LYSYL OXIDASE-LIKE 2 ASSAY AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of United States provisional application No. 61/492,210, filed June 01, 2011, United States provisional application No. 61/550,895, filed October 24, 2011, and United States provisional application No. 61/578,813, filed December 21, 2011, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0002] The entire content of the following electronic submission of the sequence listing via the USPTO EFS-WEB server, as authorized and set forth in MPEP §1730 II.B.2(a)(C), is incorporated herein by reference in its entirety for all purposes. The sequence listing is identified on the electronically filed text file as follows:

File Name	Date of Creation	Size (bytes)
246102008340Seqlist.txt	June 1, 2012	25,654 bytes

BACKGROUND

[0003] Lysyl oxidase-like 2 (LOXL2) is a protein of the extracellular matrix. Little extracellular LOXL2 is observed in healthy adult tissues, but its expression is induced in a variety of fibrotic diseases and tumors. It is secreted by activated fibroblasts, disease-associated smooth muscle cells, endothelial cells, and epithelia.

SUMMARY

[0004] The present disclosure relates to detection of lysyl oxidase-like 2 (LOXL2), *e.g.*, LOXL2 polypeptides, and use thereof in diagnostic, prognostic, and predictive methods. For example, provided are assays to detect and/or quantify LOXL2, such as assays to detect and/or quantify circulating lysyl oxidase-like 2 (LOXL2) polypeptides in an individual. Also provided are methods and uses of such assays in diagnostic, prognostic, and predictive applications and assay devices and kits for use in the same.

[0005] Provided are methods for detecting LOXL2, typically circulating LOXL2, in an individual. Among the provided methods are detection, diagnostic, prediction,

monitoring, and prognostic methods. In some examples, the methods are carried out by contacting a sample, generally a liquid sample, obtained from the individual with an antibody specific for LOXL2 and detecting binding of the antibody to polypeptide, *e.g.*, LOXL2 polypeptide, present in the sample. In some examples, the assay detects LOXL2 in the liquid sample to 300, 250, 200, 175 pg/mL or less or detects LOXL2 in the sample at a concentration of as low as 300, 250, 200, 175 pg/mL, for example, as low as from about 150 pg/mL to about 175 pg/mL, from about 125 pg/mL to about 150 pg/mL, from about 100 pg/mL to about 125 pg/mL, from about 75 pg/mL to about 100 pg/mL, from about 50 pg/mL to about 75 pg/mL, or from about 40 pg/mL to about 50 pg/mL.

[0006] In some examples, the detected LOXL2 level indicates the presence or absence of a disease or condition. In some examples, it indicates the likelihood that the individual will respond to a particular treatment for the disease, or indicates efficacy of a treatment. In some examples, such as where the methods are prognostic methods, the detected level of LOXL2 indicates the likelihood of an outcome, event, or endpoint of the disease or condition. In some aspects, the disease or condition is characterized by or associated with circulating LOXL2 or with elevated circulating LOXL2. In some aspects, the individual has the disease or condition; in some aspects, the individual is suspected of having the disease or condition. In some aspects, the methods further include determining that the individual has or does not have the disease or condition, is likely or not to respond to a particular treatment, or is likely or not to have a particular outcome or event, or that a treatment has or has not been effective.

[0007] In some examples, the individual is undergoing a treatment for the disease or condition and a detected level of LOXL2 that is lower than a level determined at an earlier time point, such as a pre-treatment level, indicates efficacy of the treatment.

[0008] The sample typically is a liquid sample, such as blood, a blood fraction, such as serum or plasma, urine, saliva, sputum, or bronchoalveolar lavage.

[0009] In some examples, the antibody includes a detectable label; exemplary labels include a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope. In some examples, the LOXL2 present in the sample is immobilized on an insoluble support by contacting the liquid sample with a second antibody specific for LOXL2 to form a second antibody-LOXL2 complex. In one example, the second antibody is immobilized on the insoluble support. In another example, the second antibody-LOXL2 complex is formed before contacting the sample with the antibody. The immobilized antibody may be polyclonal or monoclonal. In

some examples, the antibody binds LOXL2 when the LOXL2 is bound to an agent that inhibits enzymatic activity of the LOXL2, such as an allosteric inhibitor of LOXL2 enzymatic activity, *e.g.*, an anti-LOXL2 monoclonal antibody, such as one that binds an epitope within an SRCR3-4 domain.

[0010] Exemplary of the anti-LOXL2 antibodies for use in connection with the provided methods and embodiments include, for example, AB0023, AB0024, antibodies having a heavy chain variable region with an amino acid sequence as forth in SEQ ID NO: 6, 8, 10, 11, or 12, or with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO:6, 8, 10, 11, or 12, or with a CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 6, 8, 10, 11, or 12, and/or having a variable light chain region having the amino acid sequence set forth in SEQ ID NO: 7, 9, 13, or 14, or with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 7 or with a CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 7; 9, 13, or 14, such as an antibody with a heavy chain having the CDR1, CDR2, and/or CDR3 or the entire sequence of the variable region sequence set forth in SEQ ID NO: 8 and a light chain variable region with the CDR1, CDR2, and/or CDR3 or the entire sequence of the variable region sequence set forth in SEQ ID NO: 9.

[0011] In some examples, the methods further include comparing the detected level with a normal control value, where a detected level higher than a normal control value is indicative of the presence of the disease or condition, a likelihood that the individual will respond to a treatment for the disease or condition, or a likelihood of a pathological outcome. For instance, in some examples, the methods detect pathological levels of circulating LOXL2. Such methods can include comparing the detected level with a normal control or other reference value, where a detected level that is higher than a normal control or reference value is indicative of a pathology.

[0012] Also provided are methods for determining whether an individual has a disease or condition characterized by or associated with elevated circulating lysyl oxidase like-2 (LOXL2), diagnosing such a disease or condition, or making a predictive or prognostic determination regarding such a disease or condition. In examples, such methods are carried out by detecting a level of LOXL2 in a sample, *e.g.*, liquid sample, from the individual, for example, according to the assays and methods provided herein, such as those described above. Typically, a level of LOXL2 that is greater than a normal control level, reference level, or in some cases greater than baseline indicates that the individual has a

disease characterized by elevated circulating LOXL2, or indicates prognostic or predictive information about the disease or condition, such as predicting the likelihood of a particular outcome or the likelihood that the individual will respond to a particular disease treatment.

[0013] In some aspects of the provided methods, the disease or condition is fibrosis or cancer or a disease associated therewith. Examples include pulmonary fibrosis (such as idiopathic pulmonary fibrosis (IPF)), liver fibrosis, kidney fibrosis, cardiac fibrosis, myelofibrosis, cirrhosis, chronic viral hepatitis, hepatitis C virus (HCV) and hepatitis B virus (HBV). In some aspects, the disease or condition is idiopathic pulmonary fibrosis (IPF).

[0014] The methods can further include subjecting the individual to one or more further diagnostic tests, which can include pulmonary function tests, cardiac function tests, and liver function tests.

[0015] Also provided are methods for determining the likelihood that an individual having a fibrotic disease will exhibit a beneficial clinical response to a treatment for the fibrotic disease. Such methods can include determining a circulating level of lysyl oxidase like-2 (LOXL2), for example, in a liquid sample obtained from the individual, such as by the methods described above. In one aspect, a circulating level of LOXL2 that is greater than a normal control level indicates that the individual has an increased likelihood of exhibiting a beneficial clinical response to a treatment for the fibrotic disease. In some examples, reports are generated based on the determined likelihood. In some examples, the methods further include treating the individual for the fibrotic disease. In some examples, the individual has an active fibrotic disease, such as METAVIR F1 or F2 liver fibrosis, and/or an advanced stage fibrotic disease, such as METAVIR F4 liver fibrosis.

[0016] Also provided are methods for determining the efficacy of a treatment for a disease characterized by elevated lysyl oxidase like-2 (LOXL2) in an individual. In some examples, such methods are carried out by determining a circulating LOXL2 level at a time point in an individual undergoing treatment for the disease, according to the detection methods described above and herein. Typically, a level of circulating LOXL2 in the sample that is lower than a level obtained at an earlier time point, such as a pre-treatment level, from the individual indicates efficacy of the treatment. Alternatively, the level of circulating LOXL2 in the sample may increase initially followed by the clearance by the body.

[0017] Also among the provided methods are predictive and prognostic methods for idiopathic pulmonary fibrosis (IPF). In some examples, such methods are carried out by obtaining a sample from an individual; and detecting a level of LOXL2 in the sample, such

as using the methods described herein. Generally, the level of LOXL2 indicates the likelihood of an IPF disease outcome or event in the individual.

[0018] These and others of the provided methods can also include a step of comparing the detected level to a normal control level of LOXL2, where an elevated LOXL2 level compared to the normal control level indicates an increased likelihood of the occurrence of an IPF disease outcome or event in the individual. In some embodiments of the provided methods, a level of LOXL2 that is higher than a threshold baseline level correlates with the negative outcome or mortality in a subject. In one example, the threshold LOXL2 level in the sample is at least 800 picograms (pg) per milliliter (mL), at least 600 pg/mL, at least 400 pg/mL, or at least 200 pg/mL. In another example, the threshold LOXL2 level in the sample is at least 440 pg/mL. In one example, the method indicates at least a 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, or 7-fold increase in the likelihood of the IPF disease outcome in the individual compared with a subject having a LOXL2 level that is equal to the normal control LOXL2 level or baseline.

[0019] Among the IPF disease outcomes and events are IPF disease progression (such as that defined as mortality from any cause, respiratory hospitalization, or a categorical decrease in lung function), lung function decline, respiratory hospitalization, transplant-free survival, death, and responsiveness to treatment. In some cases, the methods predict an outcome, event, or endpoint, or the likelihood thereof, associated with IPF, in an individual. In some cases, the methods predict the outcome, endpoint, or likelihood thereof in an individual who has been deemed "negative" for such an output, endpoint, or likelihood by another method or assay, such as based on the Personal Clinical and Molecular Mortality index (PCMI) or level of one or more other biomarker, such as MMP7, ICAM1, IL8, VCAM1, and S100A12 (or for which such other method or assay does not detect or is incapable of detecting the outcome, event, endpoint, or likelihood thereof).

[0020] The predictive or prognostic IPF method can further include detecting a measure of IPF disease severity or functional status in the individual, selected from the group consisting of percent of predicted forced vital capacity (FVC), percent of predicted carbon monoxide diffusion capacity (DL_{CO}), 6-minute walk distance (6MWD), mean pulmonary artery pressure (mPAP), the lowest resting oxygen saturation (SpO2), the composite physiologic index (CPI), the St. George's Respiratory Questionnaire score (SGRQ), and the Transition Dyspnea Index (TDI) score, responsiveness to treatment, and biomarkers of IPF disease. In some examples, the methods further include analyzing the

LOXL2 level and/or measure of disease severity or functional status using a predictive model.

[0021] Also provided are methods for monitoring response of an individual to IPF treatment or determining the likelihood that the individual will respond to treatment. In one example, such methods are carried out by obtaining a sample from an individual undergoing treatment for IPF; and detecting a level of LOXL2 in the sample. Typically, the level of LOXL2 indicates the responsiveness of the individual to the treatment or the likelihood that the individual will respond to the treatment.

[0022] In some cases, the methods further include initiating, altering, or discontinuing an IPF treatment in the individual. In some examples, treatment is initiated, altered, or discontinued based on the information determined by the methods, such as the level or relative level of LOXL2 or the prognostic or predictive information. In some examples, the treatment is initiated prior to determination of the LOXL2 levels.

[0023] Also provided are assay devices and kits for use in the provided methods, such as for use for use in determining the level of a lysyl oxidase-like 2 (LOXL2) polypeptide in a liquid biological sample obtained from an individual. In one embodiment, such a device includes a matrix defining an axial flow path, the matrix including i) a sample receiving zone at an upstream end of the flow path that receives the fluid sample; ii) one or more test zones positioned within the flow path and downstream from the sample receiving zone, each of the one or more test zones comprising a LOXL2-specific antibody, wherein each of the LOXL2-specific antibodies is capable of binding a LOXL2 polypeptide present in the liquid sample to form an anti-LOXL2 antibody/LOXL2 complex; and iii) one or more control zones positioned within the flow path and downstream from the sample receiving zone.

[0024] The one or more control zones can be positioned between the test zones when two test zones are present. The test zones and control zones can be positioned in an alternating format within the flow path beginning with a test zone positioned upstream of any control zone. In one example, one or more of the anti-LOXL2 antibodies is immobilized on the matrix in the test zone.

[0025] In some examples, the device further includes a label zone including a labeled antibody specific for a LOXL2-specific antibody. Generally, the labeled antibody is capable of binding an anti-LOXL2 antibody present in an anti-LOXL2 antibody/LOXL2 complex to form a labeled anti-LOXL2 antibody/LOXL2, and the labeled antibody is mobilizable in the presence of liquid sample. The labeled antibody can include a label component selected

from among a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

[0026] In some examples of the devices, the matrix is positioned within a housing comprising a support and optionally a cover, wherein the housing contains an application aperture and one or more observation ports. Among the provided devices are test strips and dipstick assay devices.

[0027] Among the provided kits for determining the level of a lysyl oxidase-like 2 (LOXL2) polypeptide in a biological sample obtained from an individual are those including a first antibody specific for LOXL2 and a second antibody specific for LOXL2. The kit also can include purified LOXL2 for use in generating a standard curve. In one example, at least one of the antibodies in the kit includes a detectable label, such as a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Figure 1 depicts LOXL2 serum concentration versus Ishak fibrosis score for 87 patients with chronic hepatitis C virus (HCV) infection.

[0029] Figure 2 depicts LOXL2 levels (pg/ml) in serum samples from patients diagnosed with liver fibrosis.

[0030] Figure 3 depicts LOXL2 levels in serum samples from patients with idiopathic pulmonary fibrosis.

[0031] Figure 4 provides an amino acid sequence of human LOXL2 (SEQ ID NO:1).

[0032] Figure 5 shows an alignment of the amino acid sequences of the catalytic domains of LOXL2 proteins from human (H) (SEQ ID NO: 2), mouse (M) (SEQ ID NO: 3), rat (R) (SEQ ID NO: 4) and cynomolgus monkey (C) (SEQ ID NO: 5). Residues in the mouse, rat, and cynomolgus monkey protein, which differ from that of the human protein, are indicated by underlining.

[0033] Figure 6 shows expression of LOXL2 in human fibrotic liver tissue, as determined by Immunohistochemical (IHC) staining of liver tissues from a patient with chronic HCV infection. In the left panel (5x objective magnification), black arrows indicate areas of fibrous expansion into portal regions and tracts. White arrows indicate areas of short fibrous septa surrounding hepatic lobules. The right panel (40x objective magnification) shows LOXL2 immunoreactivity, observed in the fibrous septa (S) at the interface with hepatocytes

(H), within the perisinusoidal space (arrows), and in the myofibroblasts within the liver parenchyma (arrows).

[0034] Figure 7 shows Standard calibrator curves for LOXL2 immunoassay, with raw ECL (electrochemiluminescence) counts plotted on the y-axis and LOXL2 concentration (nM/L) plotted on the x-axis. Purified recombinant full-length LOXL2 protein was added into pooled normal human serum, followed by serial dilution in serum to create a calibrator curve. Each data point represents the mean of three replicate wells; curves for four independent plates are shown.

[0035] Figure 8 shows LOXL2 serum levels by binned baseline Ishak fibrosis score and time. Each panel shows, for the indicated time point, LOXL2 concentration (pg/mL) for two groups of patients, grouped according to Ishak Fibrosis Score (1-3 and 5-6, respectively). Three outliers (LOXL2 concentration = 5529, 6621, 8845 pg/mL), with LOXL2 concentration out of plot ranges all were from the same subject, having an Ishak fibrosis score of 5.

[0036] Figure 9 shows median within-subject LOXL2 serum levels, calculated as median LOXL2 serum concentration over weeks 4-30, for two groups of patients, grouped according to Ishak Fibrosis Score (1-3 and 5-6, respectively). The average within-subject coefficient of variation was 22 %.

[0037] Figure 10 shows median LOXL2 serum concentration (pg/mL) over time (weeks), by binned baseline ishak fibrosis score, with 95% confidence intervals. Only one subject had a change greater than or equal to 2 in Ishak fibrosis score over the 25-28 weeks between study biopsies.

[0038] Figure 11 shows median within-subject levels of LOXL2 vs. levels of Hyaluronic acid (HA) (left panel) and tissue inhibitor of metalloproteinases-1 (TIMP1) (right panel), for subjects having the indicated Ishak scores (1-6). Median within–subject expression was calculated as median expression over weeks 4 through 30. The curve was constructed using locally weighted scatter plot smoothing.

[0039] Figure 12 shows scatter plot matrices demonstrating correlation between baseline LOXL2 levels (with untransformed LOXL2 levels in panel (a) and $Log_{10}X$-transformed LOXL2 levels in panel (b)) and baseline measures of idiopathic pulmonary fibrosis (IPF) severity and functional status, as described in Example 9. In each panel, the x- and y-axis of the first row and column, respectively, represent baseline LOXL2 levels; the x- and y-axis of the second row and column, respectively, represent baseline predicted forced vital capacity (FVC); the x-and y-axis of the third row and column, respectively, represent baseline percent

of predicted carbon monoxide diffusion capacity (DL_{CO}); the x- and y-axis of the fourth row and column, respectively, represent the baseline 6-minute walk distance (6MWD); the x- and y-axis of the fifth row and column, respectively, represent the baseline composite physiologic index (CPI); the x- and y-axis of the sixth row and column, respectively, represent the baseline St. George's Respiratory Questionnaire score; and the x- and y-axis of the seventh row and column, respectively, represent the baseline Transition Dyspnea Index score. Correlation between LOXL2 and baseline measures of IPF severity and performance status are highlighted within the dark boxes at the top row of panels (a) and (b).

[0040] Figure 13 shows Kaplan Meier curves, comparing low (≤ 800 pg/mL) and high (> 800 pg/mL) LOXL2 levels for disease progression (PFS) (panel (a)) and its components: lung function decline (panel (b)), respiratory hospitalizations (panel (c)) and death (panel (d)). In each panel, the top, darker line represents patients with low (≤ 800 pg/mL) baseline serum LOXL2 levels and the lower, lighter line represents patients with high (> 800 pg/mL) baseline LOXL2 levels. All patients were treated with ambrisentan. Each y-axis shows percent of patients without the given event (with 0, 25, 50, 75, and 100 marked along the axis) and each x-axis shows time in days (with 0, 100, 200, 300, 400, 500, 600, 700, and 800 days marked along the axis).

[0041] Figure 14 shows a comparison of baseline LOXL2 distribution in the ARTEMIS-IPF subjects (14A: placebo and Ambrisentan-treated subjects combined; 14B: Ambrisentan only) and the GAP cohort subjects.

[0042] Figure 15A shows Kaplan Meier curves for all-cause mortality according to low (upper line, ≤ 440 pg/mL) versus high (lower line, > 440 pg/mL) serum LOXL2 levels at 6-months (upper left panel), 12-months (upper right panel), 18-months (lower left panel) and 24-months (lower right panel) after baseline in the GAP cohort study. Figure 15B shows Kaplan Meier curves for all-cause mortality according to low (upper line, ≤ 800 pg/mL) versus high (lower line, > 800 pg/mL) serum LOXL2 levels at 6-months (upper left panel), 12-months (upper right panel), 18-months (lower left panel) and 24-months (lower right panel) after baseline in the ARTEMIS-IPF study.

