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Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure with a Preserved Ejection Fraction: Data from the PARAMOUNT Study

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Abstract

Background: Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome that has been associated with changes in the extracellular matrix. The purpose of this study was to determine whether profibrotic biomarkers accurately reflect the presence and severity of disease, underlying pathophysiology and modify response to therapy in patients with HFpEF.

Methods and Results: Four biomarkers, soluble form of ST2(sST-2, an Interleukin-1 receptor family member), galectin-3(gal-3), matrix metalloproteinase-2(MMP-2) and collagen III n-terminal propeptide(PIIINP) were measured in the Prospective comparison of ARNI with ARB on management of heart failure with preserved ejection fraction (PARAMOUNT) trial at baseline, 12 and 36 weeks after randomization to valsartan or LCZ696. We examined the relationship between baseline biomarkers, demographic and echocardiographic characteristics, change in primary (change in n-terminal pro B-type natriuretic peptide[NT-proBNP]) and secondary (change in left atrial volume[LAV]) endpoints. The median (interquartile range) value for sST-2(33 [24.6, 48.1] ng/mL) and gal-3(17.8[14.1, 22.8] ng/mL) were higher, and MMP-2(188 [155.5, 230.6] ng/mL) lower, than in previously published referent controls; PIIINP(5.6 [4.3, 6.9] ng/mL) was similar to referent control values. All 4 biomarkers correlated with severity of disease as indicated by NT-proBNP, E/E' and LAV. Baseline biomarkers did not modify the response to LCZ696 for lowering NT-proBNP; however, LAV reduction varied by baseline level of sST2 and gal-3; patients with values < the observed median (<33 ng/mL sST-2, <17.8 ng/mL gal-3) had reduction in LAV, those above median did not. While LCZ696 reduced NT-proBNP levels of the other four biomarkers were not affected over time.

Conclusions: In HFpEF patients, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease, underlying pathophysiology, and may modify the structural response to treatment.

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Short Commentary

Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome that has been associated with changes in the extracellular matrix. The purpose of this analysis was to examine a selected portfolio of postulated profibrotic biomarkers in a defined population of HFpEF, relate these biomarkers to demographic characteristics, changes in LV structure and function, severity of disease and response to treatment. Data from this analysis support several novel and hypothesis generating findings. First, HFpEF patients have circulating biomarkers that reflect a profibrotic state. Gal-3 and sST-2 were increased and MMP-2 was decreased. In aggregate, these directional changes in these biomarkers might be expected to be associated with an increase in myocardial collagen content. Second, biomarker levels correlate with indices of disease severity. While each of the patients enrolled in PARAMOUNT had the clinical syndrome of HFpEF, those with the more severe disease had a biomarker pattern associated with a more profibrotic milieu. Third, the baseline pretreatment values of sST-2 and gal-3 may have modified the response to LCZ696, specifically reduction in Left Atrial (LA) volume. Patients who had levels of sST-2 and Gal-3 below the observed median value showed a greater LA volume response to LCZ696 than those with sST-2 and Gal-3 above the median. In HFpEF patients, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease, underlying pathophysiology, and may modify the structural response to treatment.

Introduction

A number of novel circulating biomarkers have been used to characterize the molecular and cellular changes that occur during the development of myocardial disease (1-4). In heart failure (HF) these include biomarkers that reflect hemodynamic status (such as natriuretic peptides), inflammation (such as interleukins), and collagen homeostasis (such as collagen peptides, interstitial proteases) (2). The profiles of these biomarkers appear to differ significantly in patients with HF with a reduced ejection fraction (EF) versus HF with a preserved EF (HFpEF) (5-7). The nature and extent to which biomarkers change in HFpEF as a function of disease severity, degree of left ventricular (LV) structural and functional abnormalities and demographics and co-morbid conditions have not been fully defined. In addition, whether baseline biomarkers can modify the response to treatment in HFpEF has not been examined. One potential mechanism hypothesized to play a pivotal role in the development HFpEF is a change in collagen homeostasis that results in extracellular matrix (ECM) fibrosis and the development of abnormal diastolic function (8-12). Several molecular and cellular signaling pathways that result in a profibrotic milieu have been identified in previous studies but have not been specifically examined in randomized clinical trials in HFpEF (2, 6, 7).

Patients enrolled in the Prospective comparison of ARNI with ARB on management of heart failure with preserved ejection fraction trial (PARAMOUNT) had the clinical syndrome of heart failure and evidence of increased LV filling pressures (symptoms and signs of volume overload and increased n-terminal pro B-type natriuretic peptide [NT-proBNP]) (13). We hypothesized that if changes in ECM fibrillar collagen content lead to abnormal diastolic function, these

patients should have changes in biomarkers reflecting this. The purpose of this study was to examine a selected portfolio of postulated profibrotic biomarkers in a defined population of HFpEF, relate these biomarkers to demographic characteristics, changes in LV structure and function, severity of disease and response to treatment. Given the post-hoc nature of this analysis and its modest sample size, this study was envisioned as hypothesis generating providing provocative evidence that would lead to additional, larger, more definitive studies.

Methods

Study Design

PARAMOUNT was a randomized, double-blind, parallel group, active control trial described in detail in previous publications (13). Briefly, men and women ≥ 40 years old with an LVEF ≥ 45% and a documented history of heart failure with associated signs or symptoms (dyspnea on exertion, orthopnea, paroxysmal dyspnea, and peripheral edema) were eligible. Patients were required to have NT-proBNP greater than 400 pg/mL at screening, be on diuretic therapy, and have a systolic blood pressure < 140 mm Hg, or 160 mm Hg or less if on three or more blood pressure drugs at randomization, have an estimated glomerular filtration rate (eGFR) of at least 30 mL/min per $1·73$ m² at screening, and a potassium concentration of no more than $5·2$ mmol/L. Patients were excluded if they had previous LVEF < 45% at any time, isolated right heart failure due to pulmonary disease, dyspnea due to non-cardiac causes such as pulmonary disease, anemia, or severe obesity, primary valvular or myocardial diseases, or coronary artery or cerebrovascular disease needing revascularization within 3 months of screening or likely to need revascularization during the trial. The number of patients enrolled with atrial fibrillation on ECG at screening was limited to roughly 25% of the total. The study protocol was submitted to individual sites' institutional review boards or ethics committees and all enrolled patients provided written informed consent. A data safety monitoring committee oversaw the program and reviewed trial data for patient safety at regular intervals.

Biomarkers

Plasma and serum were obtained for biomarker determination at baseline, 12, and 36 weeks after randomization. The baseline measurements were made at randomization following a

placebo run-in phase. Measurements after randomization to Valsartan or LCZ696 were made 12

and 36 weeks. NT-proBNP and matrix metalloproteinase-2 (MMP-2) were measured in plasma

and collagen III n-terminal propeptide (PIIINP) was measured in serum at Quest Diagnostics

(Valencia, CA, USA) using the Elecsys proBNP immunoassay (Roche Diagnostics, Indianapolis,

IN, USA), the Quantikine MMP-2 immunoassay (R and D Systems, Minneapolis, MN, USA)

and the UniQ PIIINP radioimmunoassay (Orion Diagnostics, Espoo, FIN). The soluble form of

ST-2 (sST-2, an Interleukin-1 receptor family member) was measured in serum at Critical

Diagnostics (San Diego, CA, USA) using their Presage® immunoassay and Galectin 3 (Gal-3)

was measured in serum at Clinical Reference Laboratories (Lenexa, KS, USA) using an enzyme-

linked immunosorbent assay (BG Medicine, Waltham, MA. USA).

Echocardiographic study

Baseline echocardiograms were analyzed in the cardiovascular imaging core laboratory at

Brigham and Women's Hospital, Boston MA, USA. All measurements were made in triplicate in

accordance with the recommendations of the American Society of Echocardiography (ASE) (14).

Left ventricular (LV) volumes, mass (LVM), relative wall thickness (RWT), mitral flow

velocities, tissue Doppler velocities, left atrial volume, LVEF were calculated according to the

recommendations of the American Society of Echocardiography (14). Left atrial strain and LV

global longitudinal strain were measured using vendor-independent 2D speckle tracking software

(15).

Statistical Analysis

Baseline characteristics were summarized by quartiles of each biomarker using counts and

percentages for binary variables and means and standard deviations for continuous variables,

with the exception of NT-proBNP, which is summarized via median [IQR] due to skewness. Tests for

trend across quartiles were conducted via chi-square trend tests, linear regression, and Cuzick's

non-parametric trend test, as appropriate. Biomarker data were presented as mean ± SEM,

geometric mean, and median [IQR] at baseline. Baseline biomarker data were compared to data

measured at 12 and 36 weeks after randomization presented as median [IQR] for the entire study

group and then divided according to treatment group (Valsartan vs. LCZ696).

