

SUMMARY OF THE INVENTION

[0016] The present application includes biomarkers, methods, reagents, devices, systems, and kits for the prediction of risk of an individual with chronic heart failure, such as stable chronic heart failure, having a Cardiovascular (CV) Event within a 90-day period, a 180-day period, or a 1-year period. The biomarkers of the present application were identified using a multiplex slow off-rate aptamer-based assay which is described in detail herein. By using the multiplex slow off-rate aptamer-based biomarker identification method described herein, this application describes a set of biomarkers that are useful for predicting the likelihood of a CV event in patients with chronic heart failure, such as stable chronic heart failure, within 90 days, 180 days, or 1 year.

[0017] In some ~~embodiment~~examples, the individual has stable chronic heart failure with preserved ejection fraction (HFpEF). In some ~~embodiment~~examples, the individual has reduced ejection fraction (HFrEF). In some ~~embodiment~~examples, the 1-year mortality prognosis of the individual is predicted.

[0018] Cardiovascular disease involves multiple biological processes and tissues. Examples of biological systems and processes associated with cardiovascular disease are inflammation, thrombosis, disease-associated angiogenesis, platelet activation, macrophage activation, liver acute response, extracellular matrix remodeling, and renal function. These processes can be observed as a function of gender, menopausal status, and age, and according to status of coagulation and vascular function. Since these systems communicate partially through protein based signaling systems, and multiple proteins may be measured in a single blood sample, the ~~invention~~disclosure provides a single sample, single assay multiple protein based test focused on proteins from the specific biological systems and processes involved in chronic heart failure.

[0019] In some ~~embodiment~~examples, methods for screening a subject for the risk of a cardiovascular event (CV) event are provided, said method comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2. In one aspect, the disclosure provides a method for screening a subject for the risk of a cardiovascular event (CV) event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2.

[0020] In some embodimentexamples, methods of predicting the likelihood that a subject will have a CV event are provided, said method comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2. In one aspect, the disclosure provides a method of predicting the likelihood that a subject will have a CV event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2.

[0021] In some embodimentexamples, at least two of the N biomarker proteins are RNAS6 and PAP1. In some embodimentexamples, at least two of the N biomarker proteins are RNAS6 and ATL2. In some embodimentexamples, at least two of the N biomarker proteins are HCC-1 and PAP1. In some embodimentexamples, at least two of the N biomarker proteins are HCC-1 and ATL2. In some embodimentexamples, at least two of the N biomarker proteins are HCC-1 and RNAS6. In some embodimentexamples, at least two of the N biomarker proteins are PAP1 and SVEP1. In some embodimentexamples, at least two of the N biomarker proteins are HCC-1 and SVEP1. In some embodimentexamples, at least two of the N biomarker proteins are RNAS6 and SVEP1. In some embodimentexamples, at least two of the N biomarker proteins are PAP1 and ATL2. In some embodimentexamples, all of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, ATL2, N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2. In some embodimentexamples, one of the N biomarker proteins is MIC-1. In some embodimentexamples, one of the N biomarker proteins is RNAS1. In any of the foregoing embodimentexamples, N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, N is 14, N is 15, or N is 16. In some embodimentexamples, the subject has heart failure with reduced ejection fraction. In some embodimentexamples, methods of predicting screening a subject for the risk of a cardiovascular event (CV) event are provided, the method comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is selected from RET and CRDL1, and at least one of the N biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2. In one aspect, the disclosure provides a method for screening a subject for the risk of a cardiovascular event (CV) event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is selected from RET and CRDL1, and at least one of the N biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2.

[0022] In some embodimentexamples, methods of predicting the likelihood that a subject will have a CV event are provided, the method comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is

selected from RET and CRDL1, and at least one of the N biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2. In one aspect, the disclosure provides a method of predicting the likelihood that a subject will have a CV event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is selected from RET and CRDL1, and at least one of the N biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2.

