myocardial infarction (Kakkar et al., *Nat. Rev. Drug Discov.* 7:827-40, 2008; Weinberg et al., *Circulation* 107:721-726, 2003; Shimpo et al., *Circulation* 109:2186-2190, 2004; Januzzi et al., *J. Am. Coll. Cardiol.* 50:607-613, 2007; Mueller et al., *Clin. Chem.* 54:752-756, 2008; Rehman et al., *J. Am. Coll. Cardiol.* 52:1458-65, 2008; Sabatine et al., *Circulation* 117:1936-1944, 2008). Elevated levels of human soluble ST2 are also predictive for death of a subject within one year (see, for example, WO 07/127749). Taken together, human soluble ST2 has been implicated in certain inflammatory diseases and the cardioprotective paracrine system, and is a predictive marker for prognosis in patients with heart failure and for death of a subject within one year.

SUMMARY

The present disclosure is based, at least in part, on the development of new antibodies specific for human soluble ST2 protein. These antibodies and antigen-binding fragments thereof are useful, e.g., for the quantitation of human soluble ST2 proteins in biological samples (e.g., clinical samples), for predicting the risk of death within one year in a subject, for determining whether to discharge or to initiate or continue therapeutic treatment (e.g., inpatient treatment) of a subject, and for selecting a subject for participation in a clinical study. Provided herein are these antibodies and antigen-binding fragments thereof, kits containing these antibodies and antibody fragments, and various methods of using these antibodies and antibody fragments.

Provided herein are isolated antibodies and antigen-binding fragments thereof that are, or bind competitively with, an antibody produced from the hybridomas deposited at the American Type Culture Collection (ATTC) and designated by Patent Deposit Designation PTA-10431 and PTA 10432. In some examples, the antibody or fragment thereof does not bind competitively with either or both of a D066-3 or a D067-3 antibody (MBL International) (described in U.S. Patent No. 7,087,396), or has a K_D for binding to human soluble ST2 equal to or less than 1.51×10^{-9} M. In some examples, the antibody or fragment thereof has a K_D for binding to human soluble ST2 equal to or less than 8.59×10^{-10} M. In some examples, the antibody or fragment thereof is chimeric or is humanized. In some examples, the fragment is selected from the group

of: a Fab fragment, a $F(ab')_2$ fragment, and a scFv fragment. In some examples, the antibody or fragment thereof is glycosylated. In some examples, the antibody or fragment thereof contains one or more complementary determining regions of the light or heavy chain of the antibody produced by the hybridoma deposited at the ATCC and designated by the Patent Deposit Designation PTA-10431 or PTA-10432. In some examples, the antibody is an antibody produced by the hybridoma deposited at the ATCC and designed by the Patent Deposit Designation PTA-10431, or an antigen-binding fragment thereof, or an antibody produced by the hybridoma deposited at the ATCC and designated by the Patent Deposit Designation PTA-10432, or an antigen-binding fragment thereof.

Also provided are isolated antibodies and antigen-binding fragments thereof that specifically bind to human soluble ST2, wherein the antibodies and antigen-binding fragments thereof were produced by a process that includes immunizing a non-human mammal (e.g., mouse, rat, rabbit, goat, cow, pig, monkey, or horse) with a recombinant human soluble ST2 isolated from a human cell (e.g., a human fibroblast, neuronal, epithelial, or endothelial cell, an embryonic or adult cell, especially a human embryonic kidney cell). In some examples, the recombinant human soluble ST2 isolated is fully glycosylated, i.e., has substantially the same glycosylation as a native endogenous human soluble ST2 present in human serum. In some examples of all of the antibodies and fragments described herein, the antibody or fragment thereof is labeled.

Also provided are cells of the hybridoma deposited at the ATCC and designated by the Patent Deposit Designation PTA-10431 and cells of the hybridoma deposited at the ATCC and designated by the Patent Deposit Designation PTA-10432.

In one aspect, the present disclosure refers to a method of identifying a subject having an increased risk of developing a cardiovascular disease, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has an increased risk of developing a cardiovascular disease.

In one aspect, the present disclosure refers to a method of identifying a subject having an increased risk of developing a cardiovascular disease, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has an increased risk of developing a cardiovascular disease.