Baseline biomarker data were compared qualitatively with referent control values. Referent

control values were presented for comparison as median [IQR]. Median [IQR] referent control

data for PIIINP and MMP-2 were taken from a previously published study in which 241 subjects

of age, sex, race distribution similar to the current study population were examined (5).

However, these well characterized subjects had no clinical, serologic, or cardiac

structural/functional abnormalities as evidenced by a normal echocardiography and 6 minute hall

walk distance. Median [IQR] referent control data for Gal-3 were taken from a previously

published study in which 1092 subjects of age, sex, race distribution similar to the current study

population were examined (33). Median [IQR] referent control data for sST-2 were aggregated

from 3 previously published study (including the Framingham study) in which subjects of age,

sex, race distribution similar to the current study population were examined (34-37). While small

differences between men and women have been seen in the biomarkers described above, since

the populations of both the current study and the referent control populations are roughly 50%

female, the referent control values listed in Table 1 represent the total population examined. In

addition, biomarker data from the current study of HFpEF patients were compared to previously

published groups of HFpEF patients (5, 38-41). Finally, Gal-3 and sST-2 data in the current

study were compared to FDA approved partition values for risk stratification; these partition

values were not specifically designed for risk stratification in HFpEF but were targeted to overall

risk in generalized populations.

Correlations between biomarkers and demographic and echocardiographic data were

performed using Spearman's correlation. Values for sST-2, gal-3 and NT-proBNP were log-

transformed because they were noticeably right-skewed. In a multivariable regression model that

included age, sex, NYHA class, history of AF, diastolic blood pressure, eGFR, log NT-pro BNP,

LV transmitral early diastolic filling velocity / LV early diastolic myocardial velocity (E/E'), and

LA volume we examined which factors were independently associated with baseline levels of

biomarkers. Variables included were on the basis of low numbers of missing values and clinical

knowledge. To examine the interaction between treatment with LCZ696 and baseline biomarker

levels on levels of NT-proBNP at 12 weeks and LA volume at 36 weeks we used a regression

model which included the effect of LCZ696, an interaction term between treatment and baseline

biomarker values and stratification variables of region and prior ACE-I or ARB use as well as

baseline NT-proBNP and LA volume respectively. Where an interaction was found this was

explored further by dividing the cohort into values above and below the observed median value

of the biomarker and stratified models of the effect of treatment on NT-proBNP and LA volume

examined. All statistical analyses were performed using STATA version 12 (Stata Corp, College

Station, TX, USA). For interaction tests a P value <0.1 was considered suggestive of an

interaction and for all other test a P value<0.05 was considered statistically significant.

Results

Demographic and Echocardiographic Data

Data from the 301 randomized patients were included in this study, 149 randomized to LCZ696, 152 to valsartan. The demographic and echocardiographic data were typical of a stable outpatient HFpEF population (31,32); elderly, female and NYHA Class II dominant, receiving multi-drug treatment, expected co-morbidities, and evidence of abnormal diastolic function with increased NT-proBNP, E/E', and LA volume. Baseline data are presented for each individual biomarker examined by quartiles (Supplemental Tables 1-4).

Baseline Biomarker Data

In these HFpEF patients, the baseline median values for gal-3 and sST-2, summarized in Table 1, were ~50% higher than the median values from previously published referent control subjects (33-41). In these HFpEF patients, the median values for MMP-2 were ~50% lower and PIIINP were similar to previously published referent control subjects (5, 42).

There were significant correlations between baseline biomarkers and demographic and echocardiographic variables (Table 2). Gal-3, sST-2 and PIIINP increased and MMP-2 decreased in association with an increase in NT-proBNP, and decreased eGFR (Figure 1A). sST-2 increased and MMP-2 decreased in association with an increase in E/E' and LA volume (Figure 1B). There was also a direct relationship between sST-2 and gal-3 (r=0.24, p<0.001, Figure 1C).

In a multivariable model after adjusting for age, sex, NYHA class, history of atrial fibrillation, diastolic blood pressure, eGFR, log NT-proBNP, E/E', and LA volume, only female sex (coef -0.27(95%CI -0.40 – -0.14), p<0.001), NYHA class (coef 0.24 (0.09 -0.39), p=0.002)

and LA volume (coef 0.003(0.0004 – 0.005),p=0.02) were statistically significantly associated with higher sST2. Only female sex (coef 0.168(0.004-0.332), p=0.04) and log NT-proBNP (coef 0.10 (0.01 - 0.19), p=0.03) were associated with baseline PIIINP. Lower diastolic blood pressure was associated with higher MMP2 levels at baseline (coef -0.014(-0.023 - -0.006), p=0.001) whereas lower eGFR was associated with higher Gal-3 (coef -0.008 (-0.011 - -0.006), p<0.001).

Relationship between Biomarkers and LCZ696 Treatment

The relationship between biomarkers and the effects of LCZ696 on the primary study endpoint (change in NT-proBNP after 12 weeks of treatment) and the secondary study endpoint (change LA volume from baseline after 36 weeks of treatment) were examined. There were no treatment interactions between LCZ696 and changes in NT-proBNP at 12 weeks (primary endpoint) for any of the four biomarkers; MMP-2 (interaction p=0.40), PIIINP (interaction p=0.14), Gal-3 (interaction p=0.32) or sST2 (interaction p=0.63). In addition, there were no treatment interactions between LCZ696 and changes in NT-proBNP at 36 weeks for any of the four biomarkers; MMP-2 (interaction p=0.09), PIIINP (interaction p=0.5), Gal-3 (interaction p=0.2) or sST2 (interaction p=0.99). However, there was an interaction between the response to treatment with LCZ696 compared to valsartan on change in LA volume at 36 weeks and baseline values of sST-2 (interaction p=0.07) and gal-3 (interaction p= 0.04). There was no interaction with PIIINP levels (interaction p=0.79) or MMP2 (interaction p= 0.61).

We further explored the interaction with sST-2 and gal-3 by dividing patients into 2 groups, for those above and below the observed baseline median value of sST-2 ($\geq$ vs. < 33.0 ng/mL) or gal-3 ($\geq$ vs. < 17.8 ng/mL). The effect on change in LA volume from baseline differed between LCZ696 and valsartan (Figure 2). In patients with a baseline value of sST-2 above the median,

36 weeks of treatment with LCZ696 did not result in a significant change in LA volume from baseline compared to valsartan (difference -1.5, 95%CI -7.8 to 4.8, p=0.6). Similarly in patients with a baseline value of gal-3 above the median, treatment with LCZ696 did not result in a significant change in LA volume from baseline compared to valsartan (difference -1.8, 95%CI -7.6 to 4.0, p=0.5). In patients with a baseline value of sST-2 or gal-3 below the median, 36 weeks of treatment with LCZ696 resulted in a statistically significantly larger change from baseline in LA volume compared to valsartan (in those with sST-2 values below median, the difference in treatment effect between LCZ696 vs. valsartan was -9.9, 95%CI -15.1 to -4.8, p < 0.0001, in those with gal-3 values below the median treatment effect between LCZ696 vs. valsartan was median difference -10.3, 95%CI -15.6 to -5.0, p < 0.0001).

For both patients with an NT-proBNP < median (difference -4.1, 95%CI -9.0, 0.7; p=0.09) and patients with an NT-proBNP > median (difference -6.0, 95%CI -12.0, 0.1; p=0.053), treatment with LCZ696 resulted in a numerically greater reduction in LA volume from baseline compared to valsartan. A formal test of the interaction between randomized treatment and baseline NT-proBNP was not statistically significant (p for interaction = 0.12). For patients with an eGFR < median (difference -2.8, 95%CI -7.7, 2.1; p=0.26) and patients with an eGFR > median (difference -8.8, 95%CI -15.0, -2.6; p=0.006), treatment with LCZ696 resulted in a numerically greater reduction in LA volume from baseline compared to valsartan. This change was statistically significant for the group with an eGFR > median. However a formal test of interaction between randomized treatment and baseline eGFR was not statistically significant (p for interaction = 0.58).

Interactions between LCZ696 and all other echocardiographic measurements of cardiac structure and function for the four biomarkers examined in this study were not performed

because they were not listed as a priori endpoints and because our previous published studies

showed that LCZ696 did not result in a change in any of these other echocardiographic

parameters (13).

There were no significant differences in any of the four biomarker values (sST-2, gal-3,

MMP-2, or PIIINP) between patients treated with Valsartan versus LCZ696 at baseline or after

12 or 36 weeks of treatment (Table 3). This was true for the patient group as a whole and for

subgroups with baseline sST-2 and gal-3 above and below the observed median values.