[0043] Figure 16 shows mean serum LOXL2 levels (pg/mL) for various groups of subjects. Figure 16A shows mean serum LOXL2 levels for baseline and week 240 samples (total of 162 samples (one baseline and one week-240 for each of 81 subjects), grouped according to Ishak fibrosis score of the corresponding subject (0, 1, 2, 3, 4, 5, 6, left-right). LOQ = level of quantification. Figure 16B shows baseline and week-240 mean serum LOXL2 levels for subjects with given Ishak stages (0, 1, 2, 3, 4, 5, 6, left-right) at baseline and week 240.

Figure 16C shows baseline, week-240, and overall serum levels of LOXL2 for patients with corresponding Ishak stages of between 1 and 3 and between 4 and 6.

[0044] Figure 17 shows the percentage of subjects in the study with each given Ishak Stage (1, 2, 3, 4, 5, 6 (individual bars left to right)) that were determined to have a given level of serum LOXL2 (pg/mL). LOD=limit of detection; LOQ=limit of quantification. Each category shown extended from the upper limit of the previous category, for example, 1500=1001-1500 pg/mL.

[0045] Figure 18 shows serum LOXL2 levels (pg/mL) at baseline and week 240 following treatment for individual CHB subjects. Figure 18A: subjects with persistent cirrhosis (n=16); Figure 18B: subjects with reversal of cirrhosis by week 240 (n=42); Figure 18C: non-cirrhotic subjects that did not experience a change in fibrotic stage (Ishak) by week 240); Figure 18D: subjects that experienced a progression to cirrhosis over the course of the study; and Figure 18E**:** non-cirrhotic subjects with greater than or equal to 2-stage reduction in fibrosis (Ishak score). LOQ (limit of quantification)=440 pg/mL, LOD (limit of detection)=180 pg/mL.

[0046] Figure 19 shows the percentage of cirrhotic CHB subjects that exhibited a histological improvement at week 240 ("Y") having given baseline serum LOXL2 levels (<1500, >1500, 1500-3000, <3000, and >3000 pg/mL) and the percentage of cirrhotic subjects determined not to have histological improvement at week 240 ("N") having the same given baseline serum LOXL2 levels.

DEFINITIONS

[0047] As used herein, the term "antibody" means an isolated or recombinant binding agent that comprises the necessary variable region sequences to specifically bind an antigenic epitope. Therefore, an antibody is any form of antibody or fragment thereof that exhibits the desired biological activity, *e.g.*, binding the specific target antigen. Thus, it is used in the broadest sense and specifically covers monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, nanobodies, diabodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments including but not limited to scFv, Fab, and Fab_2, so long as they exhibit the desired biological activity. The term "human antibody" therefore refers to antibodies containing sequences of human origin, except for possible non-human complementarity-determining regions (CDR) regions, and does not imply that the full

structure of an Ig molecule be present, only that the antibody has minimal immunogenic effect in a human.

[0048] "Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0049] "Fv" is an antibody fragment that contains a complete antigen-recognition and -binding site, and consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0050] The "Fab" fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0051] As used herein, the term "biological sample" can refer to a variety of sample types obtained from an individual that can be used in a detection, diagnostic, prognostic, or monitoring assay. A liquid biological sample can include, for example, blood, a blood fraction (e.g., serum or plasma), urine, saliva, bronchoalveolar lavage, sputum, or cerebrospinal fluid. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins.

[0052] "Axial flow" as used herein refers to lateral, vertical or transverse flow through a particular matrix or material comprising one or more test and/or control zones. The type of flow contemplated in a particular device, assay or method varies according to the structure of the device. Without being bound by theory, lateral, vertical or transverse flow may refer to flow of a fluid sample from the point of fluid contact on one end or side of a particular matrix (the upstream or proximal end) to an area downstream (or distal) of this contact. The downstream area may be on the same side or on the opposite side of the matrix from the point of fluid contact. For example, in vertical flow devices of certain embodiments of the present invention, axial flow may progress vertically from and through a first member (top to bottom) to a second member and from there on to an absorbent medium. By way of further example, and as will be appreciated by those of skill in the art, in a vertical flow device configured, for example, as a dipstick, a fluid sample may flow literally up the device, in which case however, the point of first contact of the fluid sample to the device is nonetheless considered the upstream (i.e., proximal) end and the point of termination of flow the downstream (i.e., distal) end.

[0053] As used herein the terms "upstream" and "downstream," in the context of axial flow, refer to the direction of fluid sample flow subsequent to contact of the fluid sample with a representative device of the present disclosure, wherein, under normal operating conditions, the fluid sample flow direction runs from an upstream position to a downstream position. For example, when fluid sample is initially contacted with the sample receiving zone, the fluid sample then flows downstream through the label zone and so forth.

[0054] Before embodiments of the present invention are further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0055] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included.

[0056] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of embodiments of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0057] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a LOXL2-specific antibody" includes a plurality of such antibodies and reference to "the LOXL2 polypeptide" includes reference to one or more LOXL2 polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0058] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various provided features, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the provided embodiments are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0059] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0060] The present disclosure provides an assay to detect and/or quantify LOXL2, generally circulating lysyl oxidase-like 2 (LOXL2) polypeptides in an individual. The assay is useful in diagnostic and prognostic applications, which are also provided.

[0061] Lysyl oxidase-like 2 (LOXL2) is expressed in fibrotic human liver tissue where it carries out cross-linking of collagen and other matrix components, resulting in increased stiffness, activation of pathologic fibroblasts and a dynamic process of matrix remodeling and fibrogenesis. Barry-Hamilton V, Spangler R, Marshall D, et al., "Allosteric inhibition of lysyl oxidase–like-2 impedes the development of a pathologic microenvironment," *Nat Med.* 2010. 16: 1009–1017. LOXL2 is expressed in fibrotic liver tissue from human diseases of diverse etiology, including hepatitis C infection1, non-alcoholic steatohepatitis (NASH)1, alcoholic steatohepatitis (ASH), Wilson's disease (Vadasz Z, Kessler O, Akiri G, et al., "Abnormal deposition of collagen around hepatocytes in Wilson's disease is associated with hepatocyte specific expression of lysyl oxidase and lysyl oxidase like protein-2," *J Hepatology*. 2005. 43: 499–507), and primary biliary cirrhosis2, in addition to mouse models of sclerosing cholangitis. Nakken KE, Nygard S, Haaland T, et al. "Multiple inflammatory-, tissue remodelling- and fibrosis genes are differentially transcribed in the livers of Abcb4 (– / –) mice harbouring chronic cholangitis," *Scand J Gastroent.* 2007. 42: 1245–1255.

[0062] Allosteric inhibition of LOXL2 using a monoclonal antibody is efficacious in inhibiting fibrosis in a variety of disease models, including models of liver and lung fibrosis. Inhibition of LOXL2 resulted in the down-regulation of TGFβ signaling and several key pro-fibrotic mediators (e.g. TGF-β1, CTGF, endothelin, CXCL12)1; LOXL2 is a core pathway target in fibrotic disease. Mehal WZ, Iredale J, & Friedman SL., "Expressway to the core of fibrosis," *Nat Med.* 2011. 17: 552–553.

[0063] Gradual accumulation of collagen in the hepatic parenchyma is a final common pathway of chronic liver disease. This progressive accumulation of fibrosis can ultimately lead to cirrhosis of liver and end-stage liver disease. LOXL2 catalyzes the cross linking of collagen fibrils and is a core regulatory protein of fibrogenesis. LOXL2 expression is increased in diseased liver tissue.

[0064] There is little LOXL2 expression in healthy adult tissues; and under normal (e.g., non-disease) conditions, the amount of circulating LOXL2 is low or undetectable. Under certain disease conditions, circulating LOXL2 is elevated. For example, LOXL2 can be elevated in the serum of patients with chronic liver disease, such as in chronic hepatitis C patients, with greater levels in patients with more advanced fibrosis. Detection of circulating LOXL2 is thus useful for determining whether an individual has a disease that results in elevated circulating LOXL2 levels. Such diseases include fibrosis and cancer. The present disclosure provides diagnostic methods for determining whether an individual has a disease associated with elevated circulating LOXL2 levels. Detection of circulating LOXL2 can be

followed up with other diagnostic methods, to confirm a diagnosis or to exclude the possibility that an individual has a particular disease.

[0065] It has been found that the level of circulating LOXL2 correlates with the stage of fibrosis.

[0066] It has also been found that the level of circulating LOXL2 can provide an indication as to whether an individual having fibrosis is amenable to treatment for the fibrosis and provide other prognostic and predictive information regarding disease, such as the likelihood of a particular endpoint, outcome, or event, such as disease outcome or responsiveness to treatment. The present disclosure provides methods for determining the likelihood that an individual will respond to treatment for a fibrotic disease and/or the likelihood of such and outcome, endpoint, or event.

[0067] Treatment decisions for patients with HCV infection are increasingly based on non-invasive serum tests rather than liver biopsies. However, serum tests have not been entirely optimal. *See* Castera, L., "Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease," *Best Pract Res Clin Gastroent.* 2011. 25: 291–303.

METHODS FOR DETECTING CIRCULATING LOXL2

[0068] The present disclosure provides an assay to detect and/or quantify circulating LOXL2 polypeptides in an individual. In practice, LOXL2 is detected in a liquid sample obtained from an individual being tested, where the liquid sample can be blood or a blood fraction such as plasma or serum, or other liquid sample.

[0069] In some embodiments, the provided methods and assays are useful for non-invasive surrogate measurement of the degree of liver fibrosis, such as in patients with chronic HCV infection or HBV infection.

LOXL2 polypeptide

[0070] A "LOXL2 polypeptide" refers to a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids (aa) to about 200 aa, from about 200 aa to about 300 aa, from about 300 aa to about 400 aa, from about 400 aa to about 500 aa, from about 500 aa to about 600 aa, from about 600 aa to about 700 aa, or from about 700 aa to 774 aa, of the amino acid sequence depicted in Figure 4. "LOXL2" also refers to the human LOXL2 amino acid sequence depicted in Figure 4, and naturally-occurring variants (polymorphisms) thereof.

[0071] Figure 4 depicts an amino acid sequence of human LOXL2, showing the four scavenger receptor cysteine rich (SRCR) domains. A LOXL2 polypeptide can be a full-length polypeptide or a mature (cleavage form; processed form) LOXL2 polypeptide. The predicted signal cleavage is between Ala25-Gln26. Cleavage of the signal peptide from the prepropeptide results in a LOXL2 propeptide. LOXL2 propeptide is cleaved between SRCR2 and SRCR3 (e.g., between amino acids 301 and 326 of the sequence depicted in Figure 4), leaving a LOXL2 polypeptide comprising SRCR3, SRCR4, and the lysyl oxidase (catalytic) domain.

[0072] A LOXL2 polypeptide may be enzymatically active. For example, a LOXL2 polypeptide can catalyze oxidative deamination of ε-amino groups of lysine and hydroxylysine residues, resulting in conversion of peptidyl lysine to peptidyl-α-aminoadipic-δ-semialdehyde (allysine) and the release of stoichiometric quantities of ammonia and hydrogen peroxide. This reaction most often occurs extracellularly, e.g., on lysine residues in collagen and elastin.

[0073] In some cases, the LOXL2 polypeptide that is detected using a subject LOXL2 assay is a full-length LOXL2 polypeptide without the signal sequence, e.g., including SRCR1-2, SRCR3-4, and the catalytic domain. In some instances, the LOXL2 polypeptide that is detected using a subject LOXL2 assay is a mature LOXL2 polypeptide (i.e., without the signal sequence and without SRCR1-2), including only the SRCR3-4 domain and the catalytic domain. Alternatively, or in addition to, detecting the mature LOXL2 polypeptide (SRCR3-4 and catalytic domains; without the signal sequence and SRCR1-2 domains), a subject LOXL2 assay can detect an N-terminal LOXL2 fragment, which N-terminal LOXL2 fragment includes the SRCR1-2 domains and not the SRCR3-4 or catalytic domains.

Biological samples

[0074] Suitable liquid biological samples include, but are not limited to, whole blood; blood fractions (also referred to as "blood products"), where suitable blood fractions include, but are not limited to, serum and plasma; saliva; urine; bronchoalveolar lavage; cerebrospinal fluid; sputum; and the like. The biological sample can be fresh blood or stored blood (e.g. in a blood bank) or blood fractions. The biological sample can be a liquid sample expressly obtained for an assay of the present disclosure or a liquid sample obtained for another purpose which can be subsampled for an assay of the present disclosure.

[0075] As one example, the biological sample can be whole blood. Whole blood can be obtained from the subject using standard clinical procedures. In another embodiment, the biological sample is plasma. Plasma can be obtained from whole blood samples by

centrifugation of anti-coagulated blood. Such process provides a buffy coat of white cell components and a supernatant of the plasma. In another embodiment, the biological sample is serum.

[0076] The sample can be pretreated as necessary by dilution in an appropriate buffer solution, heparinized, concentrated if desired, or fractionated by any number of methods including but not limited to ultracentrifugation, fractionation by fast protein liquid chromatography (FPLC), or precipitation. The sample can be fractionated, e.g., by an immunoaffinity method, to remove one or more non-LOXL2 proteins or other non-LOXL2 components from the sample; e.g., an anti-albumin antibody can be used to remove albumin from the sample. Any of a number of standard aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH can be used.

Anti-LOXL2 antibodies

[0077] A subject method uses antibody specific for LOXL2 to immobilize and detect LOXL2 in a liquid sample. The antibody used in a subject assay method is specific for LOXL2, e.g., the antibody binds specifically to a LOXL2 polypeptide, where specific binding refers to binding with an affinity of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, or at least about 10^{-12} M, or greater than 10^{-12} M. Non-specific binding would refer to binding with an affinity of less than about 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

[0078] A LOXL2-specific antibody does not substantially bind to any other lysyl oxidase-like polypeptide other than a LOXL2 polypeptide, e.g., a LOXL2-specific antibody does not substantially bind to a LOXL1, LOXL3, or LOXL4 polypeptide, or to a lysyl oxidase (LOX) polypeptide.

[0079] In some embodiments, a LOXL2-specific antibody binds an epitope(s) that is accessible for binding when the LOXL2 polypeptide is in a liquid biological sample, e.g., the epitope(s) bound by the LOXL2-specific antibody is surface accessible and/or not masked by one or more non-LOXL2 proteins that may be present in the liquid biological sample.

[0080] Antibodies suitable for use in a subject assay method include polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, nanobodies, diabodies, multispecific antibodies (e.g., bispecific antibodies), and antigen-binding antibody fragments.

[0081] In some cases, an anti-LOXL2 antibody used in a subject method comprises a detectable label. Suitable detectable labels include, but are not limited to, magnetic beads (e.g. Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine,

a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase, luciferase, and other enzymes commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

[0082] Where an anti-LOXL2 antibody comprises a detectable label, the anti-LOXL2 antibody can be detected by detecting a signal produced by the label (e.g., a chromophore, luminophore, etc., produced as a product of an enzyme attached to the anti-LOXL2 antibody; a signal produced directly by the label; etc.). In some cases, an anti-LOXL2 antibody does not comprise a detectable label; instead, the anti-LOXL2 antibody is detected using a secondary antibody comprising a detectable label. Suitable secondary antibodies include monoclonal and polyclonal antibodies specific for epitope(s) in the constant region domain(s) of an anti-LOXL2 antibody. A secondary antibody can comprise any of a variety of detectable labels, including, but not limited to, magnetic beads (e.g. Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase, luciferase, and other enzymes commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads. Also suitable for use as a detectable label is the SULFO-TAG™ label from MesoScale Discovery. The SULFO-TAG™ label is a ruthenium(II) tris-bipyridal tag, which can be attached to a polypeptide (e.g., a secondary antibody) via reaction of Ruthenium (II) tris-bipyridine-(4-methylsulfone) N-hydroxysuccinimide (NHS)-ester with a primary amine (e.g., a lysine side chain).

[0083] In some instances, an anti-LOXL2 antibody used in a subject assay method will be immobilized on an insoluble support. Suitable insoluble supports can comprise various materials including, but not limited to, polyvinyl difluoride (PVDF), cellulose, nitrocellulose, nylon, glass, polystyrene, polyvinyl chloride, polypropylene, silicon dioxide, polyethylene, polycarbonate, dextran, amylose, natural and modified celluloses, polyacrylamides, silica embedded in a polyacrylamide gel, agaroses, gabbros, magnetite, and the like. The insoluble support can be in any of a variety of formats (e.g., dimensions, shapes), e.g., sheets, such as used in a test strip; a dipstick assay format; a multi-well plate (e.g., such as those used in an ELISA); and the like.

[0084] Non-limiting examples of LOXL2-specific antibodies include the LOXL2-specific antibodies disclosed in U.S. Patent Publication No. 2009/0104201, and U.S. Patent Publication No. 2009/0053224.

[0085] In some instances, a suitable antibody specifically binds an epitope in the LOXL2 SRCR1 domain. In some instances, a suitable antibody specifically binds an epitope in the LOXL2 SRCR2 domain. In some instances, a suitable antibody specifically binds an epitope in the LOXL2 SRCR3 domain. In some instances, a suitable antibody specifically binds an epitope in the LOXL2 SRCR4 domain. In some instances, a suitable antibody specifically binds an epitope in the LOXL2 catalytic domain. Figure 5 provides amino acid sequences of LOXL2 catalytic domains. In some instances, a suitable antibody (e.g., a polyclonal antibody) specifically binds multiple epitopes in one, two, three, or more LOXL2 domains.

[0086] In some cases, an antibody detects a full-length LOXL2 polypeptide without the signal sequence, e.g., including SRCR1-2, SRCR3-4, and the catalytic domain. In some instances, an antibody detects mature LOXL2 polypeptide (i.e., without the signal sequence and without SRCR1-2), including only the SRCR3-4 domain and the catalytic domain. In other instances, an antibody detects an N-terminal LOXL2 fragment, which N-terminal LOXL2 fragment includes the SRCR1-2 domains and not the SRCR3-4 or catalytic domains.

[0087] For example, in some embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR3-linker-SRCR4 region, where such region is referred to as "SRCR3-4." An SRCR3-4 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 325 to 544, with amino acids 325 to 547, with amino acids 303 to 544, or with amino acids 303 to 547, of SEQ ID NO:1. Thus, e.g., in some embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 325 to 544, with amino acids 325 to 547, with amino acids 303 to 544, or with amino acids 303 to 547, of SEQ ID NO:1.

[0088] In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the linker-SRCR3-linker-SRCR4-linker region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 303 to 544, amino acids 303 to 545, amino acids 303 to 546, or amino acids 303 to 547 of SEQ ID NO:1. In certain embodiments, a subject

anti-LOXL2 antibody specifically binds an epitope within the SRCR3-linker-SRCR4-linker region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 325 to 544, amino acids 325 to 545, amino acids 325 to 546, or amino acids 325 to 547, of SEQ ID NO:1.

[0089] In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR3 region (and not within SRCR4), where an SRCR3 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 325 to 425, with amino acids 303 to 425, with amino acids 303 to 434, or with amino acids 325 to 434, of SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the linker-SRCR3 region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 303 to 425 of SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR3-linker region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 325 to 434 of SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the linker-SRCR3-linker region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 303 to 434 of SEQ ID NO:1.

[0090] In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the linker-SRCR4-linker region, e.g., in some cases a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 426 to 544, amino acids 426 to 545, amino acids 426 to 546, or amino acids 426 to 547, of SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR4 region (and not within SRCR3), where an SRCR4 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid

sequence identity with amino acids 435 to 544, amino acids 435 to 545, amino acids 435 to 546, or with amino acids 435 to 547, of SEQ ID NO:1.