Also provided are methods of quantitating a level of human soluble ST2 in a sample from a subject. The methods include contacting the sample with at least one antibody or fragment thereof described herein, and detecting binding of the antibody or fragment thereof to human soluble ST2. In some examples, the method includes the use of at least two different antibodies or fragments thereof described herein.

Also provided are methods of predicting the risk of death within one year in a subject. The methods include obtaining a sample from a subject and determining the

level of human soluble ST2 using at least one antibody or fragment thereof described herein, where an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has an increased risk of death within one year (e.g., increased risk of death within one year relative to a subject (e.g., a subject having the same disease) that has decreased level or substantially the same level of human soluble ST2 relative to the same control), and a decreased level or substantially the same level of human soluble ST2 indicates that the subject has a decreased risk of death within one year (e.g., a decreased risk of death within one year relative to a subject (e.g., a subject having the same disease) that has an increased level or substantially the same level of human soluble ST2 relative to the same control).

In one aspect, the present disclosure refers to a method of determining prognosis in a subject having heart failure or myocardial infarction, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates a poor prognosis of heart failure or myocardial infarction in the subject.

In one aspect, the present disclosure refers to a method of determining prognosis in a subject having heart failure or myocardial infarction, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates a poor prognosis of heart failure or myocardial infarction in the subject.

Also provided are methods of determining whether to discharge an inpatient or to initiate or continue treatment of a subject on an inpatient basis including obtaining a sample from a subject and determining the level of human soluble ST2 in the sample using at least one antibody or fragment described herein, where an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that inpatient treatment should be initiated or continued, and a decreased or an equal level of human soluble ST2 indicates that the subject should be considered for discharge. In some examples, the subject has at least one or more of the following symptoms: chest pain or discomfort, shortness of breath, nausea, vomiting, eructation, sweating, palpitations, lightheadedness, fatigue, and fainting.

Also provided are methods of selecting a subject for participation in a clinical study including obtaining a sample from a subject, determining the level of human soluble ST2 in the sample using at least one antibody or fragment thereof described herein, and selecting the subject for participation in a clinical study if the subject's level of human soluble ST2 relative to a reference level of human soluble ST2 indicates that the subject should be selected for participation in a clinical study. In some examples, the presence of an elevated level of human soluble ST2 indicates that the subject should be selected for participation in a clinical study.

Also provided are methods for selecting a therapeutic treatment for a subject including determining a level of human soluble ST2 in a biological sample from the

subject using at least one antibody or fragment thereof described herein, wherein the subject's level of human soluble ST2 relative to a reference level of human soluble ST2 is used to select a therapeutic treatment for the subject. In some examples, the presence of an elevated level of human soluble ST2 is used to select the therapeutic treatment for the subject.

In one aspect, the present disclosure refers to a method of selecting a therapeutic treatment for a subject, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 is used to select the therapeutic treatment for the subject.

In one aspect, the present disclosure refers to a method of selecting a therapeutic treatment for a subject, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 is used to select the therapeutic treatment for the subject.

In some examples of any of the methods described herein, the subject is undiagnosed or is not presenting with two or more (e.g., at least three, four, or five) symptoms of a disease state; the subject has been diagnosed as having a disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary syndrome, renal insufficiency, stroke, or any of the diseases described herein); or the subject has one or more of: hypertriglyceridemia, hypercholesterolemia, hypertension, and a body mass index of ≥ 30. In some examples of any of the methods described herein, the determining is performed using at least two antibodies or fragments thereof described herein.

In some examples of any of the methods described herein, the reference level of human soluble ST2 is a threshold level of human soluble ST2. In some examples, the threshold level is an average level of human soluble ST2 in a healthy patient population (e.g., a healthy male patient population or a healthy female patient population). In some examples of any of the methods described herein, the reference level is a level of human soluble ST2 present in a sample of a subject not presenting with two or more symptoms of disease, a subject not diagnosed as having a disease, or a subject not identified as being at risk for developing a disease.