Comparing baseline biomarker values to after treatment values, MMP-2 increased significantly

in both valsartan and LCZ696 treated patients at week 36 versus baseline ($p<0.001$);there were

no significant changes in the other three biomarkers comparing baseline to after treatment values.

History of atrial fibrillation was not associated with change in LA volume at 36 weeks

$p=0.12$ and there was no interaction between randomized treatment and history of atrial

fibrillation on change in LA volume $p=0.44$. In addition, for subjects with AF on ECG at

baseline, the respective p values were also non-significant at 0.39 and 0.52.

Discussion

Data from the current study support several novel and hypothesis generating findings. First, we found that HFpEF patients had values of circulating biomarkers that may reflect a profibrotic state. Gal-3 and sST-2 were increased and MMP-2 was decreased. Gal-3 and sST-2 have been shown to increase collagen synthesis in cardiac fibroblasts and MMP-2 has been shown to cause collagen degradation (2) (Figure 3). In aggregate, the directional changes in these biomarkers might be expected to be associated with an increase in myocardial collagen content. The current study adds important, novel, clinically relevant data in a group of patients in which there is a large gap in knowledge. In particular, the panel of specific biomarkers used in this study has not been examined together in previous clinical studies or randomized clinic trials (RCTs) of HFpEF patients. In addition, PARAMOUNT represents the only Phase II RCT of HFpEF patients in which the pre-specified primary endpoints have been positively improved by the therapy being tested. This provided a unique opportunity to examine the purposes proposed and test the hypotheses stated in this study.

The differences between biomarkers in the current HFpEF patients and referent controls are concordant with trends found in the very limited number of other studies that included HFpEF patients. For example, in studies including HFpEF patients sST-2 median values ranged from ~25-30 ng/ml (2, 40-42); Gal-3 median values ranged from ~12-14 ng/ml (2,38,39,42). Variations in inclusion/exclusion criteria creating differences in population characteristics, co-morbidity distribution, and severity of HF are likely responsible for small differences between studies.

For both sST-2 and Gal-3 there are FDA approved partition values that can be used in risk assessment analyses to predict morbid and mortal outcomes. When sST-2 is > 35 ng/ml or Gal-3 is > 17.8 ng/ml, there is an increase in risk. It should be noted; however, that both of these partition values were established from studies like HF-ACTION and others in which HFrEF patients were exclusively or dominantly the focus of study. It is fortuitous that the median values for these two biomarkers in the current study are identical to or very close to the FDA approved partition values. Therefore, these data lend credence to the important biomarker observations made in this study of HFpEF patients in PARAMOUNT.

Second, we found that biomarker levels correlated with indices of disease severity. The presence of more severe HFpEF is generally indicated by higher levels of NT-proBNP, diastolic function (such as E/E' and LAV) and decreased renal function (2, 5, 16-19). In the current study, there was a direct relationship between sST-2 and each of these indices of disease severity; there was an inverse relationship with MMP-2. Thus, while each of the patients enrolled in PARAMOUNT had the clinical syndrome of HFpEF, those with the more severe disease had a biomarker pattern associated with a more profibrotic milieu. While these interactions described in the current study may have been expected, they have never been previously examined in a substantially sized study of HFpEF patients. The interactions defined help to clarify signaling pathways that contribute to fibrosis-induced (and probably inflammation-induced) abnormalities in structure and function in HFpEF patients.

Third, the baseline pretreatment values of sST-2 and gal-3 may have modified the response to LCZ696, specifically reduction in LA volume but not NT-proBNP. Patients who had levels of sST-2 and Gal-3 below the observed median value showed a greater LA volume response to LCZ696 than those with sST-2 and Gal-3 above the median. These data are clearly not

conclusive; however, they do allow the generation of important new hypotheses, particularly concerning the mechanisms underlying HFpEF and the effects of LCZ696 on these mechanisms. It is possible that patients with less severe myocardial fibrosis may be more responsive to treatment, particularly over a short period of treatment. Changes in LV structural remodeling may not be detectable until treatment has been continued for at least 12 months. Confirmation of these findings in a larger patient cohort and extension of treatment duration will clearly be needed in future studies to make more definitive conclusions. These kind of analyses are planned for PARAGON-HF.

There are several possible factors that contribute to the fact that baseline biomarkers modified the response to LCZ696 on LAV but not NT-proBNP. For example, changes in natriuretic peptides and changes in left atrial structure / function may reflect different but interdependent aspects of the pathophysiologic mechanisms that underlie HFpEF. As presented in a previously published schematic, LV diastolic filling pressures can be changed rapidly as a result of intravascular volume shifts and a change in operative compliance (2). This "hemodynamic" change may be best reflected by changes in natriuretic peptides. LV diastolic filling pressures can also be changed more slowly by progressive fibrosis and a change in overall chamber compliance (2). This "structural" change may be best reflected by changes in left atrial volume. LCZ696 is likely to act on both of these mechanisms. Its direct diuretic, unloading, hemodynamic effect would be expected to rapidly reduce filling volume, operative compliance and NT-proBNP and to do so without needed to also affect structural changes in myocardial ECM collagen or LAV. Therefore, the extent of fibrosis (as reflected by sST-2 or Gal-3) should not modify the effects of LCZ696 on changes in NT-proBNP. By contrast, the effects of LCZ696 on the regression of fibrosis would be expected to occur over longer time periods. Therefore,

patients with less fibrosis (as reflected by lower values of sST-2 or Gal-3) may respond more quickly with a decrease in overall chamber compliance and a resultant decrease in LAV because there was simply less fibrosis to regress. Patients with more fibrosis may take longer than 6-9 months to respond to LCZ696; however, this finding does not necessarily signal the absence of a response. There are few studies that document the time course of regression of fibrosis in pathophysiologic processes that undergo treatments that effectively correct the pathophysiologic abnormalities. However, perhaps the best example is the effects of aortic valve replacement (AVR) in patients with aortic valve stenosis. In these studies, the complete reversal of left ventricular fibrosis was time dependent and progressive over a 2-4 year period after AVR (43). Thus, the interplay between changes in hemodynamic and structural factors (and other factors) may contribute to the findings presented in the current study.

Fourth, over the 36 week course of this study, sST-2, gal-3, MMP-2, or PIIINP did not change in either the valsartan or LCZ696 treated groups. The significance of this finding is not entirely clear. The same possible factors listed above may be applicable such as the length of the treatment period may be too short to see significant changes in these biomarkers. Conversely, it is possible that this particular set of biomarkers may have greater utility as diagnostic, prognostic and severity of illness indices rather than indices reflecting response to therapy.

Biomarkers Reflecting a Profibrotic State

Fibrillar collagen content can be altered by changes in the balance in the following processes: collagen synthesis, post-synthetic processing and degradation. Biomarkers reflecting changes in these processes or their determinants were examined in PARAMOUNT (2, 5-7). For example, Gal-3, a beta-galactoside-binding lectin, secreted by macrophages, may act to increase fibroblast

proliferation, activity, transformation into myofibroblast and increase collagen synthesis (20-30).

Likewise, soluble ST2, by acting as a decoy, prevents binding of IL-33 to membrane-bound ST2

and results in increased collagen synthesis (Figure 3). Therefore, both gal-3 and sST2 induced

increase in collagen synthesis would be expected to be reflected in an increase in levels of

collagen propeptides such as PIIINP (procollagen III N-terminal propeptide). However, in the

presence of what appear to be profibrotic stimuli, we did not see changes in PIIINP. It is likely

that the most important changes in myocardial collagen homeostasis involve a change in

collagen I rather than collagen III, thus limiting the sensitivity of PIIINP vs measurements of

collagen I propeptides. In addition to changes in synthesis, changes in degradation may affect

collagen content. Insoluble collagen fibril degradation is caused by proteases such as matrix

metalloproteinases (MMP), of which there are > 29 known members (5-11). Only MMP-2 was

measured in PARAMOUNT; lower values seen in HFpEF patients suggests decreased

degradation rates; however, measurement of this single MMP particularly without measurement

of tissue inhibitors of MMPs (TIMPs) does not fully characterize the stoichiometric balance of

this enzyme system.

Study Limitations

We readily acknowledge that comparing biomarker data from the current study with non-

simultaneous, historic, previously published referent controls imposes clear limitations. While a

referent control group was not included in the design of the PARAMOUNT study, the previously

published referent control subjects used for comparison were taken from subjects with an age,

sex, and race distribution similar to the current study population but with no evidence of active

cardiovascular disease. In addition, the methodologies used in the current study to measure

biomarkers were either identical to or equivalent to the methods used in previously published studies. The small differences in methodologies are not likely to impose significant differences between study analyses.

