Aptamer Proteomics for Biomarker Discovery in Heart Failure with Reduced Ejection Fraction

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Short Title: HFrEF Proteomics

Clinical Trial Registration:
This Study Will Evaluate the Efficacy and Safety of LCZ696 Compared to Enalapril on Morbidity and Mortality of Patients With Chronic Heart Failure (PARADIGM-HF); NCT NCT01035255
Efficacy and Safety of Aliskiren and Aliskiren/Enalapril Combination on Morbidity-mortality in Patients With Chronic Heart Failure (ATMOSPHERE); NCT00853658

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Conflicts of Interest

Drs. Zhang, Mendelson, Serrano-Fernandez, Kaiser, Yates, Chen, Turner, Patel-Murray, Beste, Laramie, Prescott, Lefkowitz, and Chutkow, Ms. Healey, and Ms. Zhao are employees of Novartis. Dr. Cunningham reports no relationships with industry. Dr. Claggett reports consulting fees from Amgen, Boehringer-Ingelheim, Cardurion, Corvia, MyoKardia, and Novartis outside the submitted work. Dr. Jacob reports salary support from Novartis and Moderna. Dr. Abraham reports consulting fees from Abbott, ARCA biopharma, Boehringer Ingelheim, Cardionomic, CVRx, Edwards Lifesciences, Respicardia, Sensible Medical, and Vectorious, and salary support from V-Wave Medical. Dr. Jhund reports consulting fees, advisory board fees, and lecture fees from Novartis; advisory board fees from Cytokinetics; and grant support from Boehringer Ingelheim. Dr. Kober reports speakers honoriaria from Novo Nordisk, Novartis, AstraZeneca and Boehringer Ingleheim; support from AstraZeneca; and personal fees from Novartis and Bristol Myers Squibb as a speaker. Dr. Packer reports consulting fees from AbbVie, Akcea, Actavis, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Cardiorentis, Daiichi Sankyo, Gilead, Johnson & Johnson, Novo Nordisk, Pfizer, Relypsa, Sanofi, Synthetic Biologics, and Theravance. Dr. Rouleau reports grants and consulting fees from Novartis and consulting fees from Abbott, AstraZeneca, MyoKardia, and Sanofi. Dr. Zile has received research funding from Novartis; and has been a consultant for Novartis, Abbott, Boston Scientific, CVRx, EBR, Endotronics, Ironwood, Merck, Medtronic, MyoKardia, and V Wave. Dr McMurray has received funding to his institution, Glasgow University, for his work on clinical trials, consulting, and other activities from Alnylam, Amgen, AstraZeneca, Bayer, Bristol Myers Squibb, Cardurion, Cytokinetics, GlaxoSmithKline, Novartis, Pfizer, and Theracos; and has received personal lecture fees from the Corpus, Abbott, Hickma, Sun Pharmaceuticals, and Medscape. Dr. Solomon reports Actelion, Alnylam, Amgen, AstraZeneca, Bellerophon, Bayer, Bristol Myers Squibb, Celladon, Cytokinetics, Eidos, Gilead, GSK, Ionis, Lilly, Mesoblast, MyoKardia, NIH/NHLBI, Neurotronik, Novartis, Novo Nordisk, Respicardia, Sanofi Pasteur, Theracos, and Us2.aI outside the submitted work; consulting fees from Abbott, Action, Akros, Alnylam, Amgen, Arena, AstraZeneca, Bayer, Boehringer-Ingelheim, Bristol Myers Squibb, Cardior, Cardurion, Corvia, Cytokinetics, DaiichiSankyo, GSK, Lilly, Merck, MyoKardia, Novartis, Roche, Theracos, Quantum Genomics, Cardurion, Janssen, Cardiac Dimensions, Tenaya, Sanofi-Pasteur, Dinaqor, Tremeau, CellProThera, Moderna, American Regent, and Sarepta; and participation on a Data Safety Monitoring Board or Advisory Board for Janssen.

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Systematically characterizing associations between circulating proteins and risk for hospitalization and death may identify novel biological pathways and improve risk prediction in heart failure (HF). Large-scale assays now enable broad proteomic investigation in clinical trials.[1]

We measured serum levels of 4076 unique proteins at baseline in a subset of patients from the ATMOSPHERE (n=1258, 487 events, 12.2 events per 100 patient-years) and PARADIGM-HF (n=1257, 287 events, 10.4 events per 100 patient-years) trials of chronic HF with reduced ejection fraction using the SomaScan modified aptamer-based proteomics assay. After quality control filters, we excluded 19 ATMOSPHERE samples and 22 PARADIGM-HF samples, and then used global median normalization to account for batch effects between assay plates. Baseline protein levels associated with the trial primary endpoint of HF hospitalization (HFH) or cardiovascular (CV) death were identified in the ATMOSPHERE discovery cohort by Cox regression (false discovery rate [FDR]<0.05), and replicated in PARADIGM-HF (Bonferroni-corrected p<0.05). Two covariate models were used: a minimal model including age, sex, treatment arm, and anticoagulant usage, and a second model adjusted for 13 additional covariates. Study protocols were approved by local institutional review boards. All subjects provided informed consent. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results.

In minimally adjusted models, 377 proteins were associated with risk of HFH or CV death (FDR<0.05) in the discovery cohort (ATMOSPHERE), and of these, 167 replicated in PARADIGM-HF (Bonferroni-adjusted p-value<0.05). Baseline protein levels marking the largest increased risk included a novel HF biomarker Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1 (SVEP1, hazard ratio 1.60 [95% CI 1.44-1.79] per

standard deviation, $p=2.0\times10^{-17}$) and known HF biomarkers Growth Differentiation Factor 15 (GDF15), Angiopoietin-2 (ANGPT2), N-terminal pro-brain natriuretic peptide (NT-proBNP), and Thrombospondin-2 (**Figure Panel A**). After adjustment for 13 additional risk factors, 64 proteins remained significantly associated with the primary endpoint in both trials (**Figure Panels B/C)**. Target specific binding of the assay for SVEP1 was supported by the measurement of two aptamers which were highly correlated ($r=0.98$, $p<2.2\times10^{-16}$), and the presence of a strong SVEP1 *cis*-pQTL in deCODE using the same platform ($p<10^{-250}$).[2]

A 64-protein proteomic risk score was derived in ATMOSPHERE using Cox LASSO regression, externally validated in PARADIGM-HF, and compared to current clinical risk prediction metrics. We re-fit a clinical risk score for the primary endpoint in ATMOSPHERE using variables from the MAGGIC score, which was developed to predict mortality.[3] In PARADIGM-HF, the ATMOSPHERE-derived proteomic score provided marginally greater discrimination (c-stat 0.70) compared with the clinical risk score (c-stat 0.64), NT-proBNP (c-stat 0.65), or high-sensitivity cardiac troponin T [hs-cTnT] (c-stat 0.65) ($p=0.001$ for all) alone, and similar discrimination to a combination of the clinical score, NT-proBNP, and hs-cTnT (c-stat 0.70) (**Figure Panel D**). Adding the clinical score, NT-proBNP, and hs-cTnT to the proteomic score did not significantly improve discrimination ($p=0.28$), suggesting the proteomic score contained most relevant information from these metrics. The proteomic score alone modestly improved 2-year continuous net reclassification index (NRI) compared to each of the clinical score (NRI 0.16, $p=0.03$), NT-proBNP (NRI 0.21, $p<0.001$), and hs-cTnT(0.16, $p=0.01$) alone, but not the combination of the clinical score, NT-proBNP, and hs-cTnT (NRI 0.04, $p=0.55$). Improvements in c-statistics compared with models including hs-cTnT may be understated because hs-cTnT was not measured in ATMOSPHERE, therefore hs-cTnT model

coefficients were derived and evaluated in PARADIGM-HF. Patients with lower proteomic risk scores had greater reductions in the primary endpoint with sacubitril/valsartan compared with enalapril in PARADIGM-HF (p-interaction=0.01).

The discovery of a novel HF biomarker, SVEP1, illustrates the value of our broad proteomic discovery approach. The magnitude of the association between baseline serum SVEP1 levels and the risk of HFH or CV death was as strong as for NT-proBNP, independent of other clinical risk factors, and consistent across the two trials and two aptamers. SVEP1 is an extracellular matrix protein expressed in vascular smooth muscle cells that promotes inflammation and atherosclerosis through integrin, notch, and fibroblast growth factor receptor signaling in animal studies.[4] The mechanism linking circulating SVEP1 and HF outcomes merits further investigation.

Improvements in discrimination and reclassification with the 64-protein score compared to the clinical score, NT-proBNP, or hs-cTnT may not be clinically significant. However, the proteomic score performed as well as these three metrics combined, and may be more convenient for clinicians than risk scores requiring online calculators.

The SomaScan proteomics platform has not been validated for all proteins in the panel However, many have been validated by mass spectrometry or presence of *cis*-pQTLs which support aptamer specificity.[2]

In conclusion, broad proteomic investigation in two HF clinical trials characterized associations between serum proteins and risk of HFH or CV death and identified SVEP1 as a new HF biomarker.

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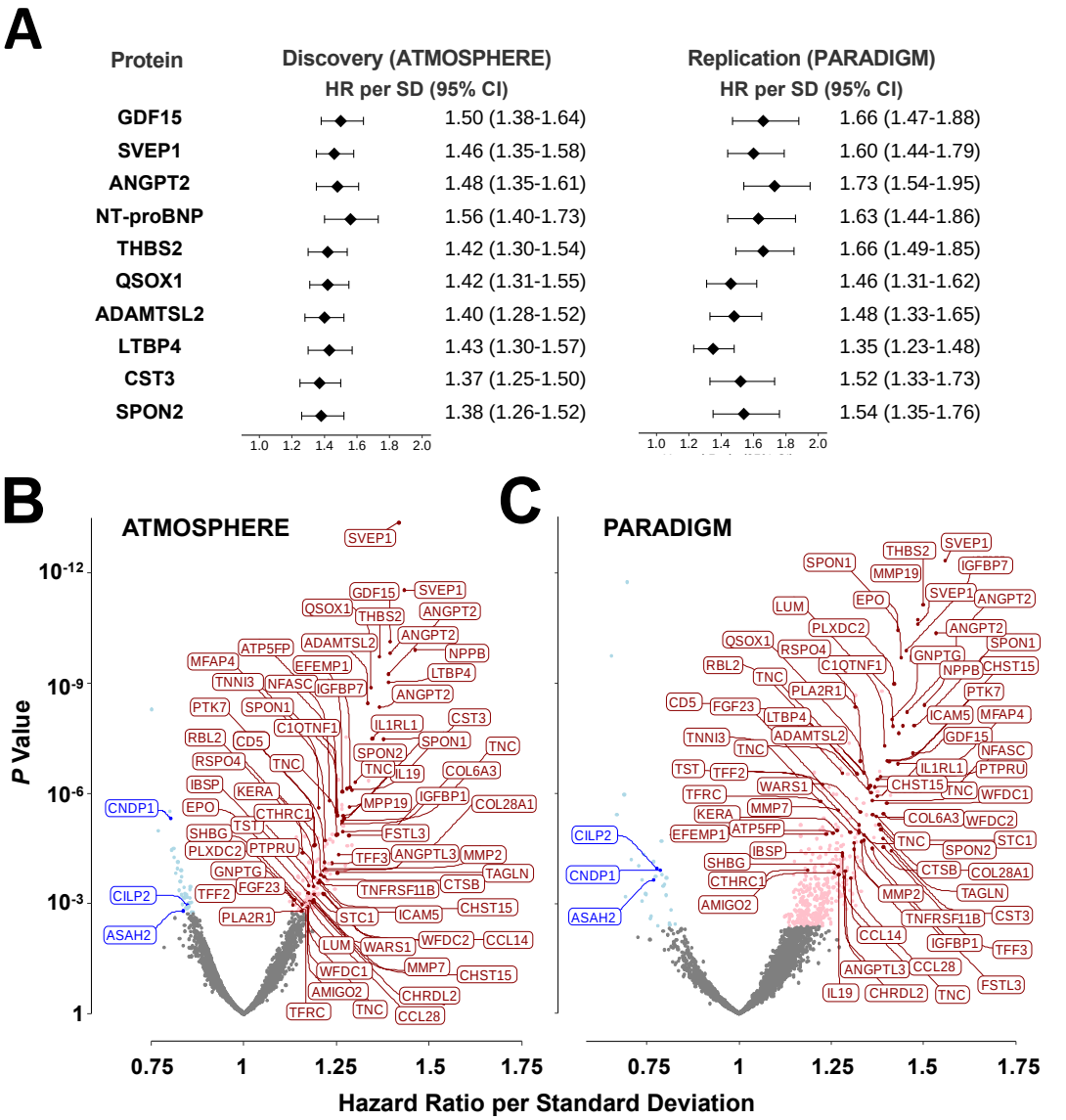
A
Protein
Discovery (ATMOSPHERE)
HR per SD (95% CI)
Replication (PARADIGM)
HR per SD (95% CI)
GDF15 1.50 (1.38-1.64) 1.66 (1.47-1.88)
SVEP1 1.46 (1.35-1.58) 1.60 (1.44-1.79)
ANGPT2 1.48 (1.35-1.61) 1.73 (1.54-1.95)
NT-proBNP 1.56 (1.40-1.73) 1.63 (1.44-1.86)
THBS2 1.42 (1.30-1.54) 1.66 (1.49-1.85)
QSOX1 1.42 (1.31-1.55) 1.46 (1.31-1.62)
ADAMTSL2 1.40 (1.28-1.52) 1.48 (1.33-1.65)
LTBP4 1.43 (1.30-1.57) 1.35 (1.23-1.48)
CST3 1.37 (1.25-1.50) 1.52 (1.33-1.73)
SPON2 1.38 (1.26-1.52) 1.54 (1.35-1.76)
1.0 1.2 1.4 1.6 1.8 2.0
B
ATMOSPHERE
C
PARADIGM
P Value
Hazard Ratio per Standard Deviation
0.75 1 1.25 1.5 1.75

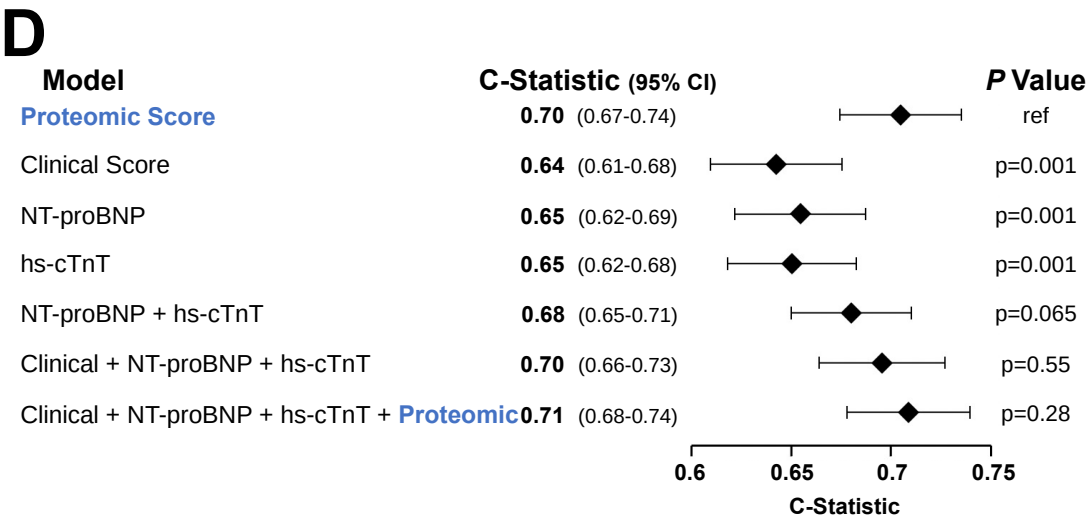
D
Model
C-Statistic (95% CI)
P Value
Proteomic Score 0.70 (0.67-0.74) ref
Clinical Score 0.64 (0.61-0.68) p=0.001
NT-proBNP 0.65 (0.62-0.69) p=0.001
hs-cTnT 0.65 (0.62-0.68) p=0.001
NT-proBNP + hs-cTnT 0.68 (0.65-0.71) p=0.065
Clinical + NT-proBNP + hs-cTnT 0.70 (0.66-0.73) p=0.55
Clinical + NT-proBNP + hs-cTnT + Proteomic 0.71 (0.68-0.74) p=0.28
0.6 0.65 0.7 0.75
C-Statistic

Figure Legend

Panel A: Top 10 Proteins Most Strongly Associated with Risk of Heart Failure Hospitalization or Cardiovascular Death. Cox regression model adjusted for age, sex, anticoagulant use, and treatment group. The order of the table was determined by p-value in the ATMOSPHERE discovery cohort. **Panels B, C: Proteins Associated with Heart Failure Hospitalization or Cardiovascular Death in Cardiovascular Risk Adjusted Model.** Proteins with significant associations at false discovery rate <0.05 in both trials are indicated by red dots (positive associations) and blue dots (negative associations) and are labelled. Faintly colored dots indicate proteins meeting false discovery rate <0.05 in only one trial. Grey dots indicate associations which were not statistically significant at false discovery rate <0.05. The cardiovascular risk factor adjusted model is adjusted for age, sex, anticoagulant use, treatment group, prior myocardial infarction, body mass index, diabetes mellitus, current smoking, time since heart failure diagnosis, prior heart failure hospitalization, systolic blood pressure, low and high-density lipoprotein concentrations, estimated glomerular filtration rate, atrial fibrillation, New York Heart Association functional class and left ventricular ejection fraction. **Panel D: Proteomic Risk Score Discrimination Compared with Current Clinical Standards.** In the PARADIGM-HF validation cohort, the proteomic risk score derived in ATMOSPHERE provided greater risk discrimination compared with a clinical risk score derived in ATMOSPHERE, NT-proBNP, or high-sensitivity troponin individually, and similar discrimination to a combination of these. P-values for differences in c-statistic compared to the proteomic score were calculated by the Somer's D method.