[0023] In some embodimentexamples, at least two of the N biomarker proteins are RET and CRDL1. In some embodimentexamples, one of the N biomarker proteins is MIC-1. In some embodimentexamples, one of the N biomarker proteins is HE4. In some embodimentexamples, one of the N biomarker proteins is Tetranectin. In some embodimentexamples, one of the N biomarker proteins is GHR. In some embodimentexamples, one of the N biomarker proteins is CA125. In some embodimentexamples, one of the N biomarker proteins is N-terminal pro-BNP. In some embodimentexamples, one of the N biomarker proteins is IGFBP-2. In some embodimentexamples, one of the N biomarker proteins is WISP-2. In some embodimentexamples, one of the N biomarker proteins is TNNT2. In some embodimentexamples, one of the N biomarker proteins is HSPB6. In some embodimentexamples, one of the N biomarker proteins is SLPI. In some embodimentexamples, one of the N biomarker proteins is MMP-12. In some embodimentexamples, all of the N biomarker proteins are selected from RET, CRDL1, Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2. In any of the foregoing amendments, N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, or N is 14. In some embodimentexamples, the subject has heart failure with preserved ejection fraction.

[0024] In various embodimentexamples, the CV event is death.

[0025] In some embodimentexamples, the risk or likelihood of the subject having a CV event within 1 year from the date that the sample was taken from the subject is screened or predicted. In some embodimentexamples, the risk or likelihood of the subject having a CV event within 180 days from the date that the sample was taken from the subject is screened or predicted. In some embodimentexamples, the risk or likelihood of the subject having a CV event within 90 days from the date that the sample was taken from the subject is screened or predicted. In some embodimentexamples, the risk or likelihood of the subject having a CV within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is high if the levels of each of at least 2 of the N biomarker proteins are abnormal relative to a control level of the respective biomarker protein. In some embodimentexamples, the risk or likelihood of the subject having a CV within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is high if the levels of each of the N biomarker proteins are abnormal relative to a control level of the respective biomarker protein. In some embodimentexamples, the risk or likelihood of the subject having a CV event within 1 year, 180 days, or 90 days from the

date that the sample was taken from the subject is calculated as a probability of survival 1 year, 180 days, or 90 days from the date that the sample was taken from the subject.

[0026] In some [embodimentexamples](#), the sample is selected from a blood sample, a serum sample, a plasma sample, and a urine sample. In some [embodimentexamples](#), the sample is a blood sample. In some [embodimentexamples](#), the method is performed in vitro.

[0027] In some [embodimentexamples](#), the method comprises contacting biomarker proteins of the sample from the subject with a set of capture reagents, wherein each capture reagent of the set of capture reagents specifically binds to one biomarker protein being detected. In some [embodimentexamples](#), two of the capture reagents bind to the same biomarker protein being detected. In some [embodimentexamples](#), two capture reagents specifically bind to SVEP1, and wherein the two capture reagents are aptamers comprising different sequences. In some [embodimentexamples](#), the method comprises contacting biomarker proteins of the sample from the subject with a set of capture reagents, wherein each capture reagent of the set of capture reagents specifically binds to a different biomarker protein being detected. In some [embodimentexamples](#), each capture reagent is an antibody or an aptamer. In some [embodimentexamples](#), each biomarker capture reagent is an aptamer. In some [embodimentexamples](#), at least one aptamer is a slow off-rate aptamer. In some [embodimentexamples](#), at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications. In some [embodimentexamples](#), each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

[0028] In some [embodimentexamples](#), the risk or likelihood of a CV event is based on the detected biomarker levels and at least one item of additional biomedical information selected from a) information corresponding to physical descriptors of the subject, b) information corresponding to a change in weight of the subject, c) information corresponding to the ethnicity of the subject, d) information corresponding to the gender of the subject, e) information corresponding to the subject's smoking history, f) information corresponding to the subject's alcohol use history, g) information corresponding to the subject's occupational history, h) information corresponding to the subject's family history of cardiovascular disease or other circulatory system conditions, i) information corresponding to the presence or absence in the subject of at least one genetic marker correlating with a higher risk of cardiovascular disease in the subject or a family member of the subject, j) information corresponding to clinical symptoms of the subject, k) information corresponding to other laboratory tests, l) information corresponding to gene expression values of the subject, and m) information

corresponding to the subject's consumption of known cardiovascular risk factors such as diet high in saturated fats, high salt, high cholesterol, n) information corresponding to the subject's imaging results obtained by techniques selected from the group consisting of electrocardiogram, echocardiography, carotid ultrasound for intima-media thickness, flow mediated dilation, pulse wave velocity, ankle-brachial index, stress echocardiography, myocardial perfusion imaging, coronary calcium by CT, high resolution CT angiography, MRI imaging, and other imaging modalities, o) information regarding the subject's medications, p) information corresponding to the age of the subject, and q) information regarding the subject's kidney function.

[0029] In some [embodimentexamples](#), the at least one item of additional biomedical information is information corresponding to the age of the subject. In some [embodimentexamples](#), the method comprises determining the risk or likelihood of a CV event for the purpose of determining a medical insurance premium or life insurance premium. In some [embodimentexamples](#), the method further comprises determining coverage or premium for medical insurance or life insurance. In some [embodimentexamples](#), the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources. In some [embodimentexamples](#), the method further comprises using information resulting from the method to enable a decision to acquire or purchase a medical practice, hospital, or company.

[0030] In some [embodimentexamples](#), a kit is provided, the kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to HCC-1, RNAS6, PAP1, SVEP1, or ATL2, and at least one of the capture reagents binds to N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, or TSP2. In one aspect, the disclosure provides a kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to HCC-1, RNAS6, PAP1, SVEP1, or ATL2, and at least one of the capture reagents binds to N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, or TSP2. In some [embodimentexamples](#), two of the capture reagents bind to SVEP1 and each of the remaining capture reagents binds to a different protein selected from HCC-1, RNAS6, PAP1, ATL2, N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2. In some [embodimentexamples](#), a kit is provided, the kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to RET or CRDL1, and at least one of the capture reagents binds to Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, or IGFBP-2. In one aspect, the disclosure provides a kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to RET or CRDL1, and at least one of the capture reagents binds to Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, or IGFBP-2. In some [embodimentexamples](#), each capture reagent binds to a different biomarker protein. In some [embodimentexamples](#), N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, N is 14, N is 15, N is 16, or N is 17. In some [embodimentexamples](#), N is 2, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14.

[0031] In some embodimentexamples, each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 1. In some embodimentexamples, each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 2. In some embodimentexamples, each of the N biomarker capture reagents is an antibody or an aptamer. In some embodimentexamples, each biomarker capture reagent is an aptamer. In some embodimentexamples, at least one aptamer is a slow off-rate aptamer. In some embodimentexamples, at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications. In some embodimentexamples, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes. In some embodimentexamples, the kit is for use in detecting the N biomarker proteins in a sample from a subject. In one aspect, the disclosure provides the kit as disclosed herein, for use in detecting the N biomarker proteins in a sample from a subject. In some embodimentexamples, the kit is for use in determining the subject's risk or likelihood of experiencing a CV event within 1 year from the date that the sample was taken from the subject, wherein the subject has heart failure. In some embodimentexamples, the CV event is death. In some embodimentexamples, the subject has heart failure with reduced ejection fraction. In some embodimentexamples, the subject has heart failure with preserved ejection fraction.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Fig. 1 shows the observed Kaplan-Meier survival probability of the training dataset of the HFrEF model, with individuals split into quartiles by predicted event probability at 365 days. The 1st to 4th quartiles are described with top line (Quartile 1), second line down (Quartile 2), third line down (Quartile 3) and bottom line (Quartile 4).

[0033] Figs. 2A-2B show Kaplan-Meier survival curves for each quartile for the validation dataset for the HFrEF model. Shaded regions in Fig. 2B represent 95% confidence intervals of the Kaplan-Meier estimates.

[0034] Fig. 3 shows the observed Kaplan-Meier survival probability of the training dataset for the HFpEF model, with individuals split into quartiles by predicted event probability at 365 days. The 1st to 4th quartiles are described with top line (Quartile 1), second line down (Quartile 2), third line down (Quartile 3) and bottom line (Quartile 4). Shaded regions represent 95% confidence intervals of the Kaplan-Meier estimates.

[0035] Fig. 4 shows Kaplan-Meier survival curves for each quartile of the validation dataset for the HFpEF model. Shaded regions represent 95% confidence intervals of the Kaplan-Meier estimates.

What is claimed is:

1. A method for screening a subject for the risk of a cardiovascular event (CV) event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2.

2. A method of predicting the likelihood that a subject will have a CV event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, and wherein a) at least two of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2; or b) at least one of the N biomarker proteins is selected from HCC-1, RNAS6, PAP1, SVEP1, and ATL2, and at least one of the N biomarker proteins is selected from N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2.

3. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are RNAS6 and PAP1; at least two of the N biomarker proteins are RNAS6 and ATL2; at least two of the N biomarker proteins are HCC-1 and PAP1; at least two of the N biomarker proteins are HCC-1 and ATL2; at least two of the N biomarker proteins are HCC-1 and RNAS6; at least two of the N biomarker proteins are PAP1 and SVEP1; at least two of the N biomarker proteins are HCC-1 and SVEP1; at least two of the N biomarker proteins are RNAS6 and SVEP1; or at least two of the N biomarker proteins are PAP1 and ATL2.

~~4. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are RNAS6 and ATL2.~~

~~5. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are HCC-1 and PAP1.~~

~~6. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are HCC-1 and ATL2.~~

~~7. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are HCC-1 and RNAS6.~~

~~8. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are PAP1 and SVEP1.~~

~~9. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are HCC-1 and SVEP1.~~

~~10. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are RNAS6 and SVEP1.~~

~~11. The method of claim 1 or claim 2, wherein at least two of the N biomarker proteins are PAP1 and ATL2.~~

~~12.4.~~ The method of any one of claims 1-~~113~~, wherein all of the N biomarker proteins are selected from HCC-1, RNAS6, PAP1, SVEP1, ATL2, N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGPT2, HE4, TAGL, RNAS1, and TSP2.

~~13.5.~~ The method of any one of claims 1-~~124~~, wherein one of the N biomarker proteins is MIC-1 or one of the N biomarker proteins is RNAS1.

~~14. The method of any one of claims 1-13, wherein one of the N biomarker proteins is RNAS1.~~

~~15.6.~~ The method of any one of claims 1-~~14-5~~, wherein N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, N is 14, N is 15, or N is 16.

~~16.7.~~ The method of any one of claims 1-~~156~~, wherein the subject has heart failure with reduced ejection fraction.

~~17.8.~~ A method for screening a subject for the risk of a ~~a~~ cardiovascular event (CV) event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is selected from RET and CRDL1, and at least one of the N biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2.

~~18.9.~~ A method of predicting the likelihood that a subject will have a CV event, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 2, wherein at least one of the N biomarker proteins is selected from RET and CRDL1, and at least one of the N

biomarker proteins is selected from Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2.

19.10. The method of claim 17-8 or claim 189, wherein at least two of the N biomarker proteins are RET and CRDL1.

20.11. The method of any one of claims 17-198-10, wherein one of the N biomarker proteins is MIC-1; one of the N biomarker proteins is HE4; one of the N biomarker proteins is Tetranectin; one of the N biomarker proteins is GHR; one of the N biomarker proteins is CA125; one of the N biomarker proteins is N-terminal pro-BNP; one of the N biomarker proteins is IGFBP-2; one of the N biomarker proteins is WISP-2; one of the N biomarker proteins is TNNT2; one of the N biomarker proteins is HSPB6; one of the N biomarker proteins is SLPI; or one of the N biomarker proteins is MMP-12.

~~21.—The method of any one of claims 17-20, wherein one of the N biomarker proteins is HE4.~~

~~22.—The method of any one of claims 17-21, wherein one of the N biomarker proteins is Tetranectin.~~

~~23.—The method of any one of claims 17-22, wherein one of the N biomarker proteins is GHR.~~

~~24.—The method of any one of claims 17-23, wherein one of the N biomarker proteins is CA125.~~

~~25.—The method of any one of claims 17-24, wherein one of the N biomarker proteins is N-terminal pro-BNP.~~

~~26.—The method of any one of claims 17-25, wherein one of the N biomarker proteins is IGFBP-2.~~

~~27.—The method of any one of claims 17-26, wherein one of the N biomarker proteins is WISP-2.~~

~~28.—The method of any one of claims 17-27, wherein one of the N biomarker proteins is TNNT2.~~

~~29.—The method of any one of claims 17-28, wherein one of the N biomarker proteins is HSPB6.~~

~~30.—The method of any one of claims 17-29, wherein one of the N biomarker proteins is SLPI.~~

~~31. — The method of any one of claims 17-30, wherein one of the N biomarker proteins is MMP-12.~~

~~32.12.~~ The method of any one of claims ~~17-34~~8-11, wherein all of the N biomarker proteins are selected from RET, CRDL1, Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, and IGFBP-2.

~~33.13.~~ The method of any one of claims ~~17 to 32~~8-12, wherein N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, or N is 14.

~~34.14.~~ The method of any one of claims ~~17-33~~8-13, wherein the subject has heart failure with preserved ejection fraction.

~~35.15.~~ The method of any one of claims 1-~~34~~14, wherein the CV event is death.

~~36.16.~~ The method of any one of claims 1-~~35~~15, wherein the risk or likelihood of the subject having a CV event within 1 year from the date that the sample was taken from the subject is screened or predicted; or the risk or likelihood of the subject having a CV event within 180 days from the date that the sample was taken from the subject is screened or predicted; or the risk or likelihood of the subject having a CV event within 90 days from the date that the sample was taken from the subject is screened or predicted.

~~37. — The method of any one of claims 1-35, wherein the risk or likelihood of the subject having a CV event within 180 days from the date that the sample was taken from the subject is screened or predicted.~~

~~38. — The method of any one of claims 1-35, wherein the risk or likelihood of the subject having a CV event within 90 days from the date that the sample was taken from the subject is screened or predicted.~~

~~39.17.~~ The method of ~~any one of claims 36-38~~claim 16, wherein the risk or likelihood of the subject having a CV within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is high if the levels of each of at least 2 of the N biomarker proteins are abnormal relative to a control level of the respective biomarker protein; the risk or likelihood of the subject having a CV within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is high if the levels of each of the N biomarker proteins are abnormal relative to a control level of the respective biomarker protein; or the risk or likelihood of the subject having a CV event within 1 year, 180 days, or 90 days from the date that the sample was

taken from the subject is calculated as a probability of survival 1 year, 180 days, or 90 days from the date that the sample was taken from the subject.

~~40. The method of any one of claims 36-38, wherein the risk or likelihood of the subject having a CV within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is high if the levels of each of the N biomarker proteins are abnormal relative to a control level of the respective biomarker protein.~~

~~41. The method of claim any one of claims 36-38, wherein the risk or likelihood of the subject having a CV event within 1 year, 180 days, or 90 days from the date that the sample was taken from the subject is calculated as a probability of survival 1 year, 180 days, or 90 days from the date that the sample was taken from the subject.~~

42.18. The method of any one of claims 1-~~41~~17, wherein the sample is selected from a blood sample, a serum sample, a plasma sample, and a urine sample.

~~43. The method of claim 42, wherein the sample is a blood sample.~~

44.19. The method of any one of claims 1-~~43~~18, wherein the method is performed *in vitro*.

45.20. The method of any one of claims 1-~~44~~19, wherein the method comprises contacting biomarker proteins of the sample from the subject with a set of capture reagents, wherein each capture reagent of the set of capture reagents specifically binds to one biomarker protein being detected.

46.21. The method of claim ~~45~~20, wherein two of the capture reagents bind to the same biomarker protein being detected, optionally wherein two capture reagents specifically bind to SVEP1, and wherein the two capture reagents are aptamers comprising different sequences.

~~47. The method of claim 46, wherein two capture reagents specifically bind to SVEP1, and wherein the two capture reagents are aptamers comprising different sequences.~~

48.22. The method of any one of claims 1-~~47~~21, wherein the method comprises contacting biomarker proteins of the sample from the subject with a set of capture reagents, wherein each capture reagent of the set of capture reagents specifically binds to a different biomarker protein being detected.

49.23. The method of any one of claims ~~45-48~~20-22, wherein each capture reagent is an antibody or an aptamer.

50.24. The method of claim ~~49~~23, wherein each biomarker capture reagent is an aptamer.

~~51.25.~~ The method of claim ~~5024~~, wherein at least one aptamer is a slow off-rate aptamer.

~~52.26.~~ The method of claim ~~5125~~, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

~~53.27.~~ The method of claim ~~5125~~ or claim ~~5226~~, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

~~54.28.~~ The method of any one of ~~the preceding claims~~ claims 1-27, wherein the risk or likelihood of a CV event is based on the detected biomarker levels and at least one item of additional biomedical information selected from

- a) information corresponding to physical descriptors of the subject,
- b) information corresponding to a change in weight of the subject,
- c) information corresponding to the ethnicity of the subject,
- d) information corresponding to the gender of the subject,
- e) information corresponding to the subject's smoking history,
- f) information corresponding to the subject's alcohol use history,
- g) information corresponding to the subject's occupational history,
- h) information corresponding to the subject's family history of cardiovascular disease or other circulatory system conditions,
- i) information corresponding to the presence or absence in the subject of at least one genetic marker correlating with a higher risk of cardiovascular disease in the subject or a family member of the subject,
- j) information corresponding to clinical symptoms of the subject,
- k) information corresponding to other laboratory tests,
- l) information corresponding to gene expression values of the subject, and
- m) information corresponding to the subject's consumption of known cardiovascular risk factors such as diet high in saturated fats, high salt, high cholesterol,
- n) information corresponding to the subject's imaging results obtained by techniques selected from the group consisting of electrocardiogram, echocardiography, carotid ultrasound for intima-media thickness, flow mediated dilation, pulse wave velocity,

ankle-brachial index, stress echocardiography, myocardial perfusion imaging, coronary calcium by CT, high resolution CT angiography, MRI imaging, and other imaging modalities,

- o) information regarding the subject's medications,
- p) information corresponding to the age of the subject, and
- q) information regarding the subject's kidney function.

~~55. The method of claim 54, wherein the at least one item of additional biomedical information is information corresponding to the age of the subject.~~

~~56.29.~~ The method of any one of ~~the preceding claims~~ claims 1-28, wherein the method comprises determining the risk or likelihood of a CV event for the purpose of determining a medical insurance premium or life insurance premium, optionally wherein the method further comprises determining coverage or premium for medical insurance or life insurance.

~~57. The method of claim 56, wherein the method further comprises determining coverage or premium for medical insurance or life insurance.~~

~~58.30.~~ The method of any one of claims 1-~~57~~29, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources or the method further comprises using information resulting from the method to enable a decision to acquire or purchase a medical practice, hospital, or company.

~~59. The method of any one of claims 1-58, wherein the method further comprises using information resulting from the method to enable a decision to acquire or purchase a medical practice, hospital, or company.~~

~~60.31.~~ A kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to HCC-1, RNAS6, PAP1, SVEP1, or ATL2, and at least one of the capture reagents binds to N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, or TSP2.

~~61.32.~~ The kit of claim ~~60~~31, wherein two of the capture reagents bind to SVEP1 and each of the remaining capture reagents binds to a different protein selected from HCC-1, RNAS6, PAP1, ATL2, N-terminal pro-BNP, RSPO4, BNP, MIC-1, FABPA, ILRL1, ANGP2, HE4, TAGL, RNAS1, and TSP2.

~~62.33.~~ A kit comprising N biomarker protein capture reagents, wherein N is at least 2, and wherein at least one of the capture reagents binds to RET or CRDL1, and at least one of the

capture reagents binds to Tetranectin, N-terminal pro-BNP, TNNT2, CA125, MIC-1, SLPI, HE4, MMP-12, HSPB6, WISP-2, GHR, or IGFBP-2.

~~63.34.~~ The kit of claim ~~6233~~, wherein each capture reagent binds to a different biomarker protein.

~~64.35.~~ The kit of claim ~~6031~~ or claim ~~6132~~, wherein N is 2, N is 3, N is 4, N is 5, N is 6, N is 7, N is 8, N is 9, N is 10, N is 11, N is 12, N is 13, N is 14, N is 15, N is 16, or N is 17; or the kit of claim 33 or claim 34, wherein N is 2, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14.

~~65.~~ ~~The kit of claim 62 or claim 63, wherein N is 2, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14.~~

~~66.36.~~ The kit of ~~any one of claims 60, 61, and 64~~claim 31 or claim 32, wherein each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 1 or the kit of claim 33 or claim 34, wherein each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 2.

~~67.~~ ~~The kit of any one of claims 62, 63, or 65, wherein each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 2.~~

~~68.37.~~ The kit of any one of claims ~~60-67~~31-36, wherein each of the N biomarker capture reagents is an antibody or an aptamer.

~~69.38.~~ The kit of claim ~~68~~37, wherein each biomarker capture reagent is an aptamer.

~~70.39.~~ The kit of claim ~~69~~38, wherein at least one aptamer is a slow off-rate aptamer.

~~71.40.~~ The kit of claim ~~70~~39, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

~~72.41.~~ The kit of claim ~~70-39~~ or claim ~~7140~~, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

~~73.42.~~ The kit of any one of claims ~~60-72~~31-41, for use in detecting the N biomarker proteins in a sample from a subject.

~~74.43.~~ The kit of claim ~~7342~~, for use in determining the subject's risk or likelihood of experiencing a CV event within 1 year from the date that the sample was taken from the subject, wherein the subject has heart failure.

~~75.44.~~ The kit of claim ~~74~~43, wherein the CV event is death.

~~76.45.~~ The kit of claim ~~74~~43 or ~~75~~44, wherein the subject has heart failure with reduced ejection fraction or the subject has heart failure with preserved ejection fraction.

~~77.~~ ~~The kit of claim 74 or 75, wherein the subject has heart failure with preserved ejection fraction.~~