Measurements of circulating biomarkers are not direct measurements of myocardial collagen homeostasis. Our analysis assumes that biomarkers are representatively excreted in a manner measureable in the circulation and that their predominant source is the myocardium. PARAMOUNT was designed with exclusion criteria that limited co-morbid conditions that would produce non-myocardial sources of circulating biomarkers that reflect changes in collagen homeostasis such a severe renal, pulmonary or hepatic fibrosis.

Conclusion

In HFpEF patients, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease, underlying pathophysiology, and may modify the structural response to treatment.

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Table 1: Biomarker Data

	PARAMOUNT-HF HFpEF Patients			Referent Controls
	Mean (SD)	Geo Mean	Median (IQR)	Median*
sST2 (ng/mL)	39.6 (24.7)	34.7	33.0 (24.6, 48.1)	20 (17, 26)
Galectin-3 (ng/mL)	19.0 (6.9)	17.9	17.8 (14.1, 22.8)	12 (9, 15)
MMP-2 (ng/mL)	198 (73)	184	188 (156, 231)	335 (323, 443)
PIIINP (ng/mL)	6.1 (3.5)	5.5	5.6 (4.3, 6.9)	6.5 (6.1, 8.2)

Abbreviation:
PIIINP = collagen III n-terminal propeptide, MMPs = matrix metaloproteinases, sST-2 = soluble form of ST2 (an Interleukin-1 receptor family member), SD = standard deviation, IQR=interquartile range. *=Referent control median [IQR] values taken from references 5, 34-37.

Table 2: Correlations Between Biomarker and Demographic/Echocardiographic Data

	ST2		Galectin		MMP2		PIIINP	
	R	P	R	P	R	P	R	P
NT-proBNP	**0.19**	**0.002**	**0.17**	**0.004**	**0.31**	**<0.001**	**0.25**	**<0.001**
eGFR	**-0.16**	**0.005**	**-0.50**	**<0.001**	**-0.19**	**0.002**	**-0.14**	**0.07**
SBP	-0.03	0.64	0.01	0.94	0.01	0.92	-0.04	0.60
E'	0.07	0.31	-0.04	0.51	0.07	0.31	0.07	0.38
E/A	0.12	0.11	-0.04	0.57	**0.24**	**0.003**	-0.02	0.84
E/E'	**0.11**	**0.09**	0.09	0.18	**0.17**	**0.01**	-0.03	0.70
LA volume	**0.25**	**<0.001**	-0.01	0.87	**0.14**	**0.03**	-0.04	0.65

Abbreviation:
PIIINP = collagen III n-terminal propeptide, MMPs = matrix metaloproteinases, sST-2 = soluble form of ST2(an Interleukin-1 receptor family member). E = early diastolic filling velocity, E' = early diastolic myocardial velocity, A = atrial contraction induced diastolic filling velocity wave, LA = left atrium, eGFR = estimated glomerular filtration rate, NT-proBNP = N-terminal pro-B-type natriuretic peptide, SBP = systolic blood pressure.

Table 3: Serial Measurements of Plasma Biomarkers

	sST2	Galectin-3	MMP-2	PIIINP
Baseline				
Sample Size:	296	294	247	178
All	33.0 [24.6,48.1]	17.8 [14.1, 22.8]	188 [156, 231]	5.6 [4.3, 6.9]
Valsartan	33.8 [25.2, 48.1]	16.9 [14.0, 22.4]	188 [156, 244]	5.6 [4.3, 6.8]
LCZ696	32.2 [24.3, 47.8]	18.9 [14.4, 23.3]	187 [150, 225]	5.5 [4.4, 7.2]
12 Weeks				
Sample Size:	262	250	244	
All	30.7 [23.4, 44.7]	17.0 [13.9, 22.1]	191 [155, 234]	
Valsartan	31.0 [23.9, 44.4]	17.1 [13.9, 21.2]	194 [150, 243]	
LCZ696	29.8 [23.3, 45.8]	16.9 [14.2, 22.2]	189 [158, 222]	
36 Weeks				
Sample Size:	211	214	241	182
All	33.4 [23.5, 48.4]	16.8 [13.8, 21.9]	253 [208, 318]	5.3 [4.2, 7.1]
Valsartan	35.2 [23.6, 45.1]	16.8 [13.8, 21.2]	261 [212, 334]	5.4 [4.1, 7.0]
LCZ696	31.4 [23.5, 50.4]	17.0 [13.8, 22.2]	248 [206, 303]	5.3 [4.2, 7.2]

Abbreviation:
All data are Median (interquartile range) and in units of ng/ml. PIIINP = collagen III n-terminal propeptide, MMPs = matrix metaloproteinases, sST-2 = soluble form of ST2 (an Interleukin-1 receptor family member).

Figure Legends

Figure 1: Panel A: Relationship between ln(sST-2) and ln(NT-proBNP) in patients with heart failure with a preserved ejection fraction; Spearman correlation r=0.19, p=0.002. **Panel B:** Relationship between ln(sST2) and left atrial volume in patients with heart failure with a preserved ejection fraction; correlation r=0.25, p=0.002. **Panel C:** Relationship between ln(sST2) and ln(Gal-3) in patients with heart failure with a preserved ejection fraction; correlation r=0.23, p<0.0001. sST-2 = soluble suppressor of tumorigenicity-2; NT-proBNP = n-terminal pro b-type natriuretic peptide; Gal-3 = Galectin 3.

Figure 2: Change from baseline in left atrial volume (LAV) produced by treatment with LCZ696 versus valsartan in those with a baseline value of sST-2 and gal-3 above and below the median. When patients were divided into 2 groups, above and below the baseline median value of sST-2 or gal-3, the effect on change in LA volume produced LCZ696 vs. valsartan differed; in patients with a baseline value of sST-2 or gal-3 above the median, treatment with LCZ696 did not result in a significant change in LA volume; in patients with a baseline value of sST-2 or gal-3 below the median, treatment with LCZ696 resulted in a decrease in LA volume after 36 weeks of treatment compared to baseline.

Figure 3: Schematic representation of mechanisms suggested by changes in circulating biomarkers. Increased gal-3 secreted by mast cells may contribute to transdifferentiation of fibroblasts to myofibroblasts. Increased sST-2 may contribute to ST-2 profibrotic signaling.

Decreased MMP-2 may contribute to less collagen degradation. In aggregate, these changes may contribute to increased myocardial ECM collagen and fibrosis and may be reflected by the changes measured in circulating biomarkers.