[0091] In some embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR1-linker-SRCR2 region, where such region is referred to as "SRCR1-2." An SRCR1-2 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 58 to 302, or 58 to 324, of the amino acid sequence depicted in SEQ ID NO:1 (Figure 4). In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 58 to 324 of the amino acid sequence depicted in SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR1 region (and not within SRCR2), where an SRCR1 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 58 to 159 of the amino acid sequence depicted in SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR1-linker region, where an SRCR1-linker region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 58 to 187 of the amino acid sequence depicted in SEQ ID NO:1. In certain embodiments, a suitable anti-LOXL2 antibody specifically binds an epitope within the SRCR1 region (and not within SRC2), where an SRCR2 region can comprise an amino acid sequence that has at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity with amino acids 188 to 302 of the amino acid sequence depicted in SEQ ID NO:1.

[0092] As one non-limiting example, a suitable antibody is monoclonal antibody AB0030, which binds specifically an epitope in the LOXL2 catalytic domain. See, e.g., US 2009/0053224, where antibody AB0030 corresponds to proBM20.

[0093] In some embodiments, a suitable antibody is one that specifically binds LOXL2 when LOXL2 is bound to an agent that inhibits LOXL2 enzymatic activity. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an "SRCR3-4" domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic

activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224. Thus, in some embodiments, a suitable anti-LOXL2 antibody: a) specifically binds an epitope within SRCR3-4; and ii) does not compete with an AB0023 antibody and/or an AB0024 antibody for binding to an epitope within SRCR3-4.

[0094] For example, in some embodiments, the antibody is an antibody having a variable heavy chain region with the following CDRs and intervening framework regions (corresponding to those of AB0023, with the sequences of CDR1, CDR2, and CDR3 underlined):

[0095] MEWSRVFIFLLSVTAGVHSQVQLQQSGAELVRPGTSVKVSCKASGYAFTYYLIEWVK QRPGQGLEWIGVINPGSGGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCARNW MNFDYWGQGTTLTVSS (SEQ ID NO: 6). In some embodiments, the antibody has a heavy chain variable region having an amino acid sequence with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO:6. In some embodiments, the antibody has a heavy chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 6.

[0096] In some embodiments, the antibody is an antibody having a variable light chain region with the following CDRs and intervening framework regions (corresponding to those of AB0023, with the sequences of CDR1, CDR2, and CDR3 underlined):

[0097] MRCLAEFLGLLVLWIPGAIGDIVMTQAAPSVSVTPGESVSISCRSSKSLLHSN GNTYLYWFLQRPGQSPQFLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGV YYCMQHLEYPYTFGGGTKLEIK (SEQ ID NO:7). In some embodiments, the antibody has a light chain variable region having an amino acid sequence with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 7. In some embodiments, the antibody has a light chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 7. In some embodiments, the antibody has a heavy chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 6 and a light chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 7.

[0098] In some embodiments, the antibody is a humanized version of such an antibody, such as one described in United States Patent Application Publication No. US 2009/0053224 (Feb. 26, 2009), such as that designated AB0024, and/or one having a heavy chain having the CDRs (CDR1, CDR2, and CDR3) of AB0024 and/or having a light chain having the CDRs (CDR1, CDR2, and CDR3) of AB0024.

[0099] For example, in one embodiment, the antibody is an antibody having a variable heavy chain region with the following CDRs and intervening framework regions (corresponding to those of AB0024, with the sequences of CDR1, CDR2, and CDR3 underlined):

[00100] QVQLVQSGAEVKKPGASVKVSCKASGYAFTYYLIEWVRQAPGQGLEWIGVINPGSGGTNYNEKFKGRATITADKSTSTAYMELSSLRSEDTAVYFCARNWMNFDYWGQGTTVTVSS (SEQ ID NO:8).

[00101] In some embodiments, the antibody has a heavy chain variable region having an amino acid sequence with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 8. In some embodiments, the antibody has a heavy chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 8.

[00102] In some embodiments, the antibody has a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 8, SEQ ID NO: 10 (QVQLVQSGAELKKPGASVKVSCKASGYAFTYYLIEWVKQAPGQGLEWIGVINPGSGGTNYNEKFKGRATLTADKSTSTAYMELSSLRSEDSAVYFCARNWMNFDYWGQGTTVTVSS), SEQ ID NO: 11 (QVQLVQSGAEVKKPGASVKVSCKASGYAFTYYLIEWVRQAPGQGLEWIGVINPGSGGTNYNEKFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCARNWMNFDYWGQGTTVTVSS), or SEQ ID NO: 12 (QVQLVQSGAEVKKPGASVKVSCKASGYAFTYYLIEWVRQAPGQGLEWIGVINPGSGGTNYNEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNWMNFDYWGQGTTVTVSS)or an amino acid having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 8, 10, 11, or 12, and/or a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 9, SEQ ID NO: 13 (DIVMTQTPLSLSVTPGQPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQFLIYRMSNLASGVPDRFSGSGSGTAFTLKISRVEAEDVGVYYCMQHLEYPYTFGGGTKVEIK), or SEQ ID NO: 14 (DIVMTQTPLSLSVTPGQPASISCRSSKSLLHSNGNTYLYWYLQKPGQSPQFLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPYTFGGGTKVEIK), or an amino acid having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 9, 13, or 14.

[00103] In some embodiments, the antibody is an antibody having a variable light chain region with the following CDRs and intervening framework regions (corresponding to those of AB0024, with the sequences of CDR1, CDR2, and CDR3 underlined):

[00104] DIVMTQTPLSLSVTPGQPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQFLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPYTFGG

GTKVEIK (SEQ ID NO: 9). In some embodiments, the antibody has a light chain variable region having an amino acid sequence with 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to SEQ ID NO: 9. In some embodiments, the antibody has a light chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 9. In some embodiments, the antibody has a heavy chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 8 and a light chain variable region with CDR1, CDR2, and/or CDR3 of the variable region sequence set forth in SEQ ID NO: 9.

[00105] Whether an agent inhibits LOXL2 enzymatic activity can be determined using any known assay. For example, an assay for LOXL2 enzymatic activity can be carried out using diaminopentane (DAP) as a substrate, or using collagen as a substrate. In both assays, enzymatic activity of LOXL2 can be measured using an assay that couples production of hydrogen peroxide (liberated by LOXL2 upon deamination of substrate) to horseradish peroxidase-catalyzed conversion of Amplex® Red (Invitrogen, Carlsbad, CA) to resorufin (a fluorescent product).

[00106] In some embodiments, a suitable anti-LOXL2 antibody inhibits enzymatic activity of a LOXL2 polypeptide. In other embodiments, a suitable anti-LOXL2 antibody does not inhibit enzymatic activity of a LOXL2 polypeptide.

[00107] Suitable anti-LOXL2 antibodies include, e.g., RPDS-1M1, RPDS-1M3, RPDS-1M8, RPDS-1M9, RPDS-1M11, RPDS-1M15, RPDS-1M17, RPDS-1M19, RPDS-1M20 (AB0030), RPDS-1M22, RPDS-1M24, RPDS-1M25, RPDS-1M27, RPDS-1M28, RPDS-1M29, RPDS-1M30, RPDS-1M31, RPDS-1M32, RPDS-2M1, RPDS-2M2, RPDS-2M3, RPDS-2M4, RPDS-2M5, RPDS-2M6, RPDS-2M7, RPDS-2M8, RPDS-2M9, RPDS-2M10, RPDS-2M11, RPDS-2M12, RPDS-2M13, RPDS-2M14, RPDS-2M15, RPDS-2M16, RPDS-2M17, RPDS-2M18, and RPDS-2M19, where such antibodies are described in U.S. Patent Application Serial No. 13/021,555. Monoclonal antibodies RPDS-1M1, RPDS-1M3, RPDS-1M8, RPDS-1M9, RPDS-1M11, RPDS-1M15, RPDS-1M17, RPDS-1M19, RPDS-1M20 (AB0030), RPDS-1M22, RPDS-1M24, RPDS-1M25, RPDS-1M27, RPDS-1M28, RPDS-1M29, RPDS-1M30, RPDS-1M31, RPDS-1M32, RPDS-2M1, RPDS-2M2, RPDS-2M3, RPDS-2M4, RPDS-2M5, RPDS-2M6, RPDS-2M7, RPDS-2M8, RPDS-2M9, RPDS-2M10, RPDS-2M11, RPDS-2M12, RPDS-2M13, RPDS-2M14, RPDS-2M15, RPDS-2M16, RPDS-2M17, RPDS-2M18, and RPDS-2M19 bind within the catalytic domain of LOXL2.

Assay formats

[00108] A subject assay for detecting circulating LOXL2 in an individual generally involves: a) contacting a liquid sample obtained from the individual with an antibody specific for LOXL2; and b) detecting binding of the antibody with LOXL2 present in the liquid sample. Suitable assay methods include an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), an immunoprecipitation assay, a lateral or axial flow assay, mass spectrometry, and the like.

[00109] A subject assay method can detect LOXL2 in a liquid sample to 175 pg/ml or less, e.g., a subject assay method can detect LOXL2 in a liquid sample to from about 150 pg/ml to about 175 pg/ml, to from about 125 pg/ml to about 150 pg/ml, to from about 100 pg/ml to about 125 pg/ml, to from about 75 pg/ml to about 100 pg/ml, to from about 50 pg/ml to about 75 pg/ml, or to from about 40 pg/ml to about 50 pg/ml. For example, a subject assay method can detect LOXL2 in a liquid sample when the LOXL2 is present in the liquid sample in a concentration of less than 10 ng/ml, e.g., in a concentration of from about 10 ng/ml to about 5 ng/ml, from about 5 ng/ml to about 1 ng/ml, from about 1 ng/ml to about 500 pg/ml, from about 500 pg/ml to about 400 pg/ml, from about 400 pg/ml to about 300 pg/ml, from about 300 pg/ml to about 200 pg/ml, from about 200 pg/ml to about 175 pg/ml, from about 200 pg/ml to about 150 pg/ml, from about 150 pg/ml to about 100 pg/ml, from about 100 pg/ml to about 75 pg/ml, from about 75 pg/ml to about 50 pg/ml, or from about 50 pg/ml to about 40 pg/ml. In some cases, a subject assay method detects LOXL2 in a liquid sample when the LOXL2 is present in the liquid sample in a concentration range of from about 175 pg/ml to about 5 ng/ml (or more than 5 ng/ml). In some cases, a subject assay method detects LOXL2 in a liquid sample when the LOXL2 is present in the liquid sample in a concentration range of from about 40 pg/ml to about 5 ng/ml (or more than 5 ng/ml). In some cases, a subject assay method detects LOXL2 in a liquid sample to a detection limit of average background plus 2.5 x SD (standard deviation of the background).

[00110] In some cases, a subject assay method involves the use of two LOXL2-specific antibodies. The two LOXL2-specific antibodies can both be monoclonal antibodies; the two LOXL2-specific antibodies can be a polyclonal antibody and a monoclonal antibody; or some other such combination.

[00111] For example, a first LOXL2-specific antibody is contacted with a liquid sample, where the first LOXL2-specific antibody forms a complex with LOXL2 present in the liquid sample. The first LOXL2-specific antibody can be immobilized on an insoluble support, such that the first LOXL2-specific antibody/LOXL2 complex is immobilized on the

insoluble support. Alternatively, the first LOXL2-specific antibody can be in solution, and the first LOXL2-specific antibody/LOXL2 complex can be insoluble, such that the first LOXL2-specific antibody/LOXL2 complex immunoprecipitates. The first LOXL2-specific antibody/LOXL2 complex can be detected using a second LOXL2-specific antibody. In some cases, the first LOXL2-specific antibody is a polyclonal antibody; and the second LOXL2-specific antibody is a monoclonal antibody.

[00112] In some embodiments, a subject assay method involves contacting a liquid sample, obtained from an individual, with an immobilized LOXL2-specific antibody, where the immobilized LOXL2-specific antibody is immobilized on an insoluble support. Any LOXL2 present in the sample will bind to the immobilized LOXL2-specific antibody, forming an immobilized anti-LOXL2/LOXL2 complex. The immobilized anti-LOXL2/LOXL2 complex can be detected using a second (non-immobilized) LOXL2-specific antibody. The second LOXL2-specific antibody can be detectably labeled, or can be detected using a detectably labeled secondary antibody.

[00113] Thus, in some embodiments, a subject method of detecting circulating LOXL2 in an individual involves: a) contacting a liquid sample obtained from the individual with a first antibody specific for LOXL2, such that the first antibody and the LOXL2 form a complex; b) contacting the LOXL2-first antibody complex with a second antibody specific for LOXL2; and c) detecting binding of the second antibody to the LOXL2-first antibody complex.

[00114] The insoluble support can be one or more wells of a multi-well plate, a test strip, a dipstick format, and the like. In any of the above-described assay formats, one or more washing steps can be carried out to remove unbound components.

[00115] An assay method of the present disclosure can detect a pathological level of circulating LOXL2 in an individual. For example, a subject assay method can involve: a) contacting a liquid sample obtained from an individual with an antibody specific for LOXL2; b) detecting binding of the antibody with LOXL2 present in the liquid sample; and c) comparing the detected level with a normal control value. A detected level that is higher than a normal control value is indicative of pathology (e.g., cancer or fibrosis).

Control values

[00116] Levels of LOXL2 in a liquid sample obtained from a test subject can be compared to a normal control value(s) or range of normal control values. The control value can be based on levels of LOXL2 in comparable samples (e.g., blood, plasma, or serum sample, or other liquid biological sample) obtained from a control population, e.g., the

general population or a select population of human subjects. For example, the select population may be comprised of apparently healthy subjects, e.g., individuals who have not previously had any signs or symptoms of fibrosis or cancer. Apparently healthy individuals also generally do not otherwise exhibit symptoms of disease. In other words, such individuals, if examined by a medical professional, would be characterized as healthy and free of symptoms of disease.

[00117] The control value can take a variety of forms. The control value can be a single cut-off value, such as a median or mean. A normal control value can be a normal control range.

[00118] In some cases, the control, normal value is below the detection limit of a subject assay method, e.g., a normal value can be less than about 175 pg/ml, less than about 150 pg/ml, less than about 100 pg/ml, less than about 75 pg/ml, less than about 50 pg/ml, or less than about 40 pg/ml.

Test subjects

[00119] As noted above, a liquid sample obtained from an individual is tested using a subject LOXL2 assay. Individuals who are suitable for testing using a subject assay include, but are not limited to, individuals who have not yet been diagnosed as having a disease, but who present with symptoms and/or complaints to a physician (e.g., individuals with an undiagnosed disorder or disease); individuals who have been diagnosed with cancer; individuals suspected of having a cancer but who have not yet been diagnosed as having cancer; individuals who are apparently healthy and who are undergoing routine screening; individuals who have been diagnosed as having fibrosis; individuals suspected of having fibrosis but who have not yet been diagnosed as having fibrosis; individuals who have been diagnosed as having a hepatitis C virus (HCV) infection, such as chronic HCV, or a hepatitis B virus (HBV) infection, such as chronic HBV (CHB); and individuals who are undergoing treatment for a cancer or a fibrotic disease.

Oncology patients

[00120] Individuals who are suitable for testing using a subject LOXL2 assay include individuals who have been diagnosed as having cancer include individuals having a benign tumor, individuals having a primary tumor, individuals having tumor metastasis, and individuals having a non-solid tumor type of cancer. Individuals who are suitable for testing using a subject LOXL2 assay include individuals who have a cancer, but who have not yet been diagnosed as having cancer. Thus, individuals who are suitable for testing using a

subject LOXL2 assay include individuals having a wide variety of cancers, including carcinomas, sarcomas, leukemias, and lymphomas.

[00121] Carcinomas include, but are not limited to, esophageal carcinoma, hepatocellular carcinoma, basal cell carcinoma (a form of skin cancer), squamous cell carcinoma (various tissues), bladder carcinoma, including transitional cell carcinoma (a malignant neoplasm of the bladder), bronchogenic carcinoma, colon carcinoma, colorectal carcinoma, gastric carcinoma, lung carcinoma, including small cell carcinoma and non-small cell carcinoma of the lung, adrenocortical carcinoma, thyroid carcinoma, pancreatic carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, renal cell carcinoma, ductal carcinoma in situ or bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical carcinoma, uterine carcinoma, testicular carcinoma, osteogenic carcinoma, epithelial carcinoma, and nasopharyngeal carcinoma, etc.

[00122] Sarcomas include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, chordoma, osteogenic sarcoma, osteosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas.

[00123] Solid tumors include, but are not limited to, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[00124] Leukemias include, but are not limited to, a) chronic myeloproliferative syndromes (neoplastic disorders of multipotential hematopoietic stem cells); b) acute myelogenous leukemias (neoplastic transformation of a multipotential hematopoietic stem cell or a hematopoietic cell of restricted lineage potential; c) chronic lymphocytic leukemias (CLL; clonal proliferation of immunologically immature and functionally incompetent small lymphocytes), including B-cell CLL, T-cell CLL prolymphocytic leukemia, and hairy cell leukemia; and d) acute lymphoblastic leukemias (characterized by accumulation of lymphoblasts). Lymphomas include, but are not limited to, B-cell lymphomas (e.g., Burkitt's lymphoma); Hodgkin's lymphoma; and the like.

[00125] Benign tumors include, e.g., hemangiomas, hepatocellular adenoma, cavernous hemangioma, focal nodular hyperplasia, acoustic neuromas, neurofibroma, bile

duct adenoma, bile duct cystanoma, fibroma, lipomas, leiomyomas, mesotheliomas, teratomas, myxomas, nodular regenerative hyperplasia, trachomas and pyogenic granulomas.

[00126] Primary and metastatic tumors include, e.g., lung cancer (including, but not limited to, lung adenocarcinoma, squamous cell carcinoma, large cell carcinoma, bronchioloalveolar carcinoma, non-small-cell carcinoma, small cell carcinoma, mesothelioma); breast cancer (including, but not limited to, ductal carcinoma, lobular carcinoma, inflammatory breast cancer, clear cell carcinoma, mucinous carcinoma); colorectal cancer (including, but not limited to, colon cancer, rectal cancer); anal cancer; pancreatic cancer (including, but not limited to, pancreatic adenocarcinoma, islet cell carcinoma, neuroendocrine tumors); prostate cancer; ovarian carcinoma (including, but not limited to, ovarian epithelial carcinoma or surface epithelial-stromal tumor including serous tumor, endometrioid tumor and mucinous cystadenocarcinoma, sex-cord-stromal tumor); liver and bile duct carcinoma (including, but not limited to, hepatocellular carcinoma, cholangiocarcinoma, hemangioma); esophageal carcinoma (including, but not limited to, esophageal adenocarcinoma and squamous cell carcinoma); non-Hodgkin's lymphoma; bladder carcinoma; carcinoma of the uterus (including, but not limited to, endometrial adenocarcinoma, uterine papillary serous carcinoma, uterine clear-cell carcinoma, uterine sarcomas and leiomyosarcomas, mixed mullerian tumors); glioma, glioblastoma, medulloblastoma, and other tumors of the brain; kidney cancers (including, but not limited to, renal cell carcinoma, clear cell carcinoma, Wilm's tumor); cancer of the head and neck (including, but not limited to, squamous cell carcinomas); cancer of the stomach (including, but not limited to, stomach adenocarcinoma, gastrointestinal stromal tumor); multiple myeloma; testicular cancer; germ cell tumor; neuroendocrine tumor; cervical cancer; carcinoids of the gastrointestinal tract, breast, and other organs; and signet ring cell carcinoma.

