixed Ab (1:1 in PBS buffer):
testing antibody (40ug/ml) + competing Ab (40ug/ml)

FIG. 4A

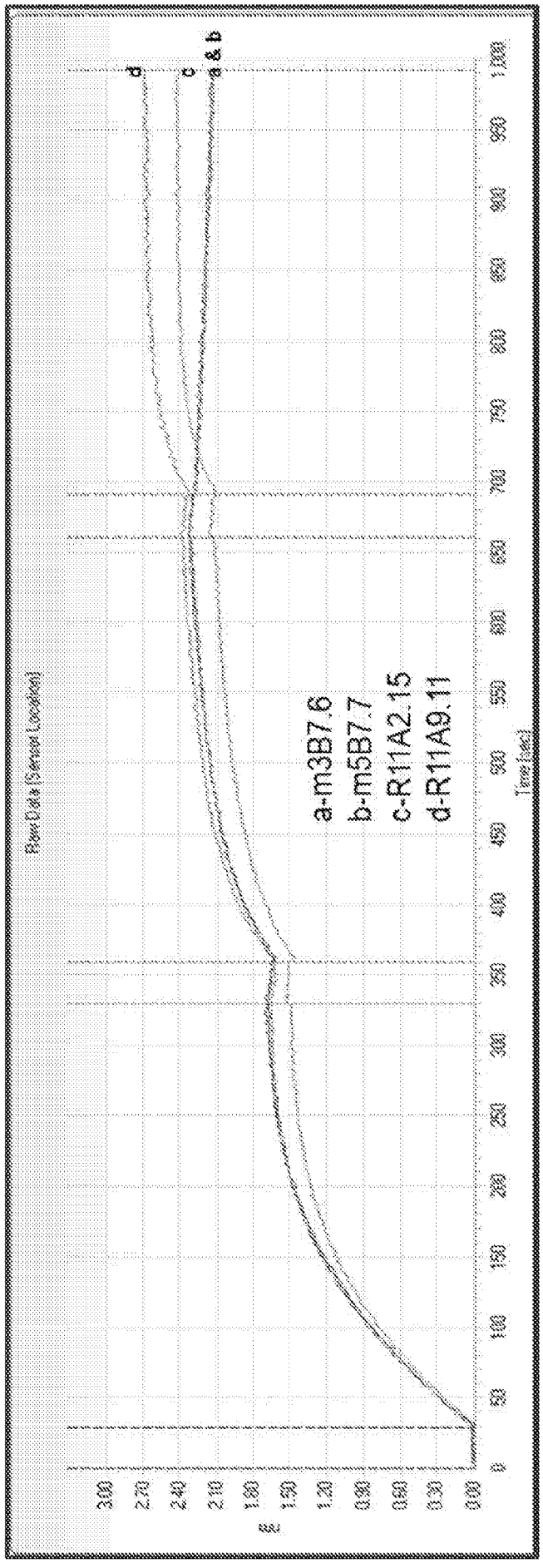
Raw Data (Sensor Location)
a-m3B7.6
b-m5B7.7
c-R11A2.15
d-R11A9.11
a & b
c
d
RU
Time (sec)

FIG. 4B

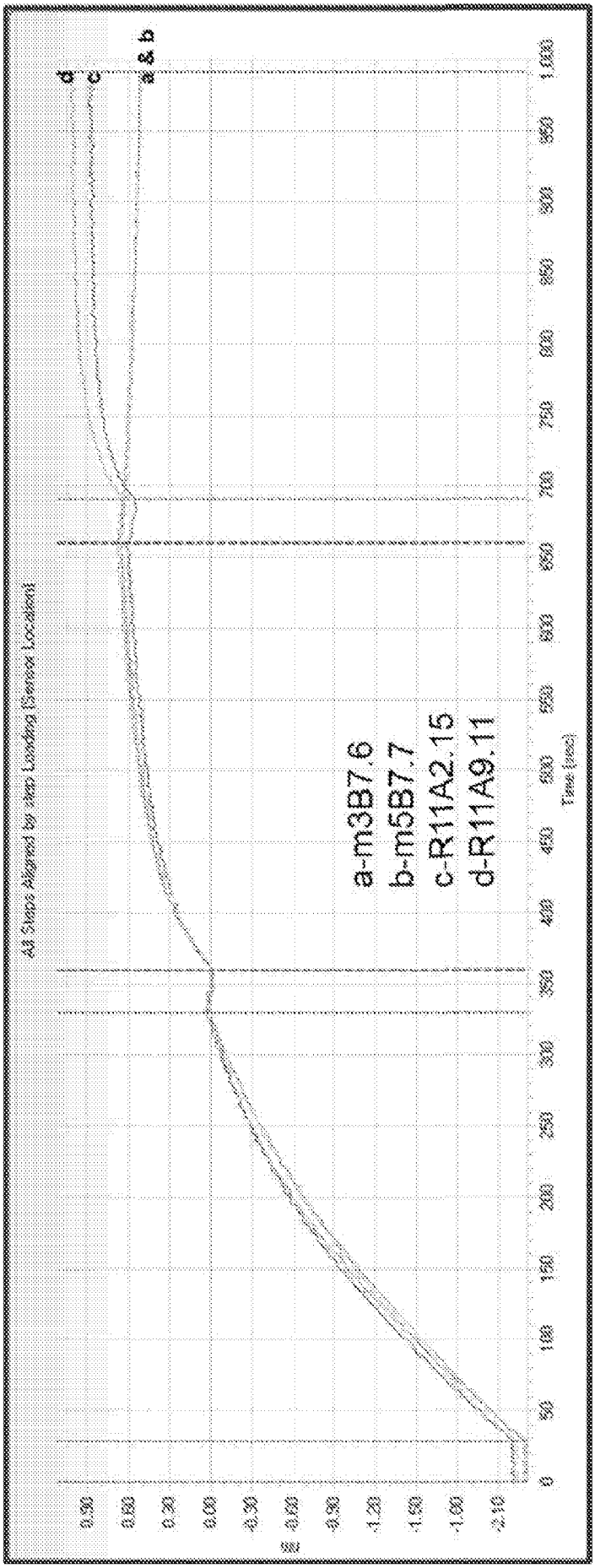
All Steps Aligned by Step Loading Stress Location
d
c
a & b
a-m3B7.6
b-m5B7.7
c-R11A2.15
d-R11A9.11
Time (sec)
SR

