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.

In one aspect, there is provided an isolated antibody or antigen-binding fragment thereof, wherein the antibody is produced by a hybridoma deposited at the American Type Culture Collection (ATCC) and designated by Patent Deposit Designation PTA-10432, and the fragment is an antigen-binding fragment of the antibody produced by a hybridoma deposited at the ATCC and designated by Patent Deposit Designation PTA-10432.

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 embodiments, 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 embodiments, 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 embodiments, the antibody or fragment thereof is chimeric or is humanized. In some embodiments, the fragment is selected from the group

of: a Fab fragment, a $F(ab')_2$ fragment, and a scFv fragment. In some embodiments, the antibody or fragment thereof is glycosylated. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments of all of the antibodies and fragments described herein, the antibody or fragment thereof is labeled.

In one aspect, there is provided a hybridoma deposited at the ATCC and designated by the Patent Deposit Designation PTA-10432.

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, there is provided a method of quantitating a level of human soluble ST2 in a sample from a subject, the method comprising:

contacting sample with (i) an antibody produced by a hybridoma deposited at the American Type Culture Collection (ATCC) and designated by the Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof, and (ii) an antibody produced by a hybridoma deposited at the ATCC and designated by Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof; and

detecting the binding of the antibody or fragment of (i) and the binding of the antibody or fragment of (ii) to human soluble ST2.

In one aspect, provided are methods of quantitating a level of human soluble ST2 in a sample from a subject, the methods comprising: contacting the sample with an antibody or fragment thereof described herein, and detecting binding of the antibody or fragment thereof to human soluble ST2. In some embodiments, 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, there is provided a method of predicting the risk of death within one year in a subject, the method comprising:

obtaining a sample from a subject; and

determining the level of human soluble ST2 in the sample using an antibody or fragment as defined herein;

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 death within one year, and a decreased or an equal level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has a decreased risk of death within one year.

In another aspect, there is provided a method of predicting the risk of death within one year in a subject, the method comprising:

determining a level of human soluble ST2 in the sample using (i) an antibody produced by the hybridoma deposited at the American Type Culture Collection (ATCC) and designed by Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof, and (ii) an antibody produced by a hybridoma desposited at the ATCC and designed by 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 death within one year, and a decreased or equal level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 indicates that the subject has a reduced risk of death within one year.

In one aspect, provided are methods of determining whether to discharge or to initiate or continue treatment of a subject on an inpatient basis, the methods comprising: obtaining a sample from a subject; and determining the level of human soluble ST2 in the sample using an 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 in the sample compared to a reference level of human ST2 in the sample indicates that the subject should be discharged. In some embodiments, 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.

In another aspect, there is provided a method of determining whether to discharge or to initiate or continue treatment of a subject on an inpatient basis, the method comprising:

obtaining a sample from a subject; and

detecting a level of human soluble ST2 in the sample using (i) an antibody produced by a hybridoma deposited at the American Type Culture Collection (ATCC) and designed by Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof, and (ii) an antibody produced by a hybridoma deposited at the ATCC and designated by 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 inpatient treatment of the subject should be initiated or continued, and a decreased or an equal level of human soluble ST2 in the sample compared to a reference level of human soluble ST2 in the sample indicates that the subject should be discharged.

In one aspect, provided are methods of selecting a subject for participation in a clinical study, the methods comprising: obtaining a sample from a subject, determining the level of human soluble ST2 in the sample using an 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 embodiments, the presence of an elevated level of human soluble ST2 indicates that the subject should be selected for participation in a clinical study.

In another aspect, there is provided a method of selecting a subject for participation in a clinical study, the method comprising:

obtaining a sample from a subject;

determining a level of human soluble ST2 in the sample using (i) an antibody produced by a hybridoma deposited at the American Type Culture Collection (ATCC) and designated by Patent Deposit Designation PTA-10431 or an antigen-binding fragment thereof, and (ii) an antibody produced by a hybridoma deposited at the ATCC and designated by Patent Deposit Designation PTA-10432 or an antigen-binding fragment thereof; 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.

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

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 embodiments, 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 embodiments, 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, there is provided a kit comprising an antibody or antigen-binding fragment thereof as defined herein.

The kits may contain at least one (e.g., two, three, four, or five) of the antibodies or antigen-binding fragments thereof described herein. Some embodiments of these kits contain two antibodies or antigen-binding fragments thereof described herein. In some embodiments 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 embodiments, the kit is provided as an enzyme-linked immunosorbent assay (ELISA). In some embodiments, the kit further contains a recombinant human soluble ST2 isolated from a human cell (e.g., a human embryonic kidney cell). In some embodiments, 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