Figure 1A
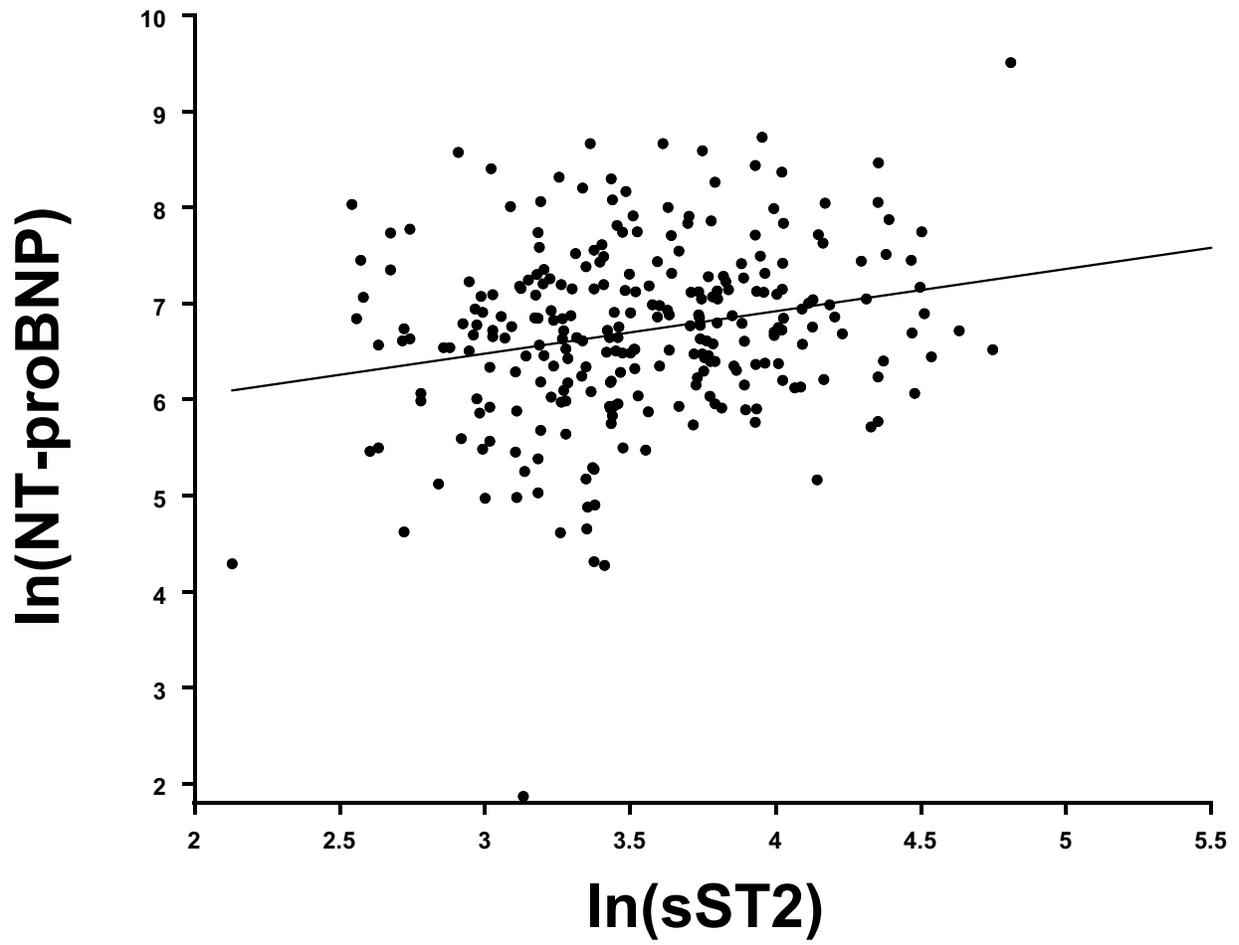
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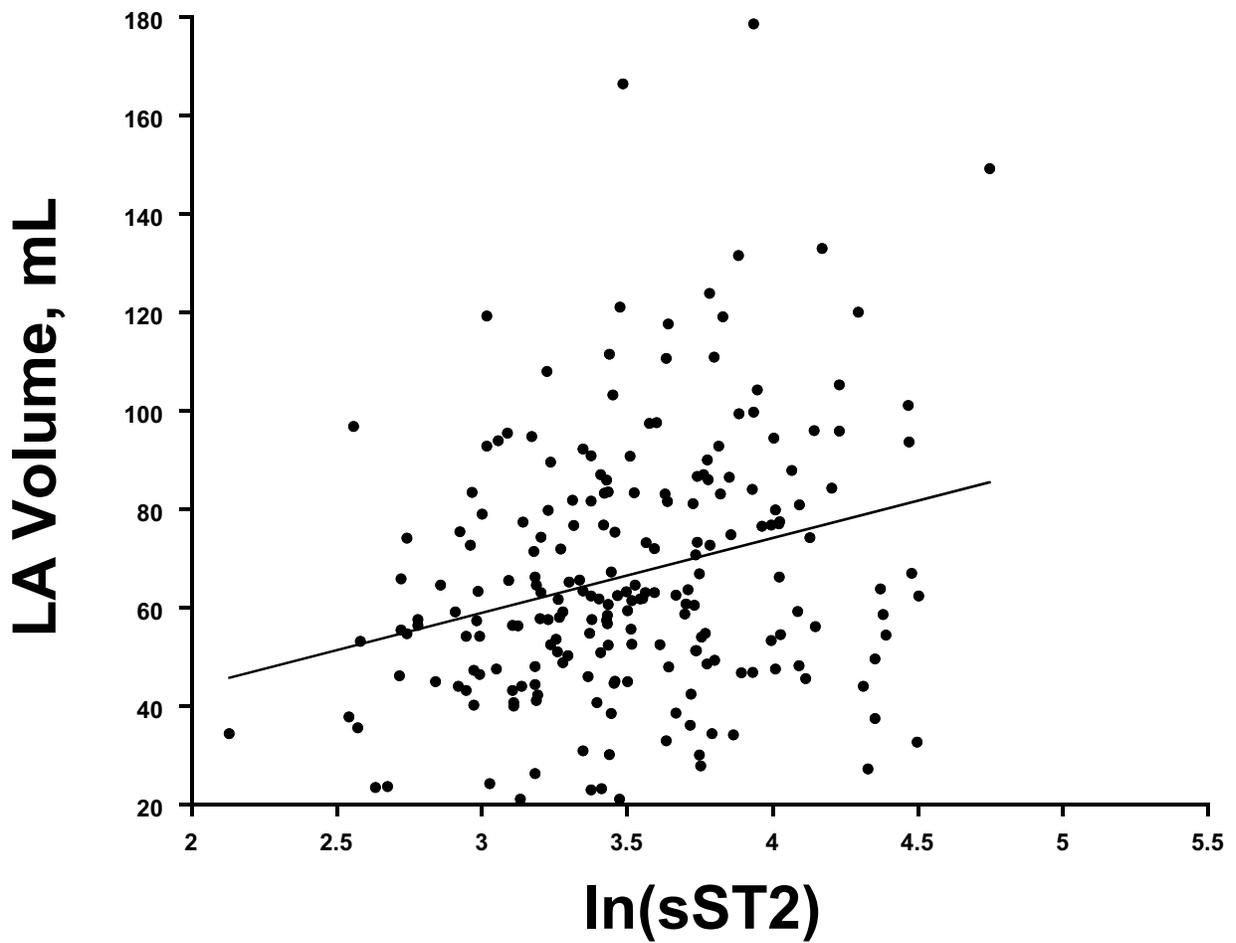
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