In any of the methods described herein, the subject has not been diagnosed as having a disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary artery disease, acute coronary syndrome, renal insufficiency, or stroke, or any of the diseases described herein). In any of the methods described herein, the sample contains blood, serum, or plasma. Any of the antibodies and fragments thereof described herein can be used in any of methods described herein.

Also provided are methods of diagnosing a disease in a subject including obtaining a sample from a subject, determining a level of human soluble ST2 in the

sample using at least one antibody or fragment thereof described herein and a level of at least one additional marker, wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 and an altered level of the at least one additional marker relative to a reference level of the at least one additional marker, indicate that the subject has the disease (e.g., cardiovascular disease, a pulmonary disease, sepsis, Kawasaki disease, or a Th2-associated disease, or any of the other diseases described herein).

Also provided are methods for determining whether a subject has a normal human soluble ST2 level (and thus is likely to be free from severe disease, e.g., cardiovascular disease, and a normal risk of death or hospitalization, e.g., within one year) including obtaining a sample from a subject and determining the level of human soluble ST2 in the sample, wherein the subject is determined to have a normal human soluble ST2 level if the level of human soluble ST2 falls in a specific range (e.g., between about 14.5 to about 25.3 ng/mL, or between about 18.1 to about 19.9 ng/mL). In some examples, a male subject is determined to have a normal human soluble ST2 level if the level of human soluble ST2 is between any range listed in Table 9. In some examples, a female subject is determined to have a normal human soluble ST2 if the level of human soluble ST2 is between any range listed in Table 9.

In one aspect, the present disclosure refers to a method of determining whether a subject has a normal soluble ST2 level, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample is determined to have a normal soluble ST2 level if the level of human soluble ST2 falls within any of the ranges listed in Table 9.

In one aspect, the present disclosure refers to a method of determining whether a subject has a normal soluble ST2 level, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample is determined to have a normal human soluble ST2 level if the level of human soluble ST2 falls within any of the ranges listed in Table 9.

Also provided are kits containing at least one (e.g., two, three, four, or five) of the antibodies or antigen-binding fragments thereof described herein. Some examples of these kits contain two antibodies or antigen-binding fragments thereof described herein. In some examples of these kits, at least one of the antibodies or fragments thereof has a K_D for binding to human soluble ST2 (e.g., recombinant human soluble ST2) equal to or less than 8.59×10^{-10} M. In some examples, the kit is provided as an enzyme-linked immunosorbent assay (ELISA). In some examples, the kit further contains a recombinant human soluble ST2 isolated from a human cell (e.g., a human embryonic kidney cell). In some examples, the recombinant human soluble ST2 is fully glycosylated.

By the term "soluble ST2" is meant a soluble protein containing a sequence at least 90% identical (e.g., at least 95%, 96%, 97%, 98%, 99%, or 100% identical) to NCBI

Accession No. NP_003847.2 (SEQ ID NO: 1) or a nucleic acid containing a sequence at least 90% identical (e.g., at least 95%, 96%, 97%, 98%, 99%, or 100% identical) to NCBI Accession No. NM_003856.2 (SEQ ID NO: 2).

By the term "elevated" or "elevation" is meant a difference, e.g., a statistically significant difference (e.g., an increase) in a determined or measured level (e.g., a human soluble ST2 protein level) compared to a reference level (e.g., a level of human soluble ST2 in a subject not having a disease, a subject not presenting with two or more symptoms of a disease, or a subject not identified as being at risk of developing a disease, or a threshold level of human soluble ST2). In some examples, the reference is a threshold level, and any level above that is considered "elevated." Additional reference levels of human soluble ST2 are described herein.

By the term "health care facility" is meant a location where a subject can receive medical care from a health care professional (e.g., a nurse, a physician, or a physician's assistant). Non-limiting examples of health care facilities include hospitals, clinics, and assisted care facilities (e.g., a nursing home).

By the term "inpatient" is meant a subject that is admitted to a medical care facility (e.g., a hospital or an assisted care facility).

By the term "inpatient treatment" is meant the monitoring and/or medical treatment of a subject that is admitted to a health care facility (e.g., a hospital or assisted care facility). For example, a subject receiving inpatient treatment may be administered one or more therapeutic agents by a health care profession or may undergo a medical procedure (e.g., surgery (e.g., organ transplant, heart bypass surgery), angioplasty, imaging (e.g., magnetic resonance imaging, ultrasound imaging, and computer tomography scanning)). In other examples, one or more marker of a disease or the severity of the condition can be periodically measured by a health care professional to assess the severity or progression of disease or the subject's condition.