FIG. 4C

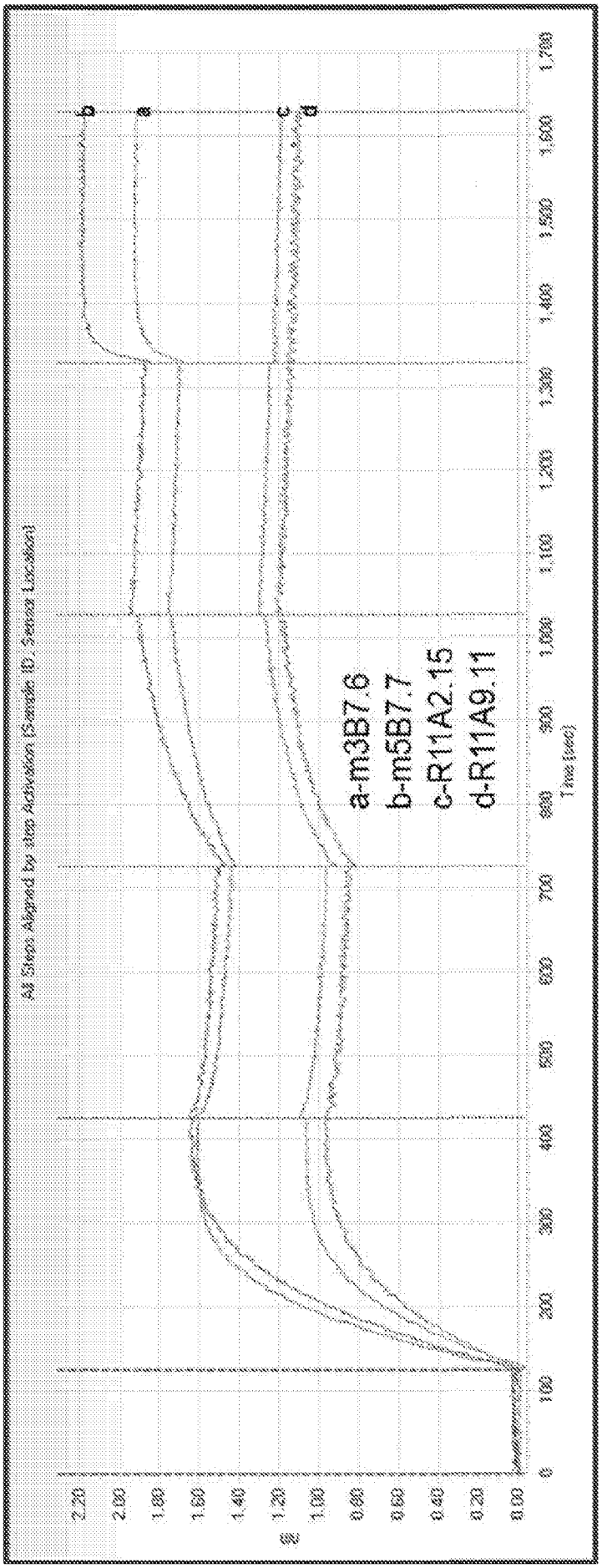
All Steps Aligned by step Activation (Sample ID, Sensor Location)
a-m3B7.6
b-m5B7.7
c-R11A2.15
d-R11A9.11
RU
Time (sec)

FIG. 4D

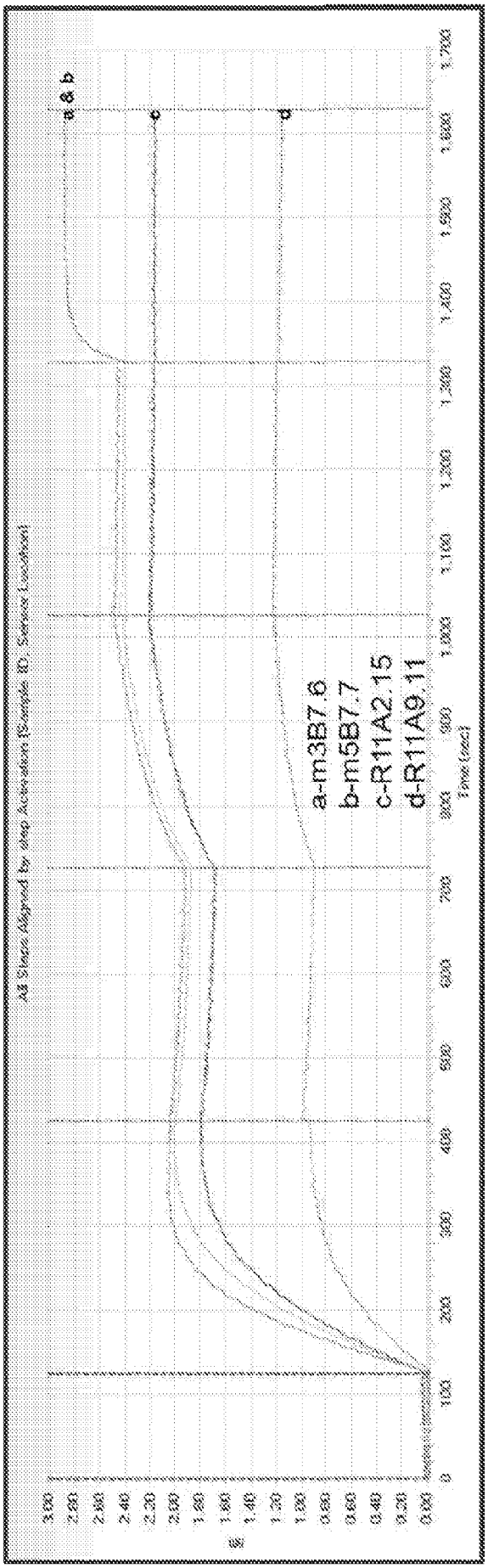
All Strain Aligned by Step Activation (Sample ID, Sensor Location)
a & b
c
d
a-m3B7.6
b-m5B7.7
c-R11A2.15
d-R11A9.11
Time (sec)

FIG. 4E

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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)

(21) International Application Number:
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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/101,697 9 January 2015 (09.01.2015) US

(71) Applicant: **MEDIMMUNE, LLC** [US/US]; One Medimmune Way, Gaithersburg, MD 20878 (US).

(72) Inventors: **CHANG, Chien-Ying**; c/o Medimmune, LLC, One Medimmune Way, Gaithersburg, MD 20878 (US). **XIAO, Xiaodong**; c/o Medimmune, LLC, One Medimmune Way, Gaithersburg, MD 20878 (US). **CHOWDHURY, Partha**; c/o Medimmune, LLC, One Medimmune Way, Gaithersburg, MD 20878 (US).

(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

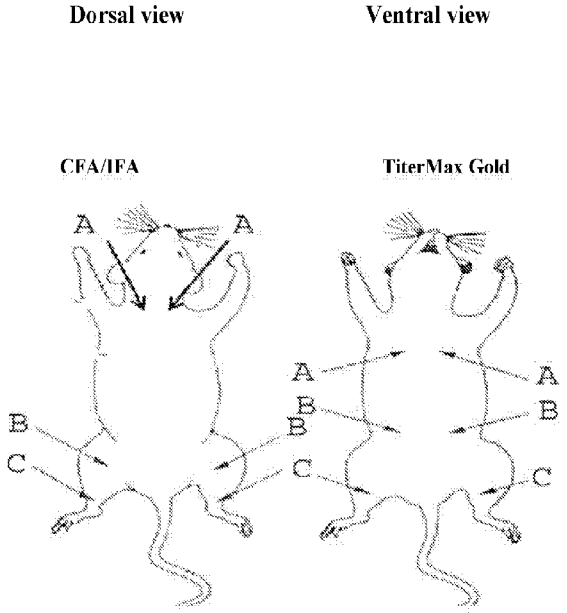

FIG. 1

(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.

WO 2016/112261 A3

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	Date of mailing of the international search report
25 June 2016	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

Form PCT/ISA/210 (second sheet) (January 2015)

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 RIIA2.15 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, ln 23-30, "mono specific antibodies which specifically bind an epitope of a human DPPIV (dipeptidyl peptidase IV/CD26) ... the anti-DPPIV antibodies E19 or E26"), and a hybridoma cells producing thereof (col 12, ln 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...

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。