[00127] In some cases, an oncology patient is one who is currently undergoing treatment for the cancer. In some instances, the treatment comprises administration of an agent that inhibits enzymatic activity of a LOXL2 polypeptide. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an "SRCR3-4" domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224.

Epithelial-to-mesenchymal transition

Individuals who are suitable for testing using a subject assay method include individuals in whom an epithelial-to-mesenchymal transition (EMT) of epithelial cells has taken place. Individuals who are suitable for testing using a subject assay method include individuals in whom desmoplasia and fibroblast activation (which are considered factors in generating a pathologic microenvironment of tumors and fibrotic disease) have occurred. Such individuals may have precancerous cells and/or be at an early stage of cancer development.

Fibrosis

[00128] Individuals who are suitable for testing using a subject LOXL2 assay method include individuals who have been diagnosed as having fibrosis (a fibrotic disease), e.g., liver fibrosis, kidney fibrosis, pulmonary fibrosis, myelofibrosis, cardiac fibrosis, or other type of fibrosis. Individuals who are suitable for testing using a subject LOXL2 assay method include individuals who have a fibrotic disease (e.g., liver fibrosis, kidney fibrosis, pulmonary fibrosis, myelofibrosis, cardiac fibrosis, or other type of fibrosis), but who have not yet been diagnosed as having the fibrotic disease.

[00129] In some cases, a suitable test subject has an advanced form of fibrosis, but might still be suitable for treatment with a treatment regimen for fibrosis. For example, a suitable test subject includes a subject with active (not end-stage) fibrosis. In some cases, a suitable test subject is one who has fibrosis, and who might be anticipated to experience rapid disease progression.

[00130] In some cases, an individual who is to be tested using a subject LOXL2 assay is one who is currently undergoing treatment for a fibrotic disease. In some instances, the treatment comprises administration of an agent that inhibits enzymatic activity of a LOXL2 polypeptide. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an "SRCR3-4" domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224.

<u>Liver fibrosis</u>

[00131] Fibrosis of the liver is implicated in the pathology of numerous hepatic diseases. Fibrosis can occur as a complication of haemochromatosis, Wilson's disease, alcoholism, schistosomiasis, viral hepatitis, bile duct obstruction, exposure to toxins, and

metabolic disorders. Left unchecked, hepatic fibrosis progresses to cirrhosis (defined by the presence of encapsulated nodules), liver failure, and death.

[00132] Liver fibrosis includes, but is not limited to, cirrhosis, and associated conditions such as chronic viral hepatitis, non-alcoholic fatty liver disease (NAFLD), alcoholic steatohepatitis (ASH), non-alcoholic steatohepatitis (NASH), primary biliary cirrhosis (PBC), biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis.

[00133] The chronic insults to the liver from such sources as parasites and viral infection (e.g. hepatitis B virus (HBV), HCV, human immunodeficiency virus (HIV), schistosomiasis) or the long term stress from alcohol consumption typically result in remodeling of the liver, presumably to encapsulate the damaged area and protect the remaining liver tissue from damage. (*Li and Friedman, Gastroenterol. Hepatol. 14:618-633, 1999*). Liver fibrosis results in extracellular matrix changes, including 3-10 fold increases in total collagen content and replacement of the low density basement membrane with high-density matrix, which impair the metabolic and synthesis function of hepatocytes, hepatic stellate cells and endothelial cells. (Girogescu, M., *Non-invasive Biochemical Markers of Liver Fibrosis*, J. Gastrointestin. Liver Dis., 15(2): 149-159 (2006)).

[00134] A number of standardized scoring systems exist which provide a quantitative assessment of the degree and severity of liver fibrosis. These include the METAVIR, Knodell, Scheuer, Ludwig, and Ishak scoring systems. Individuals with liver fibrosis include individuals with any degree or severity of liver fibrosis, based on any of the METAVIR, Knodell, Scheuer, Ludwig, and Ishak scoring systems.

[00135] The METAVIR scoring system is based on an analysis of various features of a liver biopsy, including fibrosis (portal fibrosis, centrilobular fibrosis, and cirrhosis); necrosis (piecemeal and lobular necrosis, acidophilic retraction, and ballooning degeneration); inflammation (portal tract inflammation, portal lymphoid aggregates, and distribution of portal inflammation); bile duct changes; and the Knodell index (scores of periportal necrosis, lobular necrosis, portal inflammation, fibrosis, and overall disease activity). The definitions of each stage in the METAVIR system are as follows: score: 0, no fibrosis; score: 1, stellate enlargement of portal tract but without septa formation; score: 2, enlargement of portal tract with rare septa formation; score: 3, numerous septa without cirrhosis; and score: 4, cirrhosis.

[00136] Knodell's scoring system, also called the Histology Activity Index, classifies specimens based on scores in four categories of histologic features: I. Periportal and/or bridging necrosis; II. Intralobular degeneration and focal necrosis; III. Portal inflammation;

and IV. Fibrosis. In the Knodell staging system, scores are as follows: score: 0, no fibrosis; score: 1, mild fibrosis (fibrous portal expansion); score: 2, moderate fibrosis; score: 3, severe fibrosis (bridging fibrosis); and score: 4, cirrhosis. The higher the score, the more severe the liver tissue damage. Knodell (1981) Hepatol. 1:431.

[00137] In the Scheuer scoring system scores are as follows: score: 0, no fibrosis; score: 1, enlarged, fibrotic portal tracts; score: 2, periportal or portal-portal septa, but intact architecture; score: 3, fibrosis with architectural distortion, but no obvious cirrhosis; score: 4, probable or definite cirrhosis. Scheuer (1991) J. Hepatol. 13:372.

[00138] The Ishak scoring system is described in Ishak (1995) J. Hepatol. 22:696-699. Stage 0, No fibrosis; Stage 1, Fibrous expansion of some portal areas, with or without short fibrous septa; stage 2, Fibrous expansion of most portal areas, with or without short fibrous septa; stage 3, Fibrous expansion of most portal areas with occasional portal to portal (P--P) bridging; stage 4, Fibrous expansion of portal areas with marked bridging (P--P) as well as portal-central (P--C); stage 5, Marked bridging (P--P and/or P--C) with occasional nodules (incomplete cirrhosis); stage 6, Cirrhosis, probable or definite.

Kidney fibrosis

[00139] Like liver fibrosis, kidney fibrosis can result from various diseases and insults to the kidneys. Examples of such diseases and insults include chronic kidney disease, metabolic syndrome, vesicoureteral reflux, tubulointerstitial renal fibrosis, diabetes (including diabetic nephropathy), and resultant glomerular nephritis (GN), including, but not limited to, focal segmental glomerulosclerosis and membranous glomerulonephritis, mesangiocapillary GN.

[00140] It has become recognized that metabolic syndrome is a cluster of abnormalities including diabetic hallmarks such as insulin resistance, as well as central or visceral obesity and hypertension. In nearly all cases, dysregulation of glucose results in the stimulation of cytokine release and upregulation of extracellular matrix deposition. Additional factors contributing to chronic kidney disease, diabetes, metabolic syndrome, and glomerular nephritis include hyperlipidemia, hypertension, and proteinuria, all of which result in further damage to the kidneys and further stimulate the extracellular matrix deposition. Thus, regardless of the primary cause, insults to the kidneys may result in kidney fibrosis and the concomitant loss of kidney function. (*Schena, F. and Gesualdo, L., Pathogenic Mechanisms of Diabetic Nephropathy, J. Am. Soc. Nephrol., 16: S30-33 (2005); Whaley-Connell, A., and Sower, J.R., Chronic Kidney Disease and the Cardiometabolic Syndrome, J. Clin. Hypert., 8(8): 546-48 (2006)*).

Lung fibrosis

[00141] Fibrosis of the lung includes many syndromes and diseases. Exemplary diseases include idiopathic pulmonary fibrosis (IPF), idiopathic interstitial pneumonia, and acute respiratory distress syndrome (ARDS). Lung fibrosis also includes, but is not limited to, cryptogenic fibrosing alveolitis, chronic fibrosing interstitial pneumonia, interstitial lung disease (ILD), and diffuse parenchymal lung disease (DPLD).

[00142] The pathogenesis of most lung fibroses, including the aforementioned diseases are not well understood, however all are characterized by an influx of inflammatory cells and a subsequent increase in the synthesis and deposition of collagen-rich extracellular matrix. (*Chua et al., Am J. Respir. Cell. Mol. Biol., 33:9-13 (2005); Tzortzaki et al., J. Histochem. & Cytochem., 54(6): 693-700 (2006); Armstrong et al., Am. J. Respir. Crit. Care Med., 160: 1910-1915 (1999)*).

[00143] IPF is characterized by inflammation, and eventually fibrosis, of lung tissue; although these two symptoms can also be dissociated. The cause of IPF is unknown; it may arise either from an autoimmune disorder or as a result of infection. Symptoms of IPF include dyspnea (*i.e.*, shortness of breath) which becomes the major symptom as the disease progresses, and dry cough. Death can result from hypoxemia, right-heart failure, heart attack, lung embolism, stroke or lung infection, all of which can be brought on by the disease.

[00144] In some cases, an individual who is to be tested using a subject LOXL2 assay is one who is currently undergoing treatment for IPF. In some instances, the treatment comprises administration of an agent that inhibits enzymatic activity of a LOXL2 polypeptide. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an "SRCR3-4" domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224.

Myelofibrosis

[00145] Pathogenic processes in primary myelofibrosis involve a primary megakaryocyte-weighted clonal myeloproliferation and a paraneoplastic stromal reaction that includes bone marrow fibrosis, osteosclerosis, angiogenesis, and extramedullary hematopoiesis. The bone marrow reaction includes excess deposition of extracellular matrix proteins such as fibrillar collagen, hypocellularity, activation and recruitment of bone marrow fibroblasts, excessive cytokine and growth factor production, and other changes that

result in a reduction of hematopoietic capacity. Secondary myelofibrosis can result from polycythemia rubra vera or essential thrombocytosis.

Individuals undergoing treatment

[00146] In some cases, an individual who is to be tested using a subject LOXL2 assay is one who is currently undergoing treatment for a fibrotic disease or for a cancer. In some instances, the treatment comprises administration of an agent that inhibits enzymatic activity of a LOXL2 polypeptide. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an SRCR3-4 domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224.

DIAGNOSTIC METHODS

[00147] The present disclosure provides various diagnostic methods for diseases and conditions associated with LOXL2, including diseases and conditions associated with or characterized by elevated levels of LOXL2, such as elevated circulated LOXL2. For example, provided are methods for determining whether an individual has a disease characterized by elevated circulating LOXL2. Also provided are methods for assessing the activity or severity of such a disease or condition. The diagnostic methods generally involve detecting a level of circulating LOXL2 in the individual, using a subject LOXL2 assay method, as described above. Diseases characterized by elevated circulating LOXL2 include cancer and fibrosis.

[00148] The level of LOXL2 in a given sample may be expressed in terms of concentration, by weight, or other readout of a detection assay as described herein. In one aspect, a level of circulating LOXL2 that is greater than a normal control level or other reference level indicates that the individual has a disease characterized by elevated circulating LOXL2. For example, a level of circulating LOXL2 that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, or more than 50%, higher than a normal control or other reference level, can indicate that the individual has a disease characterized by elevated circulating LOXL2. As another example, a level of circulating LOXL2 that is greater than about 40 pg/ml, greater than about 50 pg/ml, greater than about 75 pg/ml, greater than about 100 pg/ml, greater than about 150 pg/ml, greater than about 175 pg/ml, greater than about 200 pg/ml, greater than about 250 pg/ml, greater than about 300 pg/ml, greater than about 350 pg/ml, greater than about 400 pg/ml, greater

than about 450 pg/ml, greater than about 500 pg/ml, greater than about 550 pg/mL, greater than about 600 pg/mL, greater than about 650 pg/mL, greater than about 700 pg/ml, greater than about 750 pg/mL, or greater than about 800 pg/mL, can indicate that the individual has a disease characterized by elevated circulating LOXL2, and/or give prognostic or predictive information about the disease or condition, such as by indicating active disease or a particular activity level. In some cases, the level indicates active fibrogenesis in the subject. As used herein, the terms "normal control level," and "reference level," in the context of LOXL2, refer to the level of LOXL2 to which the LOXL2 level in a sample, *e.g.*, a test sample, is compared. In one example, the normal control or reference level is a level generally observed in a sample from a healthy individual, such as an individual not having the subject disease or condition, *e.g.*, LOXL2-associated disease or condition. In another example, it is a level observed in an individual having a LOXL2-associated disease or condition, such as an individual with less active disease, a relatively better prognosis, or more favorable chances associated with a particular outcome, endpoint, or event, such as survival or responsiveness to treatment. For example, the reference or normal control level may be a level observed at a particular timepoint, such as a baseline level, in a sample from an individual that ultimately showed a favorable outcome, endpoint, or event. In another example, the normal control or reference level is a level observed in a sample taken from the same individual, at a different time point compared to the sample being assayed, for example, a baseline level, prior to treatment, or a level earlier in disease progression or before disease was detected. In another example, the normal or reference level is a standard level, such as a level in a sample prepared to have a pre-defined concentration of LOXL2 or simply a pre-defined level. As used herein, "baseline" refers to an amount, level, or measurement of a particular variable at a point in time that is prior to a particular event or period, such as a point in time prior to treatment or prior to the commencement of a study monitoring disease progression. Thus, in one aspect, the reference or normal control level of LOXL2 is a baseline level, such as a baseline level from the same individual or from another individual.

Control values

[00149] Levels of LOXL2 in a liquid sample obtained from a test subject can be compared to a normal control value(s) or range of normal control values. The control value can be based on levels of LOXL2 in comparable samples (e.g., blood, plasma, or serum sample, or other liquid biological sample) obtained from a control population, e.g., the general population or a select population of human subjects. For example, the select

population may be comprised of apparently healthy subjects, e.g., individuals who have not previously had any signs or symptoms of fibrosis or cancer. Apparently healthy individuals also generally do not otherwise exhibit symptoms of disease. In other words, such individuals, if examined by a medical professional, would be characterized as healthy and free of symptoms of disease. Alternatively, the assessed values may be compared to other reference values, such as an average, mean, or median value or values observed for a population of subjects having a particular disease or condition. For example, such a reference value may be used in comparison to levels assessed for particular individuals who then are determined, for example, to have more active disease compared to the overall patient cohort from whom the reference value was obtained.

[00150] The control value can take a variety of forms. The control value can be a single cut-off value, such as a median or mean. A normal control value can be a normal control range.

Individuals to be tested

[00151] Test subjects include those listed above. Individuals who are suitable for testing using a subject assay include, but are not limited to, individuals who have not yet been diagnosed as having a disease, but who present with symptoms and/or complaints to a physician (e.g., individuals with an undiagnosed disorder or disease); individuals who have been diagnosed with cancer; individuals suspected of having a cancer but who have not yet been diagnosed as having cancer; individuals who are apparently healthy and who are undergoing routine screening; individuals who have been diagnosed as having fibrosis; individuals suspected of having fibrosis but who have not yet been diagnosed as having fibrosis; individuals who have been diagnosed as having a hepatitis C virus (HCV) or hepatitis B virus (HBV) infection (and optionally also diagnosed as having HCV infection- or HBV infection-associated liver damage); and individuals who are undergoing treatment for a cancer or a fibrotic disease.

[00152] In some cases, the individual to be tested is an individual with an undiagnosed disorder or disease, e.g., an individual who presents with symptoms and/or complaints. A subject diagnostic method can be used to determine whether such an individual might have a fibrotic disease or a cancer. A subject diagnostic method can be part of differential diagnosis; and in some cases can be used in conjunction with one or more diagnostic tests, e.g., to confirm or to rule out a diagnosis.

Generating a report

[00153] A subject diagnostic method can include generating a report that provides an indication as to whether an individual is likely to have a fibrotic disease or a cancer. Such a report can include information such as a recommendation regarding further evaluation; a recommendation regarding therapeutic drug treatment; and the like.

[00154] A subject report can further include one or more of: 1) service provider information; 2) patient data; 3) data regarding the level of LOXL2; 4) follow-up evaluation recommendations; 5) therapeutic drug treatment; and 6) other features.

Further evaluation

[00155] Based on detection of a level of LOXL2, and/or based on a report (as described above), a physician or other qualified medical personnel can determine whether further evaluation of the test subject (the patient) is required. Further evaluation can include, e.g., lung function tests (e.g., where pulmonary fibrosis is suspected); liver function tests (e.g., where liver fibrosis is suspected); and various tests for cancer, which tests may vary, depending on the type of cancer suspected.

[00156] As one example, where an individual is suspected of having a cancer, any of a variety of tests for a cancer can be performed, where such tests include, e.g., histochemical analysis of a tissue biopsy for the presence of cancerous cells; tests for the presence of a tumor associated antigen; and the like.

[00157] As another example, where an individual is suspected of having a pulmonary fibrotic disorder, the individual can be assessed for symptoms of the pulmonary fibrotic disorder. Symptoms of a pulmonary fibrotic disorder can include, but are not limited to, decreased body weight, increased lung weight, pulmonary fibrosis, pathologic lung architecture (*e.g.*, "honeycomb" lung), increased Ashcroft score, increased pulmonary collagen levels, increased number of $CD45^+$/$collagen^+$ cells, pneumocyte proliferation and expansion and increased leukocyte number in bronchioalveolar lavage (BAL) fluid. Symptoms can also include, for example, increased pulmonary levels of one or more of the following molecules: LOXL2, α-smooth muscle actin (α-SMA), transforming growth factor β-1 (TGFβ-1), stromal derived factor-1 (SDF-1) (*e.g.*, SDF-1α), endothelin-1 (ET-1) and phosphorylated SMAD2.

[00158] As a further example, where an individual is suspected of having liver fibrosis, the individual can be assessed for markers of liver function. Liver functions include, but are not limited to, synthesis of proteins such as serum proteins (e.g., albumin, clotting factors, alkaline phosphatase, aminotransferases (e.g., alanine transaminase, aspartate

transaminase), 5'-nucleosidase, γ-glutaminyltranspeptidase, etc.), synthesis of bilirubin, synthesis of cholesterol, and synthesis of bile acids; a liver metabolic function, including, but not limited to, carbohydrate metabolism, amino acid and ammonia metabolism, hormone metabolism, and lipid metabolism; detoxification of exogenous drugs; a hemodynamic function, including splanchnic and portal hemodynamics; and the like. For example, levels of serum alanine aminotransferase (ALT) are measured, using standard assays. In general, an ALT level of less than about 45 international units is considered normal. Elevated ALT levels can indicate compromised liver function. Quantitative tests of functional liver reserve can also be used to assess liver function, where such test include, e.g., indocyanine green clearance (ICG), galactose elimination capacity (GEC), aminopyrine breath test (ABT), antipyrine clearance, monoethylglycine-xylidide (MEG-X) clearance, and caffeine clearance.

Therapy

[00159] Based on detection of a level of LOXL2, and/or based on a report (as described above), a physician or other qualified medical personnel can determine whether appropriate therapeutic drug treatment is advised, e.g., to treat a fibrotic disease, to treat a cancer, etc.

[00160] For example, an individual who has been determined to have an early stage cancer, based on circulating levels of LOXL2 and optionally on further evaluation (e.g., histochemical analysis of a tissue biopsy), can be started on a cancer chemotherapeutic drug regimen and/or can be treated with radiation therapy and/or can undergo surgical removal of the cancer.

[00161] Cancer chemotherapeutic agents ("chemotherapeutics") include cytotoxic and cytostatic drugs. Chemotherapeutics may include those which have other effects on cells such as reversal of the transformed state to a differentiated state or those which inhibit cell replication. Examples of known cytotoxic agents are listed, for example, in Goodman et al., "The Pharmacological Basis of Therapeutics," Sixth Edition, A.B. Gilman et al., eds./Macmillan Publishing Co. New York, 1980. These include taxanes, such as paclitaxel and docetaxel; nitrogen such as mechlorethamine, melphalan, uracil mustard and chlorambucil; ethylenimine derivatives, such as thiotepa; alkyl sulfonates, such as busulfan; nitrosoureas, such as lomustine, semustine and streptozocin; triazenes, such as dacarbazine; folic acid analogs, such as methotrexate; pyrimidine analogs, such as fluorouracil, cytarabine and azaribine; purine analogs, such as mercaptopurine and thioguanine; vinca alkaloids, such as vinblastine and vincristine; antibiotics, such as dactinomycin, daunorubicin, doxorubicin,

and mitomycin; metal complexes, such as platinum coordination complexes, such as cisplatin; substituted urea, such as hydroxyurea; methyl hydrazine derivatives, such as procarbazine; adrenocortical suppressants, such as mitotane; hormones and antagonists, such as adrenocortisteroids (prednisone), progestins (hydroxyprogesterone caproate, acetate and megestrol acetate), estrogens (diethylstilbestrol and ethinyl estradiol), and androgens (testosterone propionate and fluoxymesterone).

[00162] As another example, an individual who has been determined to have IPF, for example, based on circulating levels of LOXL2 and optionally on further evaluation (e.g., lung function tests), can be treated with pharmaceutical treatment for IPF and/or other treatment for IPF. Primary treatment for IPF is pharmaceutical, the most common drugs used for treatment of IPF being corticosteroids (*e.g.*, prednisone), penicillamine, and various anti neoplastics (*e.g.*, cyclophosphamide, azathiporene, chlorambucil, vincristine and colchicine). Other treatments include oxygen administration and, in extreme cases, lung transplantation.

[00163] As a further example, an individual who has been determined to have liver fibrosis, based on circulating levels of LOXL2 and optionally on further evaluation (e.g., liver functions tests; tests for infection with HCV, HBV, etc.), can be treated with, e.g., an anti-viral agent, e.g., an agent suitable for treating an HCV or HBV infection or other hepatitis virus infection. For example, an HCV infection can be treated with an interferon-alpha (IFN-α), viramidine, ribavirin, levovirin, an HCV NS3 inhibitor, an HCV NS5B inhibitor, or combinations of one or more of the foregoing.

METHODS FOR MONITORING EFFICACY OF TREATMENT

[00164] The present disclosure provides methods for monitoring efficacy of treatment for a LOXL2-associated disease or condition, such as a disease characterized by elevated circulating LOXL2, the method generally involving determining a circulating LOXL2 level in the individual at a time point, using a subject LOXL2 assay. In one aspect, a level of LOXL2 in the sample that is lower than a level obtained at an earlier time point from the individual indicates efficacy of the treatment. In another aspect, a lower level compared to a control or reference sample indicates treatment efficacy. In another aspect, the level of LOXL2, *e.g.*, a high level of LOXL2, indicates that an individual will respond favorably to treatment, such as treatment with a LOXL2-targeting therapy.

[00165] For example, a circulating LOXL2 level is determined at a first time point and at a second time point in the individual, where the second time point is later than the first time point. The first time point can be before the start of treatment; and the second time point can be during treatment (e.g., after a treatment regimen has begun). The first time point

can be during treatment; and the second time point can be at a later time during treatment. The second time point can be from about 1 hour to about 1 year after the first time point, e.g., the second time point can be from about 1 hour to about 2 hours, from about 2 hours to about 4 hours, from about 4 hours to about 8 hours, from about 8 hours to about 16 hours, from about 16 hours to about 24 hours, from about 24 hours to about 36 hours, from about 36 hours to about 72 hours, from about 72 hours to about 4 days, from about 4 days to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, from about 1 month to about 3 months, from about 3 months to about 6 months, or from about 6 months to about 1 year, or more than 1 year, after the first time point.

[00166] Thus, e.g., in some embodiments, a subject method of determining efficacy of treatment for a disease characterized by elevated circulating LOXL2 comprises: a) determining the circulating level of LOXL2 in an individual at a first time point (by determining the level of LOXL2 in a liquid sample obtained from the individual at the first time point); b) determining the circulating level of LOXL2 in the individual at a second time point (by determining the level of LOXL2 in a liquid sample obtained from the individual at the second time point); and comparing the level of LOXL2 from the first and second time points.

[00167] If the circulating LOXL2 level at the second time point is lower than the circulating LOXL2 level at the first time, point, it may be concluded that the treatment for the disease characterized by elevated circulating LOXL2 was effective; in these cases, a recommendation may be made to continue with the treatment regimen. If the circulating LOXL2 level at the second time point is higher than the circulating LOXL2 level at the first time, point, it may be concluded that the treatment for the disease characterized by elevated circulating LOXL2 was not effective; in these cases, a recommendation may be made to discontinue the treatment regimen, to increase the dose of a drug used in the treatment regimen, to increase the frequency of dosing, or to administer an alternative treatment regimen. If the circulating LOXL2 level at the second time point is not significantly different than the circulating LOXL2 level at the first time, point, it may be concluded that the treatment for the disease characterized by elevated circulating LOXL2 was not effective, or that the treatment regimen should be altered; in these cases, a recommendation may be made to discontinue the treatment regimen, to increase the dose of a drug used in the treatment regimen, to increase the frequency of dosing, or to administer an alternative treatment regimen.

Test subjects

[00168] A subject method for monitoring efficacy of treatment can be used to test any of a variety of individuals, including, e.g., individuals who have been diagnosed with cancer and who are undergoing treatment for; individuals who have been diagnosed as having fibrosis and who are undergoing treatment for the fibrosis; individuals who have been diagnosed as having an HCV or HBV infection and who are undergoing treatment for the HCV or HBV infection; individuals who have been diagnosed as having HCV or HBV infection-associated liver damage, and who are undergoing treatment for the HCV or HBV infection and/or the liver damage; and the like.

[00169] In some cases, an individual who is to be tested using a subject LOXL2 assay is one who is currently undergoing treatment for a cancer. The cancer chemotherapy can be any of a variety of cytotoxic agents. Such cytotoxic agents include taxanes, such as paclitaxel and docetaxel; nitrogen such as mechlorethamine, melphalan, uracil mustard and chlorambucil; ethylenimine derivatives, such as thiotepa; alkyl sulfonates, such as busulfan; nitrosoureas, such as lomustine, semustine and streptozocin; triazenes, such as dacarbazine; folic acid analogs, such as methotrexate; pyrimidine analogs, such as fluorouracil, cytarabine and azaribine; purine analogs, such as mercaptopurine and thioguanine; vinca alkaloids, such as vinblastine and vincristine; antibiotics, such as dactinomycin, daunorubicin, doxorubicin, and mitomycin; metal complexes, such as platinum coordination complexes, such as cisplatin; substituted urea, such as hydroxyurea; methyl hydrazine derivatives, such as procarbazine; adrenocortical suppressants, such as mitotane; hormones and antagonists, such as adrenocortisteroids (prednisone), progestins (hydroxyprogesterone caproate, acetate and megestrol acetate), estrogens (diethylstilbestrol and ethinyl estradiol), and androgens (testosterone propionate and fluoxymesterone).

[00170] In some instances, the cancer treatment comprises administration of an agent that inhibits enzymatic activity of a LOXL2 polypeptide. Agents that inhibit LOXL2 enzymatic activity include an allosteric inhibitor of LOXL2 enzymatic activity. In some cases, the allosteric inhibitor is an anti-LOXL2 monoclonal antibody, e.g., an anti-LOXL2 monoclonal antibody that binds an epitope within an "SRCR3-4" domain of LOXL2. Non-limiting examples of a monoclonal antibody that inhibits LOXL2 enzymatic activity, and that binds an epitope within an SRCR3-4 domain, are AB0023 and AB0024; see, e.g., US 2009/0053224.

[00171] As another example, an individual undergoing treatment for liver fibrosis, or who is undergoing treatment for a disease that can result in liver fibrosis, is suitable for

testing using a subject method. As an example, an individual undergoing treatment for an HCV infection is suitable for testing using a subject method. For example, an HCV infection can be treated with an IFN-α, viramidine, ribavirin, levovirin, an HCV NS3 inhibitor, an HCV NS5B inhibitor, or combinations of one or more of the foregoing.

[00172] As another example, an individual undergoing treatment for IPF is suitable for testing using a subject method. Drugs commonly used to treat IPF include, e.g., corticosteroids (*e.g.*, prednisone), penicillamine, and various anti neoplastics (*e.g.*, cyclophosphamide, azathiporene, chlorambucil, vincristine and colchicine).

Control values

[00173] Levels of LOXL2 in a liquid sample obtained from a test subject can be compared to a normal control value(s) or range of normal control values or other reference values as described herein. The control value can be based on levels of LOXL2 in comparable samples (e.g., blood, plasma, or serum sample, or other liquid biological sample) obtained from a control population, e.g., the general population or a select population of human subjects. For example, the select population may be comprised of apparently healthy subjects, e.g., individuals who have not previously had any signs or symptoms of fibrosis or cancer. Apparently healthy individuals also generally do not otherwise exhibit symptoms of disease. In other words, such individuals, if examined by a medical professional, would be characterized as healthy and free of symptoms of disease.

[00174] The control value can take a variety of forms. The control value can be a single cut-off value, such as a median or mean. A normal control value can be a normal control range. In some cases, the control, normal value is below the detection limit of a subject assay method, e.g., less than about 175 pg/ml less than about 150 pg/ml, less than about 125 pg/ml, less than about 100 pg/ml, less than about 75 pg/ml, less than about 50 pg/ml, or less than about 40 pg/ml.

PROGNOSTIC METHODS

[00175] Also provided are various prognostic and predictive methods. For example, the present disclosure provides determining the likelihood that an individual having a fibrotic disease will exhibit a beneficial clinical response to a treatment for the fibrotic disease. In another example, the method determines the likelihood or risk of a particular disease output or endpoint or responsiveness to treatment. The method generally involves detecting a circulating level of LOXL2, such as in a liquid sample obtained from the individual, using a subject LOXL2 assay. In one aspect, a level of LOXL2 that is greater than a normal control or other reference level indicates that the individual has an increased

likelihood of exhibiting a beneficial clinical response to a treatment for the fibrotic disease. In another aspect, a comparatively low level indicates a relatively lower likelihood or risk of developing a particular disease outcome or endpoint, or other prognostic information. Likewise, comparatively high LOXL2 levels can indicate poorer prognosis, such as increased risk or likelihood of developing a particular disease or condition output or reaching a particular endpoint. Fibrotic diseases include pulmonary fibrosis, liver fibrosis, cardiac fibrosis, and myelofibrosis, as described above. In some cases, e.g., where the circulating LOXL2 levels indicate that the subject is likely to exhibit a beneficial clinical response to a treatment for the fibrotic disease, a subject method further involves treating the individual for the fibrotic disease.

[00176] Individuals who are suitable for testing using a subject assay method include individuals who have been diagnosed as having fibrosis, e.g., liver fibrosis, kidney fibrosis, pulmonary fibrosis, myelofibrosis, cardiac fibrosis, or other type of fibrosis. Liver fibrosis includes, but is not limited to, cirrhosis, and associated conditions such as chronic viral hepatitis (resulting from, e.g., HCV or HBV infection), NAFLD, ASH, NASH, PBC, biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis. Kidney fibrosis can result from a variety of diseases and insults, where examples of such diseases and insults include chronic kidney disease, metabolic syndrome, vesicoureteral reflux, tubulointerstitial renal fibrosis, diabetes (including diabetic nephropathy), and resultant glomerular nephritis (GN), including, but not limited to, focal segmental glomerulosclerosis and membranous glomerulonephritis, mesangiocapillary GN. Fibrosis of the lung includes many syndromes and diseases, where exemplary diseases include IPF, idiopathic interstitial pneumonia, and ARDS. Lung fibrosis also includes, but is not limited to, cryptogenic fibrosing alveolitis, chronic fibrosing interstitial pneumonia, ILD, and DPLD.

[00177] In some cases, a suitable test subject has an advanced form of fibrosis, but might still be suitable for treatment with a treatment regimen for fibrosis. For example, a suitable test subject includes a subject with active (not end-stage) fibrosis. In some cases, a suitable test subject is one who has fibrosis, and who might be anticipated to experience rapid disease progression. As an example, an individual may have an advanced stage, e.g., METAVIR F4, of liver fibrosis; an individual with METAVIR F4 fibrosis and a positive LOXL2 (e.g., greater than normal levels of LOXL2 in liquid sample, as determined using a subject LOXL2 assay) may still be a candidate for treatment for the fibrosis. A METAVIR F4 liver fibrosis patient with a negative LOXL 2 (e.g., normal levels of LOXL2 in liquid sample, as determined using a subject LOXL2 assay) may not be considered a candidate for

treatment for the fibrosis. As another example, an individual with elevated LOXL2 (e.g., greater than normal levels of LOXL2 in liquid sample, as determined using a subject LOXL2 assay) who has an early stage of liver fibrosis (e.g., METAVIR F1 or F2) may be considered a candidate for treatment for the fibrosis.

Control values

[00178] Levels of LOXL2 in a liquid sample obtained from a test subject can be compared to a normal control value(s) or range of normal control values. The control value can be based on levels of LOXL2 in comparable samples (e.g., blood, plasma, or serum sample, or other liquid biological sample) obtained from a control population, e.g., the general population or a select population of human subjects. For example, the select population may be comprised of apparently healthy subjects, e.g., individuals who have not previously had any signs or symptoms of fibrosis. Apparently healthy individuals also generally do not otherwise exhibit symptoms of disease. In other words, such individuals, if examined by a medical professional, would be characterized as healthy and free of symptoms of disease.

[00179] The control value can take a variety of forms. The control value can be a single cut-off value, such as a median or mean. A normal control value can be a normal control range. In some cases, the control, normal value is below the detection limit of a subject assay method, e.g., less than about 175 pg/ml less than about 150 pg/ml, less than about 125 pg/ml, less than about 100 pg/ml, less than about 75 pg/ml, less than about 50 pg/ml, or less than about 40 pg/ml.

Generating a report

[00180] The likelihood that a patient will exhibit a beneficial clinical response to treatment for a fibrotic disease is assessed by determining a circulating level of LOXL2. The patient's likelihood of exhibiting a beneficial clinical response to treatment for a fibrotic disease is provided in a report. The report may further include information regarding the patient's likelihood of response. For example, a subject method can further include a step of generating or outputting a report providing the results of a subject response likelihood assessment, which report can be provided in the form of an electronic medium (e.g., an electronic display on a computer monitor), or in the form of a tangible medium (e.g., a report printed on paper or other tangible medium).

[00181] A "report," as described herein, is an electronic or tangible document which includes report elements that provide information of interest relating to a subject likelihood assessment and its results. A subject report includes at least a likelihood assessment, e.g., an

indication as to the likelihood that a patient having a fibrotic disease will exhibit a beneficial clinical response to a treatment for the fibrotic disease. A subject report can be completely or partially electronically generated. A subject report can further include one or more of: 1) information regarding the testing facility; 2) service provider information; 3) patient data; 4) sample data; 5) an interpretive report, which can include various information including: a) indication; b) test data, e.g., circulating LOXL2 level; and 6) other features.

Prognostic and predictive IPF methods

[00182] In some embodiments, provided are diagnostic, prognostic, and predictive methods for idiopathic pulmonary fibrosis (IPF). As shown in the examples herein, increased expression of LOXL2 is detected in the sera of IPF patients compared with normal control samples; additionally, increased circulating LOXL2 levels indicate an active IPF phenotype and an increased risk of various disease outcomes. Higher LOXL2 expression also is detected in the lung tissue of IPF patients. Accordingly, provided are methods using LOXL2 as a marker of IPF disease, such as a marker of IPF disease activity or of the active IPF phenotype. Thus, in some embodiments of the provided methods, LOXL2 is used as a diagnostic, prognostic, and/or predictive marker for IPF. In one aspect, LOXL2 levels are used to evaluate fibrogenesis and/or various IPF stages, severity, or outcomes, such as the likelihood of particular disease outcomes or responsiveness to treatment.

[00183] In another aspect, LOXL2 levels are indicative of active disease or a level of disease activity. In another aspect, LOXL2 levels, typically serum levels, that are higher in comparison to a control or other reference sample indicate a risk of developing a particular disease outcome or developing a particular disease outcome in a particular period of time. In other aspects, LOXL2 levels indicate the likelihood that a patient will respond to a particular treatment or gives information regarding the responsiveness to ongoing treatment, such as treatment with a LOXL2 inhibitor or other treatment. Thus, in some embodiments, the methods further include initiating, discontinuing, or altering a disease treatment approach, based on the prediction or detected LOXL2 levels.

[00184] Exemplary disease outcomes that are assessed or predicted using the methods include IPF disease progression (a composite endpoint defined as one of the following: mortality from any cause), poor progression-free survival (PFS), respiratory hospitalization, decrease in lung function, *e.g.*, categorical decrease in lung function (which may be defined as either a 10% decrease in forced vital capacity (FVC) with a 5% decrease in the diffusion capacity for carbon monoxide (DL_{CO}) or a 15 % decrease in DL_{CO} with a 5% decrease in FVC), and death.

[00185] The methods generally involve obtaining a patient sample and/or determining a LOXL2 level in the sample (for example, using the methods described herein) and performing various statistical analyses based on this and other information. In one example, it is determined whether the patient or a sample has a high or low level of LOXL2, for example, a low or high circulating or serum LOXL2 level. This information can be determined, for example, by dichotomizing LOXL2 levels based on a distribution of determined LOXL2 levels in a given population, such as a collection of samples, designating cutoff points for "low" and "high" levels of LOXL2. For example, a high level of LOXL2 can be deemed a level at least or above a particular concentration in a given sample, such as greater than at or about 800 picograms (pg) LOXL2 per milliliter (mL) of sera. Alternatively, a high LOXL2 serum level may be defined based on a distribution of levels for samples within a population or based on a particular fold change compared to a control or reference sample.

[00186] In some aspects, the methods are carried out by determining LOXL2 levels in connection with other measurements, such as markers of disease severity or functional status, *e.g.*, baseline measurements of IPF, such as those reflective of IPF severity, such as percent of predicted forced vital capacity (FVC), percent of predicted carbon monoxide diffusion capacity (DL_{CO}), 6-minute walk distance (6MWD), mean pulmonary artery pressure (mPAP), the lowest resting oxygen saturation (SpO2), the composite physiologic index (CPI), the St. George's Respiratory Questionnaire score (SGRQ), and the Transition Dyspnea Index (TDI) score, responsiveness to treatment, and/or other biomarkers of disease or disease severity. Thus, in some aspects of the predictive models and methods, LOXL2 is a biomarker of IPF disease outcome integrated with measures of disease severity or functional status and/or other biomarkers.

Statistical analyses for the diagnostic, prognostic and predictive methods

[00187] In some examples, statistical analyses are carried out based on the LOXL2 level and other determinations. In one example, levels of LOXL2 are evaluated, for example, using standard histograms to evaluate untransformed or $\log_{10}x$ transformed levels of LOXL2. Statistical analyses can include determining various values, such as mean, *e.g.*, geometric mean, or median values for LOXL2 expression levels and/or baseline variables, for individual samples and/or patients, and calculating standard deviations and fold changes among various samples or conditions, and comparing expression levels and/or other variables using any of a number of well-known tests, such as the student's t-test, which, for

example, may be used to compare distribution of baseline variables and LOXL2 expression levels.

[00188] In some aspects, Pearson's Correlation (PC) is used to assess linear relationships (correlations) between pairs of values (*e.g.*, by calculating PC coefficients), such as between LOXL2 expression levels and other variables, such as baseline IPF variable(s) as described herein. Such analysis may be used to linearly separate distribution in expression patterns, by calculating PC coefficients for individual pairs of variables (plotted on x- and y- axes of individual matrices, as shown in Example 9).

Predictive modeling

[00189] In some embodiments, the predictive methods further comprise further use of statistical analysis and use of predictive models and systems. In some aspects, such models and systems are used to predict disease outcomes, endpoints, responsiveness, and/or events, based on LOXL2 levels and typically other information, such as variables indicative of disease severity and other biomarkers. For example, survival models may be used to examine the relationship between LOXL2 levels and other covariates and one or more events, endpoints, or outcomes, such as disease outcomes, *e.g.*, IPF outcome(s) and responsiveness to one or more treatment(s); such models may be used to predict the likelihood that a particular patient will have the event, endpoint, or outcome, or that such outcome will occur within a particular amount of time.

[00190] In one such example, Cox proportional hazard modeling, *e.g.*, stepwise Cox proportional hazard modeling, is carried out to examine the relationship between LOXL2 levels (and optionally other covariates, such as baseline IPF variables described herein and other variables that may be associated with disease outcomes, such as other disease biomarkers) and outcomes, such as IPF outcomes. Using well-known statistical methods, hazard ratios (HRs) are calculated, representing the relationship between the covariate, *e.g.*, LOXL2 level, and the subject outcome, endpoint, or event. Thus, in some aspects, the provided methods include using such models to predict outcomes, endpoints, and/or events, *e.g.*, IPF disease outcomes, in individual patients based on LOXL2 levels and values for other covariates. In one example, the model includes LOXL2 levels (for example, the presence or absence of "high" LOXL2 levels), 6MWD, and/or CPI.

[00191] IPF outcomes, events, and endpoints for use in such modeling include endpoints or events indicative of disease progression or severity, such as any endpoint typically specified in IPF clinical trials or treatment regimen, such IPF disease progression, lung function decline, respiratory hospitalization, and death. In some aspects, disease

progression represents a composite endpoint defined as one of the following: mortality from any cause, respiratory hospitalization, or a categorical decrease in lung function, defined as either a 10 % decrease in forced vital capacity (FVC) with a 5% decrease in the diffusion capacity for carbon monoxide (DL_{CO}) or a 15 % decrease in DL_{CO} with a 5% decrease in FVC). Lung function endpoints may be determined using pulmonary function tests. In some examples, at least two tests are used, conducted at least 4 weeks apart. Other exemplary endpoints are all cause mortality, transplant free survival, and death. The outcome can be defined as the time that elapses before such an endpoint is reached.

[00192] Receiver Operating Characteristic (ROC) Curves may be used to evaluate sensitivity versus specificity of the systems. Area Under the Curve (AUC) is computed using well-known methods.

[00193] In some examples of the predictive models, LOXL2 is significantly associated with one or more outcome or event, such as disease progression, for example, at a particular confidence interval (CI) and confidence level, such as a 95 % confidence interval, for example, based on a P-value less than a particular threshold amount, *e.g.*, 0.05. The hazard ratio may be used to determine the fold-change in risk of a particular outcome, for a given covariate, such as high LOXL2 levels. In some aspects, a given LOXL2 level is associated, *e.g.*, statistically significantly associated, with at least a 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, or 7-fold risk in developing a particular outcome, such as disease progression, hospitalization, decrease in lung function, or other outcome as described herein. The fold-change in risk, for example, can be expressed in terms of comparison to a normal subject, such as one not having an elevated level of LOXL2 or one having a "low" LOXL2 level. In one example, LOXL2 levels, *e.g.*, "high" LOXL2 levels, are statistically significantly associated with the outcome, *e.g.*, disease progression, when other covariates are included in the model, such as 6MWD and CPI.

KITS AND ASSAY DEVICES

[00194] The present disclosure provides kits and assay devices for carrying out a subject assay for circulating LOXL2.

[00195] In some embodiments, a subject kit includes: a) a first antibody specific for LOXL2; and b) a second antibody specific for LOXL2. In some cases, the first antibody is a polyclonal LOXL2-specific antibody; and the second antibody is a monoclonal LOXL2-specific antibody. In other cases, the first antibody is a monoclonal LOXL2-specific antibody; and the second antibody is a monoclonal LOXL2-specific antibody. In other cases, the first antibody is a polyclonal LOXL2-specific antibody; and the second antibody is a

polyclonal LOXL2-specific antibody. The first and/or the second antibody will in some cases comprise a detectable label. In some cases, neither the first nor the second antibody comprises a detectable label.

[00196] The first antibody will in some embodiments be immobilized on an insoluble support. Alternatively, an insoluble support is provided with the kit, and the user will effect immobilization of the first antibody onto the insoluble support. Thus, in some cases, a subject kit includes: a) a first antibody specific for LOXL2; b) a second antibody specific for LOXL2; and c) an insoluble support. The insoluble support can be provided in any of a variety of materials and formats, as described above. For example, in some instances, the insoluble support is a plastic multi-well plate, a test strip, or a dipstick.

[00197] As noted above, in some instances, neither the first nor the second antibody comprises a detectable label. In these cases, a third antibody that comprises a detectable label, and that binds to the second antibody, may be provided; such an antibody is generally referred to as a secondary antibody. The detectable label can be, e.g., a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, or a radioisotope. Thus, in some embodiments, a subject kit comprises: a) a first antibody specific for LOXL2; b) a second antibody specific for LOXL2; and c) a third antibody, where the third antibody comprises a detectable label, and binds to the second antibody. In some cases, a subject kit comprises: a) a first antibody specific for LOXL2; b) a second antibody specific for LOXL2; c) a third antibody, where the third antibody comprises a detectable label, and binds to the second antibody; and d) an insoluble support. The insoluble support can be provided in any of a variety of materials and formats, as described above. For example, in some instances, the insoluble support is a plastic multi-well plate, a test strip, or a dipstick.

[00198] A subject kit can further include purified LOXL2, for use in generating a standard curve.

[00199] A subject kit can further include one or more additional components, e.g., a buffer; a protease inhibitor; a detectable label; wash reagents; blocking agents; etc. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired.

[00200] In addition to above-mentioned components, a subject kit can include instructions for using the components of the kit to practice a subject method. The instructions for practicing a subject method are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or

plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. compact disc-read only memory (CD-ROM), digital versatile disk (DVD), diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

Assay device

[00201] The present disclosure further provides an assay device for use in detecting LOXL2 in a liquid biological sample obtained from an individual. The device can include a matrix defining an axial flow path.

[00202] The matrix can comprise: i) a sample receiving zone at an upstream end of the flow path that receives the liquid sample; ii) one or more test zones positioned within the flow path and downstream from the sample receiving zone, each of the one or more test zones comprising immobilized therein an antibody specific for LOXL2 in each of the test zones, to form an immobilized anti-LOXL2/LOXL2 complex; and iii) one or more control zones positioned within the flow path and downstream from the sample receiving zone, where the one or more control zones can include positive and/or negative controls. The test zones and control zones can be positioned in an alternating format within the flow path beginning with a test zone positioned upstream of any control zone.

[00203] The matrix can comprise: i) a sample receiving zone at an upstream end of the flow path that receives the liquid sample; ii) one or more test zones positioned within the flow path and downstream from the sample receiving zone, each of the one or more test zones comprising an antibody specific for LOXL2 in each of the test zones, to form an anti-LOXL2/LOXL2 complex; and iii) one or more control zones positioned within the flow path and downstream from the sample receiving zone, where the one or more control zones can include positive and/or negative controls. The test zones and control zones can be positioned in an alternating format within the flow path beginning with a test zone positioned upstream of any control zone. In some embodiments, the antibody specific for LOXL2 is not immobilized; and, when the anti-LOXL2 antibody binds any LOXL2 present in the sample, the anti-LOXL2 antibody/LOXL2 complex is mobilizable. For example, the anti-LOXL2

antibody/LOXL2 complex formed in a first test zone can be mobilized such that it enters a second test zone comprising an immobilized anti-LOXL2 antibody, where the anti-LOXL2 antibody/LOXL2 complex binds to the immobilized anti-LOXL2 antibody, forming an immobilized anti-LOXL2 antibody/LOXL2 complex.

[00204] In using such an assay device, in some embodiments, a labeled antibody specific for LOXL2 can first be mixed with a liquid sample before the liquid sample is applied to the sample receiving zone of the device, where such mixing results in a labeled antibody/LOXL2 complex. In these embodiments, the liquid sample comprising the labeled antibody/LOXL2 complex is applied to the sample receiving zone of the assay device. The liquid sample flows along the device until the liquid sample reaches a test zone. Antibody present in the test zone binds LOXL2 present in the labeled antibody/LOXL2 complex; and can then be detected.

[00205] The assay device can further include a label zone comprising a labeled antibody specific for LOXL2, where the labeled antibody is capable of binding LOXL2 present in an immobilized LOXL2/anti-LOXL2 antibody complex, to form a labeled LOXL2/anti-LOXL2 antibody complex, where the labeled antibody is mobilizable in the presence of liquid sample. In using such an assay device, a liquid sample which may comprise LOXL2 is applied to the sample receiving zone of the device; anti-LOXL2 antibody present in the label zone binds the LOXL2, forming labeled antibody/LOXL2 complex, which, like the labeled antibody, is mobilizable; and the labeled antibody/LOXL2 complex flows alone the device until the liquid sample reaches a test zone. Anti-LOXL2 antibody present in the test zone binds the LOXL2 present in the labeled antibody/LOXL2 complex; and can then be detected.

[00206] Alternatively, the assay device can include a label zone comprising a labeled antibody specific for an anti-LOXL2 antibody, where the labeled antibody binds to any anti-LOXL2 antibody/LOXL2 complexes formed in the test zone(s). In some cases, the labeled antibody is mobilizable.

[00207] The labeled antibody can comprise a label such as a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, or a radioisotope.

[00208] Control zones include positive control zones and negative control zones.

[00209] The matrix is generally an insoluble support, where suitable insoluble supports include, but are not limited to, polyvinyl difluoride (PVDF), cellulose, nitrocellulose, nylon, and the like. The matrix can be flexible, or can be relatively inflexible.

The matrix can be positioned within a housing comprising a support and optionally a cover, where the housing contains an application aperture and one or more observation ports. The assay device can be in any of a variety of formats, e.g., a test strip, a dipstick; etc.

EXAMPLES

[00210] The following examples are not intended to limit the scope of what the inventors regard as their invention; nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Immunoassay for detecting LOXL2 in human serum or plasma samples

MATERIALS AND METHODS

Antibodies

[00211] Rabbit polyclonal antibody ("rabbit A") was raised against recombinant purified full-length LOXL2 protein; this antibody recognizes multiple epitopes in all domains of LOXL2. Mouse monoclonal antibody, AB0030, binds to the catalytic domain of LOXL2 and recognizes both the full-length LOXL2 protein and the mature LOXL2 protein (which is cleaved between SRCR2 and SRCR3 domains).

LOXL2 immunoassay on MSD platform

[00212] Standard single-spot uncoated electrode plates from MesoScale Discovery (MSD) (cat #L15XA-3) were coated overnight at 4°C with a 30µl volume of a solution of 3µg/ml rabbit anti-human-LOXL2 polyclonal antibody formulated in phosphate-buffered saline (PBS). After coating, the wells of the plates were blocked by addition of a solution of 5% (w/v) Blocker A (MSD cat#R93AA-1) in PBS. After the blocking step, plates were washed 3 times in PBS containing 0.05% Tween-20 non-ionic detergent, using an automated plate washer. Human samples to be tested (serum or plasma) were prepared separately by diluting them 1:4 in PBS (1 part serum, 3 parts PBS). Samples were then added to each well of the plate. Samples were incubated with rotary shaking (300-600rpm) for 2-3 hours at

room temperature. After sample binding, the plates were again washed 3 times in PBS containing 0.05% Tween-20 detergent, using an automated plate washer.

[00213] The primary antibody, AB0030, is a mouse anti-human-LOXL2 monoclonal antibody that binds the LOXL2 catalytic domain. A solution of 1 μg/ml AB0030 in 2% (w/v) Blocker A in PBS was added to each well, and the plates were then incubated with rotary shaking (300-600rpm) for 1 hour at room temperature. After AB0030 binding, the plates were again washed 3 times in PBS containing 0.05% Tween-20 detergent, using an automated plate washer.

[00214] The secondary antibody is a goat-anti-mouse-IgG molecule conjugated to SulfoTag dye (MSD cat#R32AC-5). A solution of 1μg/ml secondary antibody in 2% (w/v) Blocker A in PBS was added to each well, and the plates were incubated with rotary shaking (300-600rpm) for 1 hour at room temperature. After secondary antibody binding, the plates were washed 3 times in PBS containing 0.05% Tween-20 detergent, using an automated plate washer.

[00215] 1x Read Buffer T with Surfactants (MSD cat#R92TC-2) was added to each well, followed by immediate measurement of the plate on the MSD SectorImager 2400 instrument.

[00216] Test human samples were given a relative quantitative value of LOXL2 by comparison to the calibrator curve on the same assay plate, comprised of purified recombinant human LOXL2 protein (R&D Systems) added in known concentrations to human serum or plasma pooled from normal healthy donors. Calibrator curve fitting and unknown sample interpolation were carried out using standard techniques.

LOXL2 immunoassay using standard format

[00217] Costar 3922 high-binding multi-well plates were used. Rabbit polyclonal antibody (Ab) (rabbit "A") was diluted to 0.625 μg/ml in CB2 coating buffer (Immunochemical Technologies CB2 (6248)). Diluted polyclonal Ab was added to the wells of the plate at a volume of 50 μl/well, and the plates were kept at 4°C overnight. After coating the wells with the polyclonal antibody, wells were blocked with 200 μl/ well of BB1 block solution (Immunochemical Technologies product # 640) for 1-3 hr at room temperature (RT). Following blocking, the plates were washed 3x using 200 μl per well PBS-T (PBS containing 0.05% Tween 20).

[00218] 25 μl HiSpec diluent (AbD Serotec BUF049B) was added to each well. An equal volume of test serum was then added to each well; and plates were kept at room

temperature for 2 hours. After allowing the serum samples to bind, the plates were washed three times.

[00219] The primary antibody (AB0030) was diluted to 5 μg/ml in PBS-T + 0.5% bovine serum albumin (BSA); 50 μl of the diluted primary antibody was added to each well. Plates were kept at room temperature for one hour, then washed three times with PBS-T. The secondary antibody (horse radish peroxidase (HRP)-conjugated goat anti-mouse antibody, Jackson Immunoresearch, 0.8mg/ml) was diluted 1:10,000 in PBS-T + 0.5% BSA. 50 μl of the diluted secondary antibody was added to each well. Plates were kept at room temperature for one hour, then washed three times with PBS-T.

Example 2: Serum LOXL2 measurement for estimation of liver fibrosis in patients with chronic hepatitis C virus (HCV) infection

[00220] Analysis of fibrotic liver tissues by immunohistochemistry (IHC) revealed localized LOXL2 expression at the fibrogenic interface composed of fibroblasts, neovasculature, inflammatory cells and hepatocytes, suggesting that LOXL2 is associated with active fibrogenic disease. To further explore the relationship of serum LOXL2 with fibrotic liver disease, a LOXL2-specific ELISA as described in Example 1 was used. Serum samples, along with liver biopsies, were collected from 87 patients with chronic HCV infection. Serum levels of LOXL2 and of the established biomarkers hyaluronic acid (HA) and tissue inhibitor of metalloproteinases-1 (TIMP1) were measured by immunoassay, and the histological stage of liver fibrosis was assessed for each biopsy using the Ishak scoring system. Separately, serum samples from over 30 healthy donors were also collected and assessed for serum LOXL2 levels. The correlation between the serum biomarkers and the fibrosis scores was studied using ANOVA test, as well as the Mann-Whitney U test for samples binned by fibrosis score.

RESULTS

[00221] The results are shown in Figures 1 and 2. LOXL2 protein was detected in the serum of 83% of patients with chronic HCV infection, but was not detected in serum from any normal healthy donors. There was a positive correlation between serum levels of HA, TIMP1, and LOXL2 and stage of fibrosis. The serum results were consistent with the IHC analysis, which revealed high levels of LOXL2 protein in areas of active fibrosis, compared to low or undetectable levels in samples from non-infected or healthy individuals.

Example 3: Serum LOXL2 in IPF patients

[00222] Serum samples from 15 patients with a diagnosis of idiopathic pulmonary fibrosis (IPF) were tested for LOXL2. The results are shown in Figure 3. Individual patient identification numbers are shown. Ten of 15 patients tested positive; the other 5 were below the limit of detection and are reported as "not detected." Age-matched normal subjects were also tested; all were negative ("not detected"; below the limit of detection) for serum LOXL2.

Example 4: Serum LOXL2 in oncology patients

[00223] Eight cancer patients being treated with anti-LOXL2 (AB0024) antibody for the cancer were studied. Patient identification ("Pt ID"); cancer diagnosis; dose level of anti-LOXL2 antibody; time to progression; and LOXL2 expression, as examined by immunohistochemistry in a sample (~5 μm section) of fixed tissue isolated from the original primary tumor or related sample, are provided in Table 1, below.

Table 1

Pt ID	Diagnosis	Dose level (mpk)	Time to progression	LOXL2 expression
001	Renal cell	1	44 days	Vascular
002	Colorectal	1	Stable (~7 months)	Positive desmoplastic
003	Endometrial mixed mullerian	1	57 days	Minimal; not desmoplastic
004	Breast	3	38 days	Minimal; patchy
005	Colorectal	3	56 days	Positive desmoplastic
006	Melanoma	3	42 days	positive
007	Colon SC	10	57 days	
008	Prostate	10	30 days	Positive desmoplastic
009	Ovarian/breast	10	51 days	Weak, not desmoplastic

[00224] Blood samples were obtained at Day 1 on which anti-LOXL2 treatment began (sample taken before anti-LOXL2 treatment); and on days 29 and 57 following the beginning of anti-LOXL2 treatment.

RESULTS

[00225] LOXL2 was detected in plasma of 8 of 8 patients, and in serum samples of 5 of 8 patients, at all time points available. AB0024 administration did not clear or mask the LOXL2 signal.

Example 5: LOXL2 expression in liver tissue from patients with chronic HCV infection, non-alcoholic steatohepatitis (NASH)1, and alcoholic steatohepatitis (ASH)Immunohistochemical (IHC) staining demonstrated LOXL2 expression in liver tissues from a patient having chronic HCV infection. Snap-frozen human tissue samples were obtained from Cureline (Burlingame, CA) and Asterand (Detroit, MI) and serial sections were stained with anti-LOXL2.

RESULTS

[00226] Results from sections obtained from a patient with chronic HCV infection are shown in Figure 6, showing LOXL2 protein expression in the liver tissue of this patient. In the left panel of Figure 6 (5x objective magnification), black arrows indicate areas of fibrous expansion into portal regions and tracts. White arrows indicate areas of short fibrous septa surrounding hepatic lobules. The right panel of Figure 6 (40x objective magnification) shows LOXL2 immunoreactivity, observed in the fibrous septa (S) at the interface with hepatocytes (H), within the perisinusoidal space (arrows), and in the myofibroblasts within the liver parenchyma (arrows). The results show that in this study, LOXL2 was expressed in liver tissues of patients with chronic HCV infection, and that the expression is measurable by embodiments of the provided assays. In another IHC study, a strong localization of LOXL2 expression in liver tissue at the active disease interface in NASH, HCV-associated fibrosis, and ASH, but not in healthy liver (data not shown).

Example 6: Calibrator standards for LOXL2 immunoassay in human serum matrix

[00227] Using the LOXL2 immunoassay described in Example 1 (sandwich mmunoassay developed on the MesoScale Discovery platform), LOXL2 was not detected in serum from healthy individuals. To create a calibrator curve, purified recombinant full-length LOXL2 protein was added into pooled normal human serum, followed by serial dilution in serum.

RESULTS

[00228] The results are shown in Figure 7. Each data point represents the mean of 3 replicate wells; curves for 4 independent plates are shown.

[00229] Table 2 shows the characteristics of calibrator standards in human serum matrix. In Table 2, lower limit of detection (LLOD) is the mean + 2.5*stdev of the blank wells (raw values, extrapolated); Lower limit of quantitation (LLOQ) is the lowest calibrator standard with relative error < 30% and coefficient of variation < 30% for the raw

measurements. Intra-assay and inter-assay precision were determined using incurred samples.

Table 2: LOXL2 immunoassay: characteristics of calibrator standards in human serum matrix

Assay characteristic	Result
Accuracy (relative error)	<15%
Intra-assay precision	3.5%
Inter-assay precision	15.5%
Recovery after freeze/thaw cycle	70% one cycle, >70% 2 or more cycles
Lower limit of detection (LLOD)	150-200 pg/ml
Lower limit of quantitation (LLOQ)	180-550 pg/ml
Upper limit of quantitation (ULOQ)	Not determined

Example 7: Increased serum LOXL2 levels in subjects with liver cirrhosis as compared to those with mild to moderate liver fibrosis

[00230] Patient serum samples were collected from twenty-six adults with chronic hepatitis C infection enrolled in the placebo arm of a clinical trial. Subjects were grouped by Ishak fibrosis scores (1-3: mild to moderate fibrosis; 5-6: cirrhosis). Demographic characteristics of the subjects are shown in Table 3.

Table 3: Demographic characteristics of HCV subjects

Characteristic	Ishak Score 1-3 (n=14)	Ishak Score 5-6 (n=12)	All (n=26)
Age*	53 (50.5, 56.0)	55 (47.8, 55.0)	53.5 (49.3, 55.8)
Sex Male Female	 9 (64.3%) 5 (35.7%)	 9 (75.0%) 3 (25.0%)	 18 (69.2%) 8 (30.8%)
Race White Black	 11 (78.6%) 3 (21.4%)	 10 (83.3%) 2 (16.7%)	 21 (80.8%) 5 (19.2%)

Baseline Ishak Fibrosis Score (n)					
F1	F2	F3	F4	F5	F6
3	6	5	0	7	5

*Median and inter-quartile range (25%,75%) reported

[00231] Serum samples were taken at six time points, relative to the study baseline: weeks 4, 8, 16, 24, 26, and 30. Paired liver biopsies (screening and week 24) were evaluated by a central pathologist in a blinded fashion. *See* Manns M, Palmer R, Flisiak E, et al., *J*

Hepatology. 2011, 54 Supplement 1: S55–S56. Serum LOXL2 was measured using the LOXL2 immunoassay described in Example 1 (sandwich mmunoassay developed on the MesoScale Discovery platform).

[00232] For statistical analysis, subjects were grouped by Ishak fibrosis scores (1-3: mild to moderate fibrosis; 5-6: cirrhosis). No subject in the study was observed to have a baseline Ishak fibrosis score of 4. Serum samples with detectable LOXL2 below the assay lower limit of quantitation (LLOQ) were set to the LLOQ. Differences in biomarkers levels were descriptively and graphically summarized. 95% confidence intervals (CI) were constructed through 10,000 bootstraps of the median using sampling with replacement with the observed sample sizes per group. P-values were calculated using Wilcoxon rank sum tests when comparing groups within a time point and by a repeated measures linear model with a within-subject random effect when comparing groups across all time points.

RESULTS

[00233] Figure 8 shows LOXL2 serum levels by binned baseline Ishak fibrosis score and time. Each panel shows, for the indicated time point (weeks 4, 8, 16, 24, 26, 30), LOXL2 concentration (pg/mL) for two groups of patients, grouped according to Ishak Fibrosis Score (1-3 and 5-6, respectively). Three outliers (LOXL2 concentration = 5529, 6621, 8845 pg/ml), with LOXL2 concentration out of plot ranges all were from the same subject, having an Ishak fibrosis score of 5.

[00234] Figure 9 shows the median within-subject LOXL2 serum levels, calculated as median LOXL2 serum concentration over weeks 4-30, for the two groups of patients, grouped according to Ishak Fibrosis Score (1-3 and 5-6, respectively). The average within-subject coefficient of variation was 22 %.

[00235] Figure 10 shows median LOXL2 serum concentration (pg/mL) over time (weeks), by binned baseline ishak fibrosis score, with 95% confidence intervals. Only one subject had a change greater than or equal to 2 in Ishak fibrosis score over the 25-28 weeks between study biopsies.

[00236] Table 4 shows the median LOXL2 concentration (pg/mL) for each time-point, with p-values showing statistical significance of the increase in subjects with liver cirrhosis compared to those with mild to moderate liver fibrosis.

Table 4: Statistical significance of LOXL2 serum levels according to binned fibrosis score

Time point	Median LOXL2 conc. (pg/ml)		P-value
	Ishak F1-F3	Ishak F5-F6	
Week 4	641	1684	0.0149

Week 8	786	1700	0.0091
Week 16	814	1457	0.0407
Week 24	881	1616	0.0596
Week 26	865	1763	0.0716
Week 30	711	1118	0.5890
Overall	810	1591	**0.0275**

[00237] These results confirm the ability of an embodiment of provided immunoassays to measure serum concentrations of LOXL2 protein. The results also demonstrate that in this study, serum LOXL2 protein levels were significantly increased in subjects with liver cirrhosis as compared with those with mild to moderate liver fibrosis, and that the increase is measurable in serum using embodiments of the provided assays.

Example 8: Serum LOXL2 levels correlated with serum hyaluronic acid TIMP1 levels in subjects with chronic HCV infection

[00238] The immunoassay and statistical analysis was carried out as in Example 7. Additionally, hyaluronic acid (HA) and TIMP1 were measured using commercial immunoassay kits. The association between the biomarkers (LOXL2 and HA or TIMP1) was assessed using Spearman rank correlation.

RESULTS

[00239] Figure 11 shows median within-subject levels of LOXL2 vs. levels of Hyaluronic acid (HA) (left panel) and tissue inhibitor of metalloproteinases-1 (TIMP1) (right panel), for subjects having the indicated Ishak scores (1-6). Median within–subject expression was calculated as median expression over weeks 4 through 30. The curve was constructed using locally weighted scatter plot smoothing.

[00240] These results demonstrate that in this study, serum LOXL2 levels were correlated with serum HA and TIMP1 levels, as measured using an embodiment of the provided immunoassay.

Example 9: LOXL2 baseline levels in IPF patients

A. ARTEMIS-IPF Patients

[00241] Serum samples were collected from subjects participating in the ARTEMIS-IPF trial. This was a randomized, double-blind, placebo-controlled, event-driven trial. Subjects were randomized in a 2:1 ratio to receive ambrisentan, a selective antagonist of the ET_A receptor, or placebo. This study was terminated prematurely; 660 subjects were enrolled.

[00242] Baseline variables reflective of IPF severity and functional status were collected. The baseline variables included percent of predicted forced vital capacity (FVC), percent of predicted carbon monoxide diffusion capacity (DL_{CO}), 6-minute walk distance (6MWD), mean pulmonary artery pressure (mPAP), the lowest resting oxygen saturation (SpO2), the composite physiologic index (CPI), the St. George's Respiratory Questionnaire score (SGRQ), and the Transition Dyspnea Index (TDI) score. The mPAP was obtained via a right heart catheterization, which was required of all study subjects at baseline. The CPI was a validated multidimensional model incorporating FVC, the forced expiratory volume in one-second (FEV_1) and DL_{CO} to estimate the extent of fibrosis seen on a computed tomographic scan of the patient's chest. The primary endpoint was time to IPF disease progression, a composite endpoint defined as one of the following: mortality from any cause, respiratory hospitalization, or a categorical decrease in lung function, defined as either a 10% decrease in forced vital capacity (FVC) with a 5% decrease in the diffusion capacity for carbon monoxide (DL_{CO}) or a 15 % decrease in DL_{CO} with a 5% decrease in FVC. Lung function endpoints were confirmed by two pulmonary function tests conducted at least 4 weeks apart.

[00243] The baseline levels of LOXL2were quantified in triplicate using an immunoassay developed on the MesoScale Discovery platform using anti-LOXL2 antibodies described in Example 1.

[00244] Standard histograms were used to evaluate untransformed and $\log_{10}X$ transformed LOXL2 baseline levels. Student's T-test was used to compare distribution of baseline variables. Pearson's correlation coefficient was used to examine the relationship between LOXL2 baseline levels and baseline variables. Stepwise Cox proportional hazard modeling was used to examine the relationship between LOXL2 baseline levels and IPF outcomes. Receiver operating curves were used to estimate the area under the curve.

Results

[00245] Serum samples from 69 subjects in the intent-to-treat cohort were available for the analysis. In comparison to the 423 subjects from ARTEMIS-IPF for whom no serum samples were available, there were no statistically significant differences in baseline measures of IPF severity or functional status (Table 5). However, among the 69 subjects, there were statistically significant differences in baseline measures of IPF severity and functional status when comparing the ambrisentan and the placebo treatment groups (Table 6). Subjects in the ambrisentan group had lower baseline DL_{CO} (p=0.035), lower baseline 6MWD (p=0.004), higher baseline mPAP (p=0.016), higher baseline CPI (p=0.05) and

higher baseline SGRQ (p=0.011). The mean baseline LOXL2 level was higher for the ambrisentan subjects (p=0.026).

[00246] Analysis of the distribution of LOXL2 baseline levels showed 8 subjects having LOXL2 levels of less than about 88 pg/mL, 34 subjects having LOXL2 levels of about 88 to about 440 pg/mL, and 28 subjects having LOXL2 levels of more than about 440 pg/mL. The median LOXL2 level was about 325 pg/mL with an interquartile range of about 147 pg/mL to about 770 pg/mL, and minimum of about 18 pg/mL and maximum of about 5,400 pg/mL.

[00247] Based on Pearson's correlation coefficient, correlation was weak between LOXL2 baseline levels and these baseline measures of IPF severity and functional status. Figure 12 shows scatter plot matrices representing the relationship between LOXL2 baseline levels and FVC, DL_{CO}, 6MWD, CPI, SGRQ, and TDI. Correlations between LOXL2 and baseline severity measures were highlighted within the dark boxes at the top row of panels (a) and (b). The correlation coefficients between LOXL2 and the individual baseline severity measures were as follows: -0.21 (FCV), -0.11 (DL_{CO}), 0.03 (6MWD), 0.10 (mPAP), -0.07 (SpO_2), 0.14 (CPI), 0.06 (SGRQ), and -0.05 (TDI). Whereas $Log_{10}X$ transformation of the LOXL2 baseline levels normalized the distribution, correlation between LOXL2 and baseline measures of IPF severity and functional status remained weak (Figure 12b).

[00248] Given the majority of the baseline LOXL2 levels were less than about 800 pg/mL, the LOXL2 baseline levels were dichotomized as $\leq$ 800 pg/mL ("low") versus > 800 pg/mL ("high") for the remainder of the analysis. Of the 28 subjects having LOXL2 baseline levels of more than about 440 pg/mL, 12 had low LOXL2 baseline levels of about 440-800 pg/mL and were grouped into the low group; and 16 had LOXL2 baseline levels of more than 800pg/mL and were grouped into the high group.

[00249] Comparison of disease progression between the "high" and "low" LOXL2 baseline level groups is shown in Figure 13. Because there were only two patients having "high" LOXL2 baseline lines in the placebo group (neither of which had any events), Figure 13 compares only "low" and "high" LOXL2 baseline levels in the ambrisentan group. Results indicated that high LOXL2 baseline level was associated with more disease progression events (Figure 13a) and that high LOXL2 baseline levels were associated with more lung function decline events (Figure 13b), more respiratory hospitalizations (Figure 13c) and more deaths (Figure 13d).

[00250] Additionally, as shown in Table 7, Cox proportional hazard modeling indicated that presence of a high LOXL2 baseline level was associated with a 5-fold

increase in risk for disease progression (hazard ratio [HR] 4.95, 95% confidence interval [CI] 1.52-16.18, p=0.008), a 7-fold increase in risk for lung function decline (HR 7.36, 95% CI 1.16=46.74, p=0.034), and a 5-fold increase in risk for respiratory hospitalization (HR 4.85, 95% CI 1.09-21.68, p=0.039). All of these statistical models were adjusted for treatment assignment and baseline 6MWD and CPI score. High baseline LOXL2 levels were not significantly associated with a significant increase in risk for death (HR 1.59, 95% CI 0.24-10.53, p=0.633).

[00251] Samples were also analyzed for levels of MMP7, ICAM1, IL8, VCAM1, and S100A12. None of these proteins was significantly associated with the treatment outcomes. The results showed that high baseline LOXL2 levels were associated with a 5-7 fold increase in risk for IPF disease progression, but not death.

[00252] Table 5. Comparison of baseline IPF severity and functional status according to availability of serum in ARTEMIS-IPF

Baseline Measures of IPF Severity	No Serum N=423	Serum N=69	P-value
Mean % FVC (SD)	69 (14)	70 (12)	0.649
Mean % DLCO (SD)	43 (14)	42 (11)	0.487
Mean 6MWD m (SD)	416 (120)	399 (116)	0.256
Mean PAP mmHg (SD)	20 (7)	20 (6)	0.920
Mean lowest SpO2 % (SD)	88 (6)	88 (6)	0.825
Mean CPI (SD)	52 (11)	53 (9)	0.784
Mean SGRQ (SD)	39 (20)	38 (18)	0.605
Mean TDI (SD)	7 (2)	8 (2)	0.588

[00253] Table 6. Comparison of baseline IPF severity and functional status according to treatment assignment in ARTEMIS-IPF among subjects with baseline serum available

Baseline Measures of IPF Severity	Ambrisentan N=49	Placebo N=20	P-value
Mean % FVC (SD)	68 (12)	73 (12)	0.128
Mean % DLCO (SD)	40 (11)	47 (9)	0.035

Mean 6MWD m (SD)	373 (109)	461 (110)	0.004
Mean PAP mmHg (SD)	22 (6)	18 (5)	0.016
Mean lowest SpO_2 % (SD)	87 (6)	87 (5)	0.166
Mean CPI (SD)	54 (9)	49 (8)	0.050
Mean SGRQ (SD)	42 (19)	29 (15)	0.011
Mean TDI (SD)	7 (2)	8 (2)	0.083
Mean LOXL2 (SD)	903 (1172)	295 (288)	0.026

[00254] Table 7. Levels of baseline LOXL2 in IPF patients and its relationship with study endpoints.

Endpoints	# of Events		Hazard Ratio (95% CI) for High LOXL2	P-value
	Low LOXL2	High LOXL2		
Disease Progression	10	8	4.95 (1.52-16.18)	0.008
Lung Function Decline	5	4	7.36 (1.16-46.74)	0.034
Respiratory Hospitalization	6	6	4.85 (1.09-21.68)	0.039
Death	5	4	1.59 (0.24-10.53)	0.633

B. GAP cohort IPF Patients

[00255] Serum LOXL2 levels were assessed in subjects in a second clinical IPF prospective follow-up study, which assessed disease progression in 111 IPF subjects (deemed the GAP cohort) who had no history of other lung illnesses. All GAP cohort subjects were diagnosed with IPF according to ATS/ERS guidelines, confirmed by surgical lung biopsy or radiographic findings of subpleural honeycomb changes, traction bronchiectasis, and minimal alveolar filling in patients over 55 years of age and without a defined etiology. Pulmonary function testing revealed a forced vital capacity of 40-70% predicted. Subjects were able to receive all ongoing care and follow-up at a clinical facility.

[00256] At the initial visit, each participant had a blood draw, pulmonary function testing, 6-minute walk test (6MWT), echocardiogram, and CT scan, and several questionnaires designed to measure how the patient was feeling. At follow-up visits in 3-8

month intervals, blood samples were collected and PFTs, questionnaires, and 6MWTs were repeated. The median FVC, FEV1, and DLCO were 65.7 ± 17.5%, 76.8 ± 18.7%, and 47.3 ± 17.9% of the predicted values, respectively.

[00257] Baseline serum levels of LOXL2 were quantified as described above for the ARTEMIS-IPF subjects. Standard histograms were used to evaluate LOXL2 baseline serum levels at the natural log format. LLOD of 180 pg/mL and LLOQ of 440 pg/mL were determined experimentally.

[00258] LOXL2 levels for the GAP cohort were normalized to the ARTEMIS-IPF data after natural log transformation using a regression method. The results are shown in Figure 14.

[00259] Time to all-cause mortality was assessed, with a lung transplant considered a death event (most lung transplant patients died). A classification and regression trees (CART) method was applied as an unbiased approach to select the optimal threshold or cut-off point for dichotomization of the baseline serum LOXL2 levels. In the GAP cohort, when Log(LOXL2) was the only variable, CART analysis selected 440 pg/mL (6.08 at natural log scale) as the cut-off point.

[00260] Table 8A shows baseline and demographic characteristics for subjects in the GAP cohort, and Table 8B shows correlation among various baseline values in this cohort.

[00261] Table 8A: GAP Cohort Baseline and demographic characteristics

Variable	N	Mean (Std)	Median (Min, Max)
Sex	M: 74 (67%) F: 37 (33%)		
Age (Years)	111	67 (9.3)	67 (3, 84)
FVC %Predicted	73	66 (18)	64 (34, 113)
FEV_1 %Predicted	73	77 (19)	74 (37, 129)
DLCO %Predicted	73	48 (18)	46 (14, 109)
CPI	73	52 (13)	52 (12, 78)
6 Min Walk Distance	17	912 (420)	890 (100, 1555)
LOXL2	111	1495 (2307)	717 (90, 15708)
LOG (LOXL2)	111	7 (1)	7 (5, 10)
LOG (LOXL2) *	111	6 (1)	6 (5, 9)

* Normalized LOXL2 through a regression method

[00262] Table 8B: Correlation among baseline variables

	Age	FVC % pred.	FEV1 % pred.	DLCO % pred.	CPI
Log LOXL2	-0.7	-0.03	-0.06	-0.28	-0.24
Age		0.07	0.23	0.02.	0.05
FVC % pred.			0.93	0.38	-0.61
FEV1 % pred.				0.47	-0.60
DLCO % pred.					-0.95

[00263] The correlation between the dichotomized LOXL2 levels and all cause mortality was evaluated using Cox proportional hazard modeling and Kaplan-Meier survival plots at six (6) months, twelve (12) months, eighteen (18) months, and twenty-four (24) months after baseline. The correlation between baseline LOXL2 levels and hospitalization and lung function decline was not evaluated as data was not available.

[00264] Analysis of the distribution of baseline LOXL2 levels showed a skewed distribution toward the lower spectrum, similar to that observed for the ARTEMIS-IPF cohort. The median baseline LOXL2 level was 716.5pg/mL (interquartile range 358.3 pg/ml, 1446.6 pg/ml). Correlation was weak between LOXL2 and baseline demographics and baseline clinical indicators of IPF severity (correlation coefficients for age -0.07, FVC -0.03, DLCO -0.28). No additional clinical indicators of disease severity were available for further analysis.