By the term "reference level" is meant a threshold level or a level in a control subject or control patient population. A reference level will depend on the assay performed and can be determined by one of ordinary skill in the art. A reference level can be a baseline level or a level in the same patient measured at an earlier or later point

in time. Some non-limiting examples of reference levels of human soluble ST2 include the level of human soluble ST2 in a subject that: has not been diagnosed as having a disease; does not present with at least two or more symptoms of a disease; does not have high risk CVD; does not have renal failure; does not have hypertriglyceridemia, hypercholesterolemia, hypertension, and/or a body mass index of < 30 (e.g., a BMI under 25); is not at risk of developing a disease; and/or does not suffer from a disease associated with increased ST2 levels (e.g., cardiovascular disease, a pulmonary disease, sepsis, Kawasaki disease, or a Th2-associated disease, or any of the other diseases described herein). Additional control patient populations are described herein. Additional examples of reference levels of human soluble ST2 are threshold levels of human soluble ST2. Reference levels of human soluble ST2 can be determined using methods known in the art; some exemplary levels are described herein.

In some examples, a ratio of two human soluble ST2 levels in a subject is calculated. The reference ratio can be compared to a reference ratio of human soluble ST2 levels measured in a subject (e.g., any of the control subjects described herein or the same subject), for example, a reference ratio can be a ratio of the levels of human soluble ST2 before and after onset of a disease (e.g., cardiac disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary syndrome, and angina), renal insufficiency, or stroke, or any of the other diseases associated with increased human soluble ST2 levels as described herein) symptoms; a ratio of the levels of human soluble ST2 before and after diagnosis with disease (e.g., cardiac disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary syndrome, and angina), renal insufficiency, or stroke, or any of the other diseases described herein); a ratio of the levels of ST2 before and after therapeutic treatment for a disease ((e.g., cardiac disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary syndrome, and angina), renal insufficiency, or stroke, or any of the other diseases described herein); a ratio of the human soluble ST2 levels at two different time points during therapeutic treatment (e.g., inpatient or outpatient treatment) for a disease (e.g., cardiac disease (e.g., heart failure, coronary artery disease, cardiovascular disease, acute coronary syndrome, and angina), renal insufficiency, or stroke, or any of the other

diseases described herein); or a ratio of the human soluble ST2 levels before and after a cardiac event (e.g., a myocardial infarction).

In some examples, the ratio of the levels of human soluble ST2 measured in a subject can be compared to a threshold reference ratio. Reference ratios of human soluble ST2 can be determined using methods known in the art; reference ratios of human soluble ST2 can be calculated using the data described herein. For example, reference ratio of human soluble ST2 may be between about 0.7 and about 1.1, or about 1.

By the term "therapeutic treatment" or "treatment" is meant the administration of one or more pharmaceutical agents to a subject or the performance of a medical procedure on the body of a subject (e.g., surgery, such as organ transplant or heart surgery). Non-limiting examples of pharmaceutical agents that can be administered to a subject include nitrates, calcium channel blockers, diuretics, thrombolytic agents, digitalis, renin-angiotensin-aldosterone system (RAAS) modulating agents (e.g., beta-adrenergic blocking agents, angiotensin-converting enzyme inhibitors, aldosterone antagonists, renin inhibitors, and angiotensin II receptor blockers), and cholesterol-lowering agents (e.g., a statin). The term therapeutic treatment also includes an adjustment (e.g., increase or decrease) in the dose or frequency of one or more pharmaceutical agents that a subject can be taking, the administration of one or more new pharmaceutical agents to the subject, or the removal of one or more pharmaceutical agents from the subject's treatment plan.

As used herein, a "subject" is a mammal, e.g., a human.

As used herein, a "biological sample" includes one or more of blood, serum, plasma, urine, and body tissue. Generally, a biological sample is a sample containing serum, blood, or plasma.

As used herein, the term "antibody" refers to a protein that generally contains heavy chain polypeptides and light chain polypeptides. Antigen recognition and binding occurs within the variable regions of the heavy and light chains. Single domain antibodies having one heavy chain and one light chain, and heavy chain antibodies devoid of light chains, are also known. A given antibody comprises one of five different types of heavy chains, called alpha, delta, epsilon, gamma, and mu, the categorization of

hybridoma cell. In additional examples, the antibody may be produced from a transgenic animal that contains the locus for a human heavy chain immunoglobulin and a human light chain immunoglobulin, or contains a nucleic acid that encodes the heavy and light chains of a specific human antibody.

"Complementarity-determining region" or "CDR" as the terms are used herein refer to short polypeptide sequences within the variable region of both heavy and light chain polypeptides that are primarily responsible for mediating specific antigen recognition. CDRs have been described by Kabat, et al., *J. Biol. Chem.* 252, 6609-6616, 1977; Chothia et al., *J. Mol. Biol.* 196:901-917, 1987; and MacCallum et al., *J. Mol. Biol.* 262:732-745, 1996. There are three CDRs (termed CDR1, CDR2, and CDR3) within each VL and each VH.

"Fragment" or "antibody fragment" as the terms are used herein refer to a polypeptide derived from an antibody polypeptide molecule (e.g., an antibody heavy and/or light chain polypeptide) that does not comprise a full-length antibody polypeptide, but that still comprises at least a portion of a full-length antibody polypeptide that is capable of binding to an antigen. Antibody fragments can comprise a cleaved portion of a full length antibody polypeptide, although the term is not limited to such cleaved fragments. Antibody fragments can include, for example, Fab fragments, F(ab')2 fragments, scFv (single-chain Fv) fragments, linear antibodies, monospecific or multispecific antibody fragments such as bispecific, trispecific, and multispecific antibodies (e.g., diabodies, triabodies, tetrabodies), minibodies, chelating recombinant antibodies, tribodies or bibodies, intrabodies, nanobodies, small modular immunopharmaceuticals (SMIP), binding-domain immunoglobulin fusion proteins, camelized antibodies, and VHH containing antibodies. Additional examples of antigen-binding antibody fragments are known in the art.

"Framework region" as the term is used herein refers to amino acid sequences within the variable region of both heavy and light chain polypeptides that are not CDR sequences, and are primarily responsible for maintaining correct positioning of the CDR sequences to permit antigen binding. Although the framework regions themselves typically do not directly participate in antigen binding, as is known in the art, certain

residues within the framework regions of certain antibodies can directly participate in antigen binding or can affect the ability of one or more amino acids in CDRs to interact with antigen.

"Humanized antibody" as the term is used herein refers to an antibody that has been engineered to comprise one or more human framework regions in the variable region together with non-human (e.g., mouse, rat, or hamster) complementarity-determining regions (CDRs) of the heavy and/or light chain. In some examples, a humanized antibody comprises sequences that are entirely human except for the CDR regions. Humanized antibodies are typically less immunogenic to humans, relative to non-humanized antibodies, and thus offer therapeutic benefits in certain situations. Humanized antibodies are known in the art, and suitable techniques for generating humanized antibodies are also known. See for example, Hwang et al., *Methods* 36:35, 2005; Queen et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:10029-10033, 1989; Jones et al., *Nature* 321:522-25, 1986; Riechmann et al., *Nature* 332:323-27, 1988; Verhoeyen et al., *Science* 239:1534-36, 1988; Orlandi et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:3833-3837, 1989; U.S. Patent Nos. 5,225,539; 5,530,101; 5,585,089; 5,693,761; 5,693,762; and 6,180,370; and WO 90/07861.

As used herein, the term "Th2-associated disease" refers to a disease associated with an abnormal type-2 T helper cell (Th2) response.

As used herein, the term "cardiovascular disease" refers to a disorder of the heart and blood vessels, and includes disorders of the arteries, veins, arterioles, venules, and capillaries.

As used herein, the term "pulmonary disease" refers to a disorder of the lungs.

By the term "additional marker" is meant a protein, nucleic acid, lipid, or carbohydrate, or a combination (e.g., two or more) thereof, that is diagnostic of the presence of a particular disease. The methods described herein for diagnosing a subject as having a disease can include detecting a level of soluble human ST2 and at least one additional marker in a sample from the subject. Several additional markers useful for the diagnosis of disease are known in the art (e.g., proANP, NT-proANP, ANP, proBNP, NT-proBNP, BNP, troponin, CRP, creatinine, Blood Urea Nitrogen (BUN), liver function

enzymes, albumin, and bacterial endotoxin; and those markers described in U.S. Patent Application Nos.: 2007/0248981; 2011/0053170; 2010/0009356; 2010/0055683; 2009/0264779; each of which is hereby incorporated by reference).

By the term "hypertriglyceridemia" is meant a triglyceride level that is greater than or equal to 180 ng/mL (e.g., greater than or equal to 200 ng/mL).

By the term "hypercholesterolemia" is meant an increased level of at least one form of cholesterol or total cholesterol in a subject. For example, a subject with hypercholesterolemia can have a high density lipoprotein (HDL) level of ≥ 40 mg/dL (e.g., ≥ 50 mg/dL or ≥ 60 mg/mL), a low density lipoprotein (LDL) level of ≥ 130 mg/dL (e.g., ≥ 160 mg/dL or ≥ 200 mg/dL), and/or a total cholesterol level of ≥ 200 mg/dL (e.g., 240 mg/dL).

By the term "hypertension" is meant an increased level of systolic and/or diastolic blood pressure. For example, a subject with hypertension can have a systolic blood pressure that is ≥ 120 mmHg (e.g., ≥ 140 mmHg or ≥ 160 mmHg) and/or a diastolic blood pressure that is ≥ 80 mmHg (e.g., ≥ 90 mmHg or ≥ 100 mmHg).

By the term "healthy subject" is meant a subject that does not have a disease (e.g., cardiovascular disease or pulmonary disease). For example, a healthy subject has not been diagnosed as having a disease and is not presenting with one or more (e.g., two, three, four, or five) symptoms of a disease state.

By "risk of death" is meant the risk of death in a subject from a disease or complications associated with a disease compared to a reference population (e.g., a healthy control population). The term risk of death as used herein excludes intentional or accidental death, e.g., death by blunt or crushing trauma, such as a car accident.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure. Other suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other

references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is an image showing the results of SDS-PAGE gel analysis of fractions from the recombinant human soluble ST2 protein purification. Lanes (10 µL per sample lane) are as follows: 1 - MWM (5 µL); 2 - His ladder (3 µL); 3 - untransfected negative control; 4 - supernatant before purification; 5 - column flow through; 6 - binding buffer wash 1; 7 - 5 mM wash 2; 8 - 5 mM wash 3; 9 - 200 mM eluate fraction 2; 10 - 200 mM eluate fraction 3; 11 - 200 mM eluate fraction 4; 12 - 200 mM eluate fraction 5; 13 - 200 mM eluate fraction 6; 14 - 200 mM eluate fraction 7; and 15 - 0.3 µg soluble ST2.

FIG. 2 shows a Western blot of purification fractions detecting the histidine tag in the recombinant human soluble ST2 protein. Lanes (10 µL per lane) are as follows: 1 - MWM (5 µL); 2 - His ladder (3 µL); 3 – supernatant before purification; 4 - column flow through; 5 - binding buffer wash 1; 6 - binding buffer wash 2; 7 – 5 mM wash 2; 8 – 5 mM wash 3; 9 – 200 mM eluate fraction 2; 10 – 200 mM eluate fraction 3; 11 – 200 mM eluate fraction 4; 12 – 200 mM eluate fraction 5; 13 – 200 mM eluate fraction 6; 14 – 200 mM eluate fraction 7; and 15 - 0.3 µg soluble ST2.