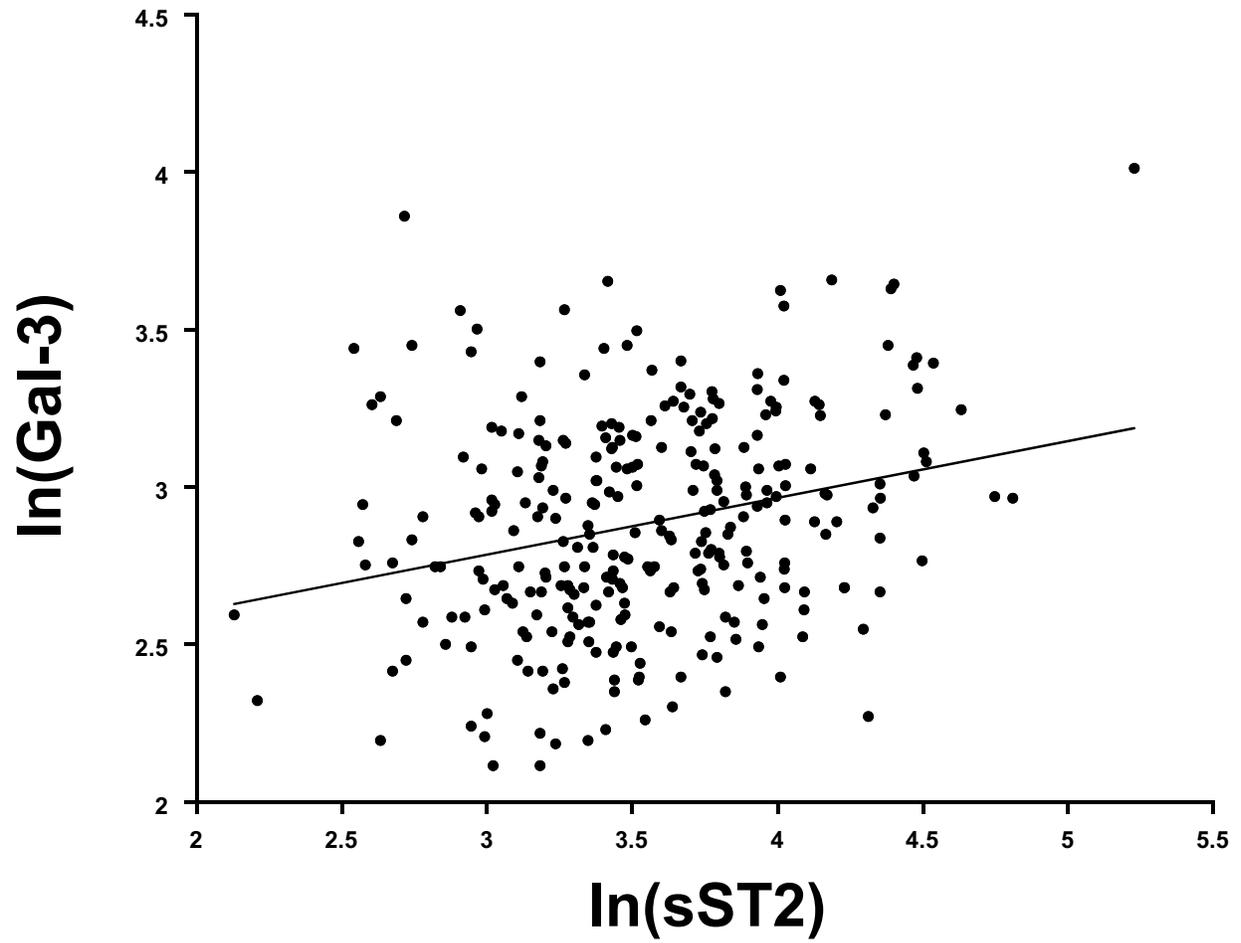
Figure 2
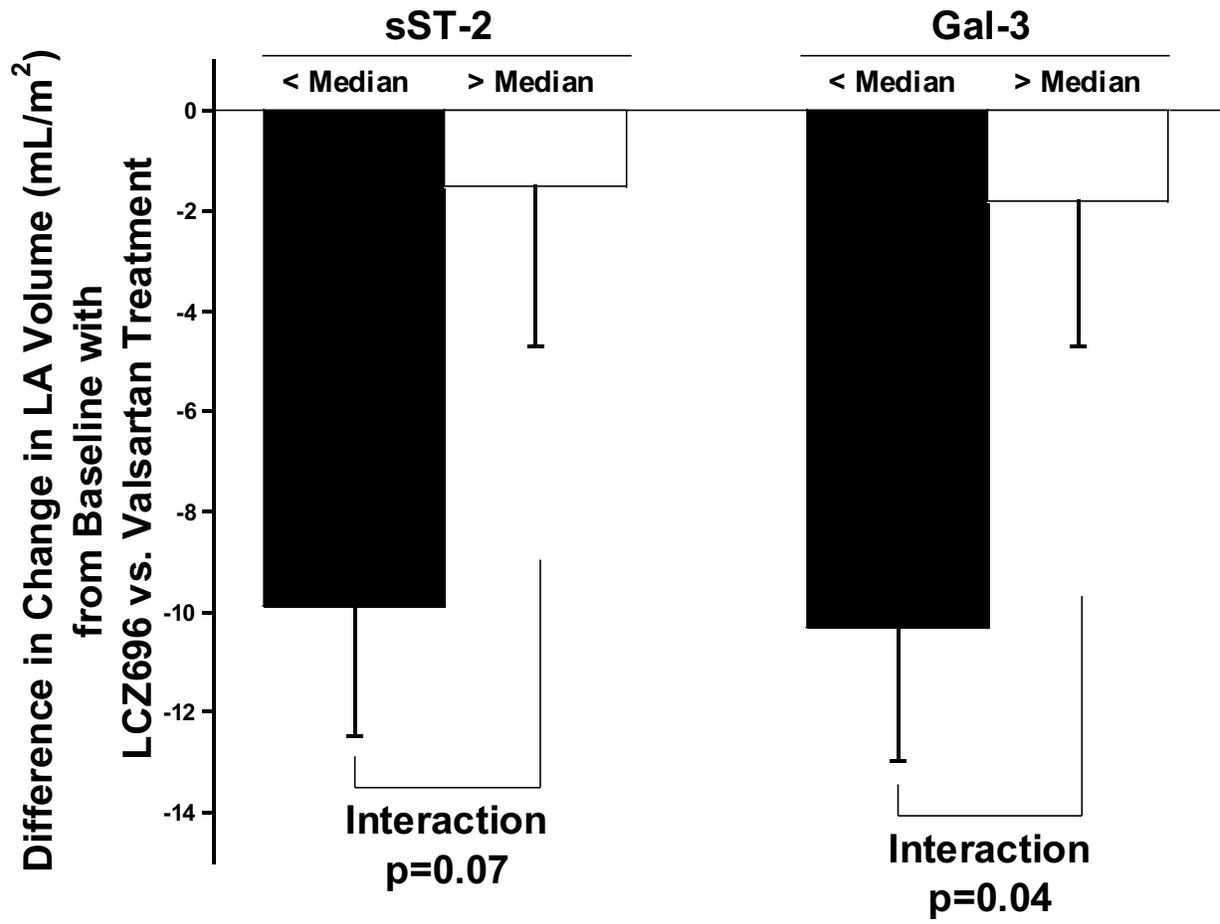
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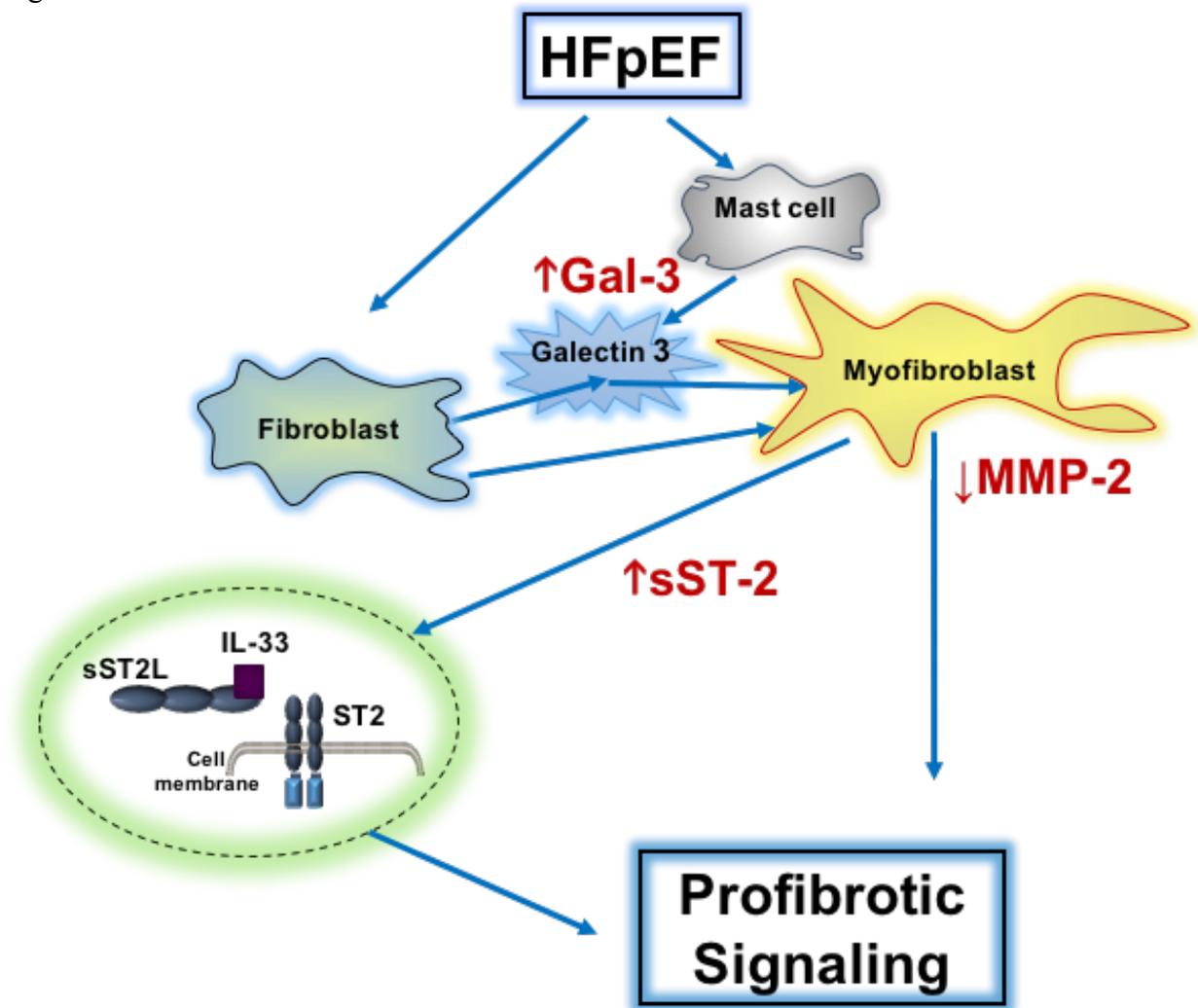
SUPPLEMENTAL MATERIAL FOR

Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure with a Preserved Ejection Fraction: Data from the PARAMOUNT Study

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Supplemental TABLE 1. Baseline Characteristics According to GALECTIN-3 by Quartiles

	Q1, N=74		Q2, N=73		Q3, N=74		Q4, N=73		P for trend
Age, years	68 ± 10		73 ± 8		72 ± 9		72 ± 9		0.020
Women (%)	36	(48.6%)	39	(53.4%)	41	(55.4%)	49	(67.1%)	0.027
NYHA Class I	1	(1.4%)	1	(1.4%)	0	(0.0%)	0	(0.0%)	0.12
NYHA Class II	61	(82.4%)	58	(79.5%)	60	(81.1%)	54	(74.0%)	
NYHA Class III	12	(16.2%)	14	(19.2%)	14	(18.9%)	19	(26.0%)	
Previous admission for heart failure	24	(32.4%)	32	(43.8%)	26	(35.1%)	40	(54.8%)	0.023
History of atrial fibrillation	31	(41.9%)	29	(39.7%)	29	(39.2%)	33	(45.2%)	0.72
Atrial fibrillation at screening ECG	20	(27.0%)	20	(27.4%)	20	(27.0%)	23	(31.5%)	0.58
History of hypertension	66	(89.2%)	72	(98.6%)	66	(89.2%)	72	(98.6%)	0.13
History of diabetes	25	(33.8%)	24	(32.9%)	29	(39.2%)	33	(45.2%)	0.11
History of myocardial infarction	14	(18.9%)	17	(23.3%)	19	(25.7%)	9	(12.3%)	0.41
NT-proBNP (pg/mL), median [Q1, Q3]	693.0 [368.0, 1377.0]		867.0 [499.0, 1341.0]		909.5 [512.0, 1269.5]		962.0 [628.0, 1960.5]		0.014
Heart rate (bpm)	67.15 ± 11.76		68.03 ± 11.66		69.77 ± 12.18		72.26 ± 15.71		0.012
Body mass index (kg/m^2)	29.90 ± 5.60		29.60 ± 5.22		30.47 ± 5.87		30.00 ± 6.38		0.70
Systolic BP (mmHg)	134.65 ± 15.06		136.29 ± 14.17		136.10 ± 13.11		133.87 ± 13.66		0.73
Diastolic BP (mmHg)	79.31 ± 8.65		77.73 ± 9.79		77.88 ± 7.61		74.95 ± 10.93		0.008
eGFR (mL/min per 1.73 m²)	76.18 ± 20.31		68.40 ± 15.46		65.10 ± 17.35		51.13 ± 19.61		<0.001
ACE inhibitors or ARBs	67	(90.5%)	68	(93.2%)	73	(98.6%)	67	(91.8%)	0.47
Beta blockers	58	(78.4%)	61	(83.6%)	59	(79.7%)	53	(72.6%)	0.33
Aldosterone antagonists	9	(12.2%)	16	(21.9%)	19	(25.7%)	18	(24.7%)	0.05
E' (cm/s)	7.77 ± 2.92		7.02 ± 2.48		7.43 ± 3.05		7.64 ± 2.46		0.57
E/E'	11.41 ± 4.55		13.40 ± 6.89		12.74 ± 6.42		12.70 ± 5.69		0.17
LA volume index (mL/m²)	35.46 ± 12.54		35.90 ± 14.93		37.08 ± 15.57		34.50 ± 11.35		0.84
LV end diastolic volume (mL)	118.37 ± 29.41		115.44 ± 33.84		107.42 ± 26.09		107.70 ± 23.68		0.11
LV ejection fraction (%)	56.65 ± 7.00		57.89 ± 9.71		59.62 ± 7.16		58.81 ± 6.77		0.06
LV mass index (g/m²)	73.23 ± 17.35		81.46 ± 25.00		77.92 ± 21.16		77.39 ± 20.80		0.29
Relative wall thickness	0.35 ± 0.05		0.37 ± 0.07		0.37 ± 0.08		0.38 ± 0.08		0.004
Tricuspid regurgitant velocity (m/s)	2.43 ± 0.33		2.50 ± 0.36		2.60 ± 0.32		2.47 ± 0.49		0.31
LA strain (%)	22.10 ± 9.31		19.84 ± 7.15		22.34 ± 8.68		19.83 ± 6.24		0.40
LV global longitudinal strain (%)	-14.57 ± 3.52		-14.56 ± 3.20		-15.16 ± 3.48		-14.20 ± 3.09		0.79

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 2. Baseline Characteristics According to MMP-2 Quartiles

	Q1 N=62	Q2 N=62	Q3 N=62	Q4 N=61	P for trend
Age, years	68 ± 10	72 ± 9	73 ± 8	73 ± 8	0.003
Women (%)	36 (58.1%)	32 (51.6%)	38 (61.3%)	34 (55.7%)	0.92
NYHA Class I	0 (0.0%)	1 (1.6%)	1 (1.6%)	0 (0.0%)	0.30
NYHA Class II	53 (85.5%)	51 (82.3%)	46 (74.2%)	49 (80.3%)	
NYHA Class III	9 (14.5%)	10 (16.1%)	15 (24.2%)	12 (19.7%)	
Previous admission for heart failure	26 (41.9%)	20 (32.3%)	24 (38.7%)	26 (42.6%)	0.76
History of atrial fibrillation	22 (35.5%)	30 (48.4%)	29 (46.8%)	27 (44.3%)	0.38
Atrial fibrillation at screening ECG	14 (22.6%)	22 (35.5%)	16 (25.8%)	21 (34.4%)	0.32
History of hypertension	58 (93.5%)	58 (93.5%)	57 (91.9%)	57 (93.4%)	0.89
History of diabetes	19 (30.6%)	21 (33.9%)	22 (35.5%)	27 (44.3%)	0.12
History of myocardial infarction	17 (27.4%)	16 (25.8%)	10 (16.1%)	9 (14.8%)	0.04
NT-proBNP (pg/mL), median [Q1, Q3]	573.0 [346.5, 1022.5]	766.5 [387.0, 1273.0]	973.5 [645.0, 1590.0]	1154.0 [744.0,1853.0]	<0.001
Heart rate (bpm)	69.87 ± 12.70	67.59 ± 11.40	68.53 ± 15.40	68.02 ± 11.90	0.53
Body mass index (kg/m^2)	30.34 ± 5.61	30.19 ± 5.24	31.17 ± 6.15	30.11 ± 5.50	0.92
Systolic BP (mmHg)	135.13 ± 14.22	136.55 ± 14.61	133.15 ± 12.00	137.16 ± 15.22	0.74
Diastolic BP (mmHg)	80.27 ± 9.17	78.62 ± 10.01	76.57 ± 8.45	74.00 ± 10.05	<0.001
eGFR (mL/min per 1.73 m²)	71.82 ± 21.46	66.18 ± 17.27	65.97 ± 22.51	59.52 ± 16.78	0.001
ACE inhibitors or ARBs	60 (96.8%)	56 (90.3%)	57 (91.9%)	57 (93.4%)	0.56
Beta blockers	47 (75.8%)	50 (80.6%)	50 (80.6%)	54 (88.5%)	0.09
Aldosterone antagonists	14 (22.6%)	11 (17.7%)	16 (25.8%)	13 (21.3%)	0.86
E' (cm/s)	6.85 ± 2.74	7.44 ± 2.91	6.92 ± 2.59	8.45 ± 2.80	0.17
E/E'	11.44 ± 5.18	11.72 ± 5.38	14.86 ± 6.77	12.79 ± 6.35	0.013
LA volume index (mL/m²)	33.13 ± 13.09	35.78 ± 13.58	37.12 ± 14.13	38.03 ± 13.32	0.042
LV end diastolic volume (mL)	114.89 ± 31.94	115.91 ± 26.66	106.07 ± 24.37	114.51 ± 31.76	0.31
LV ejection fraction (%)	57.47 ± 8.54	57.86 ± 8.95	59.19 ± 7.48	59.87 ± 6.08	0.028
LV mass index (g/m²)	75.58 ± 20.12	78.31 ± 21.32	75.04 ± 20.53	79.58 ± 21.49	0.98
Relative wall thickness	0.37 ± 0.06	0.37 ± 0.07	0.37 ± 0.05	0.38 ± 0.09	0.48
Tricuspid regurgitant velocity (m/s)	2.41 ± 0.29	2.35 ± 0.15	2.58 ± 0.45	2.64 ± 0.45	0.004
LA strain (%)	22.57 ± 7.70	21.24 ± 8.72	20.72 ± 6.92	20.55 ± 8.61	0.22
LV global longitudinal strain (%)	-15.25 ± 3.85	-15.03 ± 2.97	-14.42 ± 3.18	-15.18 ± 3.09	0.68

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 3. Baseline Characteristics According to PIIINP Quartiles