[00265] The results showed that a threshold 440 pg/ml baseline serum LOXL2 level was correlated with the risk for all-cause mortality. Presence of a baseline LOXL2 level higher than 440 pg/mL in the serum was associated with more deaths at 12-, 18-, and 24-months after baseline (Figure 15A and B).

[00266] Multivariate Cox proportional hazard modeling (covariates included age and sex) suggested that presence of a baseline LOXL2 level higher than 440 pg/mL was associated with a 2.3-fold increase in risk for death at 12-, 18-, and 24-months after baseline (see Table 9A and B).

[00267] Table 9A: Event rates and hazard ratios for subjects with low (≤440 pg/mL) versus high (>440 pg/mL) baseline LOXL2 levels at 6-, 12-, 18-, and 24-months after baseline in GAP cohort.

Time after Baseline	Event Rate		Hazard ratio* (95% CI)	P-value
	Low LOXL2	High LOXL2		
6 months	5/52 (10%)	10/59 (17%)	1.76 (0.60, 5.22)	0.3051

12 months	10/52 (19%)	23/59 (39%)	2.27 (1.05, 6.98)	0.0319
18 months	12/52 (23%)	26/59 (44%)	2.22 (1.12, 4.43)	0.0231
24 months	14/52 (27%)	30/59 (51%)	2.31(1.22, 4.37)	0.0105

*Models include age and sex as covariates

[00268] Table 9B: Event rates and hazard ratios for subjects with low (≤440 pg/ml) versus high (>440 pg/ml) baseline LOXL2 levels at 6-, 12-, 18-, and 24-months after baseline.

Time after Baseline	Event Rate		Hazard ratio (95% CI)	P-value
	Low LOXL2	High LOXL2		
6 months	2/36 (6%)	3/13 (23%)	5.08 (0.85, 30.47)	0.0756
12 months	5/36 (14%)	3/13 (23%)	1.90 (0.45, 7.99)	0.3796
18 months	5/36 (14%)	3/13 (23%)	1.90 (0.45, 7.99)	0.3796
24 months	5/36 (14%)	4/13 (31%)	2.11(0.54, 8.24)	0.2846

[00269] For a subset of the subjects, additional serum samples were collected prospectively. Over the duration of the study, two (2) samples were collected from 60 subjects, three (3) samples were collected from 42 subjects, four (4) samples were collected from 31 subjects, five (5) samples were collected from 17 subjects, six (6) samples were collected from 12 subjects, seven (7) samples were collected from seven (7) subjects, and eight (8) samples were collected from two (2) subjects. None of the samples were collected in association with an acute exacerbation.

[00270] Multivariate Cox proportional hazards modeling (with covariates including age and sex) was used, incorporating LOXL2 levels in each of the samples as a time-dependent continuous variable, to evaluate the relationship between serum LOXL2 levels and all-cause mortality. Serum LOXL2 levels measured over time were associated with the risk for mortality (p=0.003). In the GAP cohort, for each 2.7-fold increase in serum LOXL2 level drawn at any time during the study, the risk for mortality increased by 1.63 fold (95% confidence interval 1.19-2.25).

[00271] Table 10 shows results of a multivariate analysis with serum LOXL2 levels at various times after baseline.

[00272] Table 10: Multivariate analysis according to low (≤440 pg/mL) versus high (>440 pg/mL) serum LOXL2 levels at 6-, 12-, 18- and 24-months after baseline

Response Variable	Model Term	Hazard Ratio (95 % CI)	p-value
Time to death 6 month	Log LOXL2 (≤ or > 6.08)	1.8 (0.6, 5.2)	0.305
	Sex	*0.5 (0.1 1.8)	0.299
	Age (continuous)	1.0 (1.0, 1.1)	0.931
Time to death 12 months	Log LOXL2 (≤ or > 6.08)	2.3 (1.1, 7.0)	0.032

	Sex	*0.4 (0.2, 0.9)	0.037
	Age (continuous)	1.0 (1.0, 1.0)	0.647
Time to death 18 months	Log LOXL2 (≤ or > 6.08)	2.2 (1.1, 4.4)	0.023
	Sex	*0.5 (0.2, 1.0)	0.052
	Age (continuous)	1.0 (1.0, 1.0)	0.848
Time to death 24 months	Log LOXL2 (≤ or > 6.08)	2.3 (1.2, 4.4)	0.011
	Sex	*0.4 (0.2, 1.0)	0.026
	Age (continuous)	1.0 (1.0, 1.0)	0.808

*Hazard ratio favors female patients

[00273] The results of the GAP cohort were similar to those of the ARTEMIS-IPF study described above. Both studies showed that a baseline serum LOXL2 level that was higher than the threshold level was associated with an increased risk of negative outcome in IPF patients.

Example 10: Baseline serum LOXL2 levels in patients with Chronic Hepatitis B (CHB)

[00274] Serum LOXL2 levels were assessed in subjects with chronic hepatitis B (CHB) and liver fibrosis, both before treatment and after 240 weeks of treatment with 300 mg tenofovir disoproxil fumarate (TDF). Liver biopsies were taken from 348 human subjects with CHB, prior to treatment and after 240 weeks of treatment with TDF. The biopsies were scored by pathologists using the Ishak scale for assessment of fibrosis. In the study, 96.3% of the subjects exhibited improvement in, or no progression of, liver fibrosis. Of the 96 subjects who began the study with biopsy-proven cirrhosis, 74% had regression of cirrhosis after 240 weeks of treatment.

[00275] Serum LOXL2 levels were retrospectively assessed by ELISA at baseline and at week 240 for 81 of the 348 subjects, including several subjects exhibited an improvement in fibrosis score. At week 240 following treatment, 42 of these 81 subjects had cirrhosis regression, 16 had persistent cirrhosis, 2 had progressed to cirrhosis over the course of treatment, 18 were non-cirrhotic subjects with no change in fibrosis, and 3 were non-cirrhotic subjects with at least a 2-point reduction in fibrosis as measured by Ishak.

[00276] Baseline serum LOXL2 levels were elevated in 91% of the 81 CHB subjects and in 97% of cirrhotic subjects. As shown below, the patients with cirrhosis (Ishak score 5 or 6) had elevated median LOXL2 serum levels at baseline compared to the patients with less severe liver fibrosis. This observation is similar to the LOXL2 serum levels observed in patients with chronic Hepatitis C infection. Moreover, the histology study showed that LOXL2 protein was concentrated at the sites of active fibrogenesis (data not shown). These results suggest that the patients with cirrhosis are still undergoing active fibrogenesis in the

liver. Also, over the course of 240 weeks of treatment, 72% of the 60 patients with baseline cirrhosis showed a regression or improvement of their Ishak fibrosis score. Also, these patients had a lower median serum LOXL2 level at week 240 compared to baseline. The results suggest that both overall fibrosis and fibrogenesis were reduced by anti-viral treatment.

[00277] Figure 16A shows that serum LOXL2 levels (pg/mL) correlated with fibrosis score and Figures 16B and 16C show that serum baseline LOXL2 levels (pg/mL) correlated with baseline Ishak fibrosis score. At 240 weeks after treatment, mean serum LOXL2 levels had been reduced and no longer correlated with Ishak fibrosis score. See also Table 11.

[00278] Table 11: Mean Serum LOXL2 levels compared to Ishak Stage at baseline and week 240 after initiation of treatment

	N	Baseline	N	Week 240
All subjects (mean LOXL2 (pg/mL))	81	2678.6	81	748.9
Ishak Stage 0-3 (mean LOXL2 (pg/mL))	18	510.2	56	746.8
Ishak Stage 4-6 (mean LOXL2 (pg/mL))	63	3298.2	25	753.5

[00279] As shown in Figure 17, all subjects having a baseline Ishak stage between 1 and 3 had a serum LOXL2 level below 1500 pg/mL and 49 % of subjects with a baseline Ishak stage between 4 and 6 had serum LOXL2 levels above 1500 pg/mL.

[00280] 79% of the 81 subjects experienced a reduction in serum LOXL2 levels. The 11% of subjects (each with a baseline level below the limit of quantitation) had no change in LOXL2 levels.

[00281] Figure 18 show baseline and week-240 serum LOXL2 levels (pg/mL) for individual subjects in the following groups: subjects with persistent cirrhosis at week 240 (n=16, Figure 18A); subjects with reversal of cirrhosis by week 240 (n=42, Figure 18B); non-cirrhotic subjects that did not experience a change in fibrotic stage (Ishak) by week 240 (n=18, Figure 18C); subjects that experienced a progression to cirrhosis over the course of the study (Figure 18D); and non-cirrhotic subjects with greater than or equal to 2-stage reduction in fibrosis by week 240 (Figure 18E).

[00282] Table 12 compares baseline and week 240 serum LOXL2 levels (pg/mL) in subjects with persistent cirrhosis at week 240, subjects with reversed cirrhosis at week 240, and non-cirrhotic subjects that experienced no change in fibrotic change over the course of the study ("Non-Cirrhotic No Δ").

[00283] Table 12: Change in serum LOXL2 levels in different CHB subject groups

	Persistent Cirrhosis	Reversed Cirrhosis	Non-Cirrhotic No Δ

	(n=16)		(n=42)		(n=18)	
	Baseline	Wk 240	Baseline	Wk 240	Baseline	Wk 240
Mean	9124.1	603.8	1355.0	922.6	798.4	436.8
Median	1863	LOQ	1073	< LOQ	< LOQ	< LOQ
< LoQ	2 (13%)	8 (50%)	4 (10%)	29 (69%)	10 (56%)	14 (78%)
< LoD	1 (6%)	2 (13%)	1 (2%)	13 (31%)	4 (22%)	8 (44%)
< 1000	5 (31%)	14 (88%)	20 (48%)	35 (83%)	13 (72%)	15 (83%)
> 3000	5 (31%)	0 (0%)	2 (5%)	3 (7%)	1 (6%)	0 (0%)
Decrease		14 (88%)		37 (88%)		9 (50%)
Increase		0 (0%)		5 (12%)		2 (11%)

[00284] As shown in Table 12, 88% of cirrhotic subjects had a reduction in LOXL2 levels. Additionally, baseline serum LOXL2 levels were determined to be the highest in those subjects who at week 240 had persistent cirrhosis.

[00285] Figure 19 shows the percentage of cirrhotic subjects determined to have a histological improvement at week 240 ("Y") having given baseline serum LOXL2 levels (<1500, >1500, 1500-3000, <3000, and >3000 pg/mL) and the percentage of cirrhotic subjects determined not to have histological improvement at week 240 ("N") having the same given baseline serum LOXL2 levels. As shown, cirrhotic subjects having a baseline serum LOXL2 level less than 1500 pg/mL had an 88% chance of regression. Cirrhotic subjects having a baseline serum LOXL2 level between 1500 pg/mL and 3000 pg/mL had a 70% chance of regression, while cirrhotic subjects having a baseline serum level above 3000 pg/mL had only a 29% chance of regression. Thus, among cirrhotic patients, baseline serum LOXL2 levels below 1500 pg/mL were associated with an 88% likelihood of regression, while baseline serum LOXL2 levels above 3000pg/mL were associated with a 29% likelihood of regression.

[00286] Baseline serum LOXL2 levels correlated more with week 240 Ishak fibrosis stage than with Baseline fibrosis stage. This suggests high serum LOXL2 levels reflected active fibrogenesis.

[00287] The results of this study demonstrated that serum LOXL2 levels were elevated in patients with CHB and were highest in those with the most fibrosis, demonstrating a general correlation between serum LOXL2 and fibrosis score. Serum LOXL2 levels reflected active disease and active fibrogenesis (for example, given that

higher baseline levels were associated with higher fibrosis stages at week 240). Treating the underlying CHB resulted in a decline in LOXL2 in most patients, suggesting downregulation of fibrogenesis. There was a decrease in serum LOXL2 after 5 years even in patients with unchanged fibrosis scores that were clinically doing well. The results demonstrate serum LOXL2 level as a marker of active disease and that high LOXL2 is predictive of lack of regression.

[00288] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto

CLAIMS

What is claimed is:

1. A method for detecting, predicting, or monitoring a disease or condition, the method comprising:

a) contacting a liquid sample obtained from an individual with an antibody specific for lysyl oxidase-like 2 (LOXL2); and

b) detecting binding of the antibody with LOXL2 present in the liquid sample, thereby detecting a level of LOXL2 in the liquid sample,

wherein the detected level of LOXL2 indicates the presence or absence of the disease or condition in the individual or the likelihood of a response to a treatment for the disease or condition by the individual.

2. A prognostic method for a disease or condition, the method comprising:

a) contacting a liquid sample obtained from an individual having the disease or condition with an antibody specific for lysyl oxidase-like 2 (LOXL2); and

b) detecting binding of the antibody with LOXL2 present in the liquid sample, thereby detecting LOXL2 in the liquid sample,

wherein the level of LOXL2 detected indicates the likelihood of an outcome, event, or endpoint of the disease or condition.

3. The method of claim 1 or claim 2, wherein:

the individual is undergoing a treatment for the disease or condition; and

a level of LOXL2 detected in step (b) that is lower than a level determined at an earlier time point in the individual indicates the efficacy of the treatment.

4. The method of claim 3, wherein the level determined at the earlier time point is a pre-treatment level.

5. The method of any of claims 1-4, wherein the liquid sample is blood, a blood fraction, urine, saliva, sputum, or bronchoalveolar lavage.

6. The method of any of claims 1-5, wherein the antibody specific for LOXL2 comprises a detectable label.

7. The method of any of claims 1-6, further comprising immobilizing the LOXL2 present in the liquid sample on an insoluble support, wherein the immobilizing is carried out by contacting the liquid sample with a second antibody specific for LOXL2 to form a second antibody-LOXL2 complex, wherein the second antibody is immobilized on the insoluble support.

8. The method of claim 7, wherein the immobilizing is carried out prior to step (a).

9. The method of any of claims 1-8, wherein the antibody in step (a) is capable of binding to LOXL2 when the LOXL2 is bound to an agent that inhibits enzymatic activity of the LOXL2.

10. The method of claim 9, wherein the agent is an allosteric inhibitor of LOXL2 enzymatic activity.

11. The method of claim 10, wherein the allosteric inhibitor is an anti-LOXL2 monoclonal antibody.

12. The method of claim 11, wherein the anti-LOXL2 monoclonal antibody binds to an epitope within an SRCR3-4 domain of LOXL2.

13. The method of any of claims 1-12, further comprising:

c) comparing said detected level with a normal control value, wherein a detected level that is higher than a normal control value is indicative of the presence of the disease or condition or a likelihood that the individual will respond to a treatment for the disease or condition.

14. The method of any of claims 1-13, wherein the disease or condition is a fibrotic disease or cancer.

15. The method of claim 14, wherein the disease or condition is a fibrotic disease and a circulating level of LOXL2 that is greater than a normal control level indicates that the individual is likely to exhibit a beneficial clinical response to a treatment for the fibrotic disease.

16. The method of claim 14 or claim 15, wherein the disease or condition is pulmonary fibrosis, liver fibrosis, kidney fibrosis, cardiac fibrosis, or myelofibrosis, cirrhosis, chronic viral hepatitis, hepatitis C virus (HCV) or hepatitis B virus (HBV).

17. The method of claim 16, wherein the disease or condition is idiopathic pulmonary fibrosis (IPF).

18. The method of claim 17, wherein the detected level indicates the likelihood of an IPF disease outcome, endpoint, or event in the individual.

19. The method of claim 18, wherein the IPF disease outcome, endpoint, or event is IPF disease progression, lung function decline, respiratory hospitalization, transplant-free survival, death, or responsiveness to treatment.

20. The method of any of claims 17-19, wherein the method further comprises detecting a measure of IPF disease severity or functional status in the individual, the measure selected from the group consisting of percent of predicted forced vital capacity (FVC), percent of predicted carbon monoxide diffusion capacity (DL_{CO}), 6-minute walk distance (6MWD), mean pulmonary artery pressure (mPAP), lowest resting oxygen saturation (SpO2), composite physiologic index (CPI), St. George's Respiratory Questionnaire score (SGRQ), Transition Dyspnea Index (TDI) score, responsiveness to treatment, and biomarkers of IPF disease.

21. The method of any of claims 1-20, further comprising analyzing the LOXL2 level using a predictive model.

22 The method of any of claims 1-21, further comprising initiating, altering, or discontinuing treatment for the disease or condition in the individual.

23. The method of any of claims 1-22, further comprising subjecting the individual to one or more further diagnostic tests.

24. The method of claim 23, wherein the one or more further diagnostic tests is a pulmonary function test or a liver function test.

25. The method of any of claims 1-24, wherein the detected level indicates that the individual has an active fibrotic disease or an advanced stage fibrotic disease.

26. The method of claim 25, wherein the active fibrotic disease is METAVIR F1 or F2 liver fibrosis, or the advanced stage fibrotic disease is METAVIR F4 liver fibrosis.

27. An assay device for use in determining the level of a lysyl oxidase-like 2 (LOXL2) polypeptide in a liquid biological sample obtained from an individual, the device comprising: a matrix defining an axial flow path, the matrix comprising:

i) a sample-receiving zone at an upstream end of the flow path that receives the fluid sample;

ii) one or more test zones positioned within the flow path and downstream from the sample receiving zone, each of said one or more test zones comprising a LOXL2-specific antibody, wherein each of said LOXL2-specific antibodies is capable of binding to a LOXL2 polypeptide present in a liquid sample to form an anti-LOXL2 antibody/LOXL2 complex; and

iii) one or more control zones positioned within the flow path and downstream from the sample receiving zone.

28. The assay device of claim 27, wherein, when the one or more test zone comprises at least two test zones, at least one of the one or more control zones is positioned between two test zones.

29. The assay device of claim 28, wherein the at least two test zones and at least one control zone are positioned in an alternating format within the flow path beginning with a test zone positioned upstream of any control zone.

30. The assay device of claim 28 or 29, wherein one or more of said anti-LOXL2 antibodies is immobilized on the matrix in the test zone.

31. The assay device of any of claims 27-30, further comprising a label zone, comprising a labeled antibody specific for a LOXL2-specific antibody, wherein:

the labeled antibody is capable of binding to an anti-LOXL2 antibody present in an anti-LOXL2 antibody/LOXL2 complex, to form a labeled anti-LOXL2 antibody/LOXL2, and

the labeled antibody is mobilizable in the presence of liquid sample.

32. The assay device of claim 31, wherein the labeled antibody comprises a label component selected from the group consisting of a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

33. The assay device of any of claims 27-32, wherein the matrix is positioned within a housing comprising a support and optionally a cover, wherein the housing contains an application aperture and one or more observation ports.

34. The assay device of any of claims 28-33, wherein the device is a test strip.

35. The assay device of any of claims 28-34, wherein the device is a dipstick assay device.

36. A kit for determining the level of a lysyl oxidase-like 2 (LOXL2) polypeptide in a biological sample obtained from an individual, the kit comprising:

a) a first antibody specific for LOXL2; and

b) a second antibody specific for LOXL2.

37. The kit of claim 36, further comprising purified LOXL2 for use in generating a standard curve.

38. The kit of claim 36 or claim 37, wherein at least one of said antibodies comprises a detectable label.

39. The kit of claim 38, wherein the detectable label comprises a chemiluminescent agent, a particulate label, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

40. The method of any of claims 1-26, wherein the contacting and detecting are carried out using the assay device of any of claims 27-35 or the kit of claims 36-39.