FIGs. 3A-3C show a Coomassie gel (FIG. 3A) and two Western blots of purified recombinant soluble ST2 comparing anti-ST2 commercial antibody D066 (MBL International) (FIG. 3B) with the hexa-histidine antibody (FIG. 3C). Lanes (10 µL/sample lane) are as follows: 1 - MWM; 2 - His ladder; 3 - Serum ST2-His 1000 ng; 4 - Serum ST2-His 500 ng; 5 - Serum ST2-His 200 ng; 6 - Serum ST2-His 100 ng; 7 - Serum ST2-His 50 ng; 8 - Serum-free ST2-His 1000 ng; 9 - Serum-free ST2-His 500 ng; 10 - Serum-free ST2-His 200 ng; 11 - Serum-free ST2-His 100 ng; and12 - Serum-free ST2-His 50 ng.

FIG. 4 is a line graph showing the results of an antigen sensitivity assessment of the 7E4 and 9F8 antibodies.

WHAT IS CLAIMED IS:

1. A method of determining prognosis in a subject having heart failure or myocardial infarction, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates a poor prognosis of heart failure or myocardial infarction in the subject.

2. A method of determining prognosis in a subject having heart failure or myocardial infarction, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates a poor prognosis of heart failure or myocardial infarction in the subject.

3. A method of selecting a therapeutic treatment for a subject, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 is used to select the therapeutic treatment for the subject.

4. A method of selecting a therapeutic treatment for a subject, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 is used to select the therapeutic treatment for the subject.

5. The method of claim 3 or 4, wherein the therapeutic treatment is selected from the group consisting of: nitrates, calcium channel blockers, diuretics, thrombolytic agents, digitalis, renin-angiotensin-aldosterone system (RAAS) modulating agents, and cholesterol-lowering agents.

6. The method of any one of claims 3-5, wherein the reference level of human soluble ST2 is a threshold level of human soluble ST2.

7. The method of claim 6, wherein the threshold level of human soluble ST2 is an average level of human soluble ST2 in a healthy patient population.

8. The method of claim 7, wherein the healthy patient population is a healthy male patient population.

9. The method of claim 7, wherein the healthy patient population is a healthy female patient population.

10. The method of any one of claims 3-5, wherein the reference level is a level of human soluble ST2 present in a sample of a subject not presenting with two or more symptoms of disease.

11. The method of any one of claims 3-5, wherein the reference level is a level of human soluble ST2 present in a sample of a subject not diagnosed as having a disease.

12. A method of determining whether a subject has a normal soluble ST2 level, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample is determined to have a normal soluble ST2 level if the level of human soluble ST2 falls within any of the ranges listed in Table 9.

13. A method of determining whether a subject has a normal soluble ST2 level, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein the level of human soluble ST2 in the sample is determined to have a normal human soluble ST2 level if the level of human soluble ST2 falls within any of the ranges listed in Table 9.

14. The method of claim 12 or 13, wherein a normal soluble ST2 level in the subject indicates that the subject is likely to be free from severe disease and has a normal risk of death or hospitalization within one year.

15. A method of identifying a subject having an increased risk of developing a cardiovascular disease, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has an increased risk of developing a cardiovascular disease.

16. A method of identifying a subject having an increased risk of developing a cardiovascular disease, the method comprising:

determining a level of human soluble ST2 in a sample that has been obtained from the subject using an isolated antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof,

wherein an elevated level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has an increased risk of developing a cardiovascular disease.

17. The method of claim 15 or 16, wherein the cardiovascular disease is heart failure or coronary artery disease.

18. The method of any one of claims 3, 4, and 15-17, wherein the subject is undiagnosed or is not presenting with two or more symptoms of disease.

19. The method of any one of claims 3, 4, and 15-17, wherein the subject has one or more of: hypertriglyceridemia, hypercholesterolemia, hypertension, and a body mass index of ≥ 30.

20. The method of any one of claims 3, 4, and 15-17, wherein the subject has one or more of the following symptoms: chest pain or discomfort, shortness of breath, nausea, vomiting, eructation, sweating, palpitations, lightheadedness, fatigue, and fainting.

21. The method of any one of claims 1-20, wherein the sample comprises blood, serum, or plasma.

22. The method of any one of claims 1-21, wherein the antigen-binding fragment is a Fab fragment or a $F(ab')_2$ fragment.

23. The method of any one of claims 1-21, wherein the antibody or the antigen-binding fragment is glycosylated.

24. The method of any one of claims 1-21, wherein the antibody or the antigen-binding fragment is labeled.