	Q1 N=45		Q2 N=44		Q3 N=44		Q4 N=45		P for trend
Age, years	70 ± 10		70 ± 11		72 ± 8		73 ± 9		0.06
Women (%)	22	(48.9%)	25	(56.8%)	25	(55.6%)	27	(61.4%)	0.28
NYHA Class I	0	(0.0%)	0	(0.0%)	1	(2.2%)	1	(2.3%)	0.88
NYHA Class II	37	(82.2%)	34	(77.3%)	36	(80.0%)	34	(77.3%)	
NYHA Class III	8	(17.8%)	10	(22.7%)	8	(17.8%)	9	(20.5%)	
Previous admission for heart failure	18	(40.0%)	14	(31.8%)	20	(44.4%)	9	(20.5%)	0.15
History of atrial fibrillation	20	(44.4%)	19	(43.2%)	23	(51.1%)	20	(45.5%)	0.74
Atrial fibrillation at screening ECG	15	(33.3%)	13	(29.5%)	17	(37.8%)	12	(27.3%)	0.76
History of hypertension	44	(97.8%)	40	(90.9%)	40	(88.9%)	43	(97.7%)	0.88
History of diabetes	23	(51.1%)	10	(22.7%)	14	(31.1%)	14	(31.8%)	0.12
History of myocardial infarction	15	(33.3%)	13	(29.5%)	7	(15.6%)	8	(18.2%)	0.142
NT-proBNP (pg/mL), median [Q1, Q3]	511.0 [369.0, 1018.0]		796.0 [517.0, 1267.0]		1107.0 [572.0,1800.0]		967.0 [625.0, 1507.0]		0.001
Heart rate (bpm)	67.73 ± 12.54		65.09 ± 9.77		68.11 ± 11.91		73.36 ± 15.13		0.020
Body mass index (kg/m^2)	30.40 ± 4.38		30.32 ± 5.18		29.90 ± 5.84		31.79 ± 6.67		0.32
Systolic BP (mmHg)	135.47 ± 12.81		133.42 ± 13.05		137.43 ± 13.96		133.87 ± 11.51		0.93
Diastolic BP (mmHg)	79.79 ± 8.76		77.58 ± 9.56		78.32 ± 10.12		76.88 ± 9.76		0.21
eGFR (mL/min per 1.73 m²)	72.20 ± 18.41		70.14 ± 20.06		65.82 ± 18.07		63.79 ± 17.54		0.019
ACE inhibitors or ARBs	40	(88.9%)	43	(97.7%)	43	(95.6%)	41	(93.2%)	0.50
Beta blockers	37	(82.2%)	34	(77.3%)	40	(88.9%)	35	(79.5%)	0.89
Aldosterone antagonists	9	(20.0%)	11	(25.0%)	12	(26.7%)	6	(13.6%)	0.54
E' (cm/s)	7.33 ± 2.70		7.58 ± 2.73		7.57 ± 2.98		8.19 ± 2.90		0.30
E/E'	11.74 ± 5.66		13.39 ± 7.19		13.06 ± 5.72		11.03 ± 4.85		0.56
LA volume index (mL/m²)	32.74 ± 12.62		38.87 ± 13.36		37.76 ± 13.05		33.83 ± 16.06		0.83
LV end diastolic volume (mL)	122.27 ± 31.17		108.15 ± 27.74		116.64 ± 31.47		108.56 ± 22.50		0.16
LV ejection fraction (%)	57.16 ± 8.41		57.87 ± 6.81		58.82 ± 9.70		60.45 ± 7.71		0.033
LV mass index (g/m²)	74.45 ± 23.32		75.40 ± 17.25		73.08 ± 20.08		75.05 ± 20.04		0.96
Relative wall thickness	0.35 ± 0.08		0.35 ± 0.06		0.37 ± 0.06		0.37 ± 0.06		0.17
Tricuspid regurgitant velocity (m/s)	2.42 ± 0.35		2.53 ± 0.36		2.51 ± 0.36		2.52 ± 0.30		0.68
LA strain (%)	22.78 ± 7.44		18.80 ± 7.71		21.96 ± 8.89		23.31 ± 8.26		0.52
LV global longitudinal strain (%)	-15.39 ± 3.17		-14.67 ± 2.96		-15.22 ± 3.22		-15.55 ± 2.77		0.72

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 4. Baseline Characteristics According to sST-2 Quartiles

	Q1 N=74		Q2 N=74		Q3 N=74		Q4 N=74		P for trend
Age, years	69 ± 10		70 ± 10		71 ± 8		74 ± 8		0.004
Women (%)	47	(63.5%)	45	(60.8%)	38	(51.4%)	36	(48.6%)	0.036
NYHA Class I	0	(0.0%)	1	(1.4%)	1	(1.4%)	0	(0.0%)	0.043
NYHA Class II	66	(89.2%)	55	(74.3%)	59	(79.7%)	54	(73.0%)	
NYHA Class III	8	(10.8%)	18	(24.3%)	14	(18.9%)	20	(27.0%)	
Previous admission for heart failure	28	(37.8%)	29	(39.2%)	33	(44.6%)	34	(45.9%)	0.25
History of atrial fibrillation	17	(23.0%)	32	(43.2%)	33	(44.6%)	39	(52.7%)	<0.001
Atrial fibrillation at screening ECG	7	(9.5%)	20	(27.0%)	22	(29.7%)	33	(44.6%)	<0.001
History of hypertension	66	(89.2%)	72	(97.3%)	73	(98.6%)	67	(90.5%)	0.66
History of diabetes	26	(35.1%)	21	(28.4%)	34	(45.9%)	32	(43.2%)	0.10
History of myocardial infarction	20	(27.0%)	14	(18.9%)	13	(17.6%)	14	(18.9%)	0.22
NT-proBNP (pg/mL), median [Q1, Q3]	773.5 [355.0, 1247.5]		715.5 [393.0, 1341.0]		957.0 [601.0, 1322.0]		1014.0 [603.0,1800.0]		0.002
Heart rate (bpm)	69.23 ± 13.28		67.59 ± 10.67		70.18 ± 14.45		70.01 ± 13.46		0.47
Body mass index (kg/m^2)	28.57 ± 5.26		30.52 ± 6.23		30.61 ± 5.38		30.24 ± 6.09		0.09
Systolic BP (mmHg)	134.34 ± 13.40		137.82 ± 13.19		134.34 ± 13.62		134.84 ± 15.93		0.79
Diastolic BP (mmHg)	77.78 ± 7.73		80.02 ± 10.24		77.79 ± 8.03		73.79 ± 10.60		0.004
eGFR (mL/min per 1.73 m²)	69.12 ± 21.46		68.47 ± 19.88		62.32 ± 20.74		61.19 ± 18.14		0.005
ACE inhibitors or ARBs	72	(97.3%)	69	(93.2%)	67	(90.5%)	68	(91.9%)	0.15
Beta blockers	55	(74.3%)	58	(78.4%)	61	(82.4%)	59	(79.7%)	0.34
Aldosterone antagonists	9	(12.2%)	13	(17.6%)	22	(29.7%)	18	(24.3%)	0.021
E' (cm/s)	7.10 ± 2.69		7.43 ± 2.77		7.72 ± 2.60		7.54 ± 2.99		0.30
E/E'	10.98 ± 3.87		12.57 ± 6.00		13.29 ± 6.85		13.70 ± 6.63		0.06
LA volume index (mL/m²)	31.67 ± 11.70		35.10 ± 12.93		36.40 ± 13.94		39.83 ± 14.90		<0.001
LV end diastolic volume (mL)	114.79 ± 29.12		106.59 ± 22.81		111.27 ± 29.73		120.46 ± 33.43		0.35
LV ejection fraction (%)	57.88 ± 9.10		58.83 ± 7.20		58.46 ± 7.25		57.33 ± 7.59		0.71
LV mass index (g/m²)	76.34 ± 18.92		77.54 ± 21.03		79.36 ± 25.66		77.16 ± 18.49		0.76
Relative wall thickness	0.37 ± 0.07		0.36 ± 0.06		0.37 ± 0.08		0.37 ± 0.06		0.61
Tricuspid regurgitant velocity (m/s)	2.38 ± 0.29		2.54 ± 0.31		2.52 ± 0.40		2.58 ± 0.44		0.42
LA strain (%)	23.94 ± 8.92		20.15 ± 7.80		21.04 ± 7.53		19.78 ± 7.46		0.025
LV global longitudinal strain (%)	-14.56 ± 3.61		-14.19 ± 3.02		-15.06 ± 3.51		-14.85 ± 3.21		0.39

Abbreviations:

sST-2 = soluble form of ST2(an Interleukin-1 receptor family member), ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular