Galectin-3 Inhibition with Modified Citrus Pectin in Hypertension

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Twitter post: First RCT of Galectin-3 inhibition: no reduction in collagen biomarkers in patients with hypertension and elevated Gal-3 levels.

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HIGHLIGHTS

- Galectin-3 is a beta-galactoside binding leptin that regulates inflammation and fibrosis, and is highly predictive of heart failure events and mortality.
- In a randomized placebo-controlled trial of modified citrus pectin (MCP), Gal-3 inhibition did not influence collagen markers, echocardiographic measures, or vascular function.
- Baseline Gal-3 levels were higher in women compared with men.
- Consistent with previous studies, higher Gal-3 levels were associated with diabetes and reduced glomerular filtration rate.

SUMMARY

We investigated the effect of Galectin-3 inhibition with modified citrus pectin (MCP) on markers of collagen metabolism in a proof-of-concept randomized placebo-controlled trial of participants with elevated Gal-3 levels and hypertension. While higher Gal-3 levels were associated with female sex, diabetes, and reduced glomerular filtration rate in cross-sectional analyses, treatment with MCP did not change collagen markers. The effect of Gal-3 inhibition among individuals with HF warrants further investigation.

ABBREVIATIONS

A= late mitral inflow velocity on Doppler
AP= augmentation pressure
AIx= augmentation index
CITP= C-terminal telopeptide of type I collagen
DBP= diastolic blood pressure
DT= deceleration time of early mitral inflow on Doppler
E= early mitral inflow velocity on Doppler
E'= early mitral annular velocity on tissue Doppler
eGFR= estimated glomerular filtration rate
Gal-3= Galectin-3
FS= fractional shortening
LAD= left atrial end-systolic diameter
LVDD= left ventricular end-diastolic dimension
LVEF= left ventricular ejection fraction
LVM= left ventricular mass
LVSD= left ventricular end-systolic dimension
LVWT= left ventricular wall thickness
MCP= Modified citrus pectin
PICP= C-terminal propeptide of type I procollagen
PIIINP= C-terminal propeptide of type III procollagen
PWV= pulse-wave velocity
RWT= relative wall thickness
SBP= systolic blood pressure
TIMP-1/MMP-1= (tissue inhibitor of) matrix metalloproteinase type I
UACR= urine albumin to creatinine ratio

Key words: Galectin-3, Cardiac Fibrosis, Heart Failure

INTRODUCTION

Heart failure (HF) is an important public health concern, with a lifetime risk of 1 in 5 for both men and women at age 40 (1). HF development is often a clinically inconspicuous process, characterized by progressive cardiac remodeling that is not diagnosed until late in the disease course. Accordingly, most available therapies are implemented during the symptomatic phase of HF when extensive remodeling has already occurred with limited benefit, garnering considerable interest in treatment strategies that target individuals prior to the symptom onset. Indeed, in a prevention trial of patients with asymptomatic left ventricular (LV) dysfunction, treatment with enalapril was associated with a reduction in mortality that was sustained over 12 years (2,3).

Cardiac fibrosis, a pathologic phenomenon caused by numerous conditions including hypertension (HTN), ischemia, and aging, contributes to the pathophysiology of HF (4). Galectin-3 (Gal-3) is a beta-galactoside binding leptin that plays an important role in regulating inflammation and fibrosis. Gal-3 is known to be upregulated in a number of human fibrotic diseases including liver cirrhosis (5), pulmonary fibrosis (6), and most recently, cardiac fibrosis (7). We and others have previously shown that elevated circulating Gal-3 levels are associated with incident HF events and mortality in the community (8,9). Most recently, we found that longitudinal changes in Gal-3 were highly predictive of HF independent of baseline Gal-3 levels (10). Among individuals with existing HF, Gal-3 has been approved as a prognostic biomarker of HF and the recommendation that the measurement of Gal-3, alone or in a multi-marker strategy, may provide additional risk stratification (11).

Modified citrus pectin (MCP), a soluble dietary fiber found in citrus fruit, is a direct Gal-3 inhibitor via binding of the carbohydrate recognition domain of Gal-3 (12,13). In an animal model of acute kidney injury, MCP was shown to decrease Gal-3 expression and renal fibrosis

(14). While MCP has been tested in human clinical trials of solid tumors and lead intoxication (15-17), its role in attenuating cardiovascular disease (CVD) has not been previously investigated. In this context, we conducted a randomized placebo-controlled trial of MCP in patients with HTN and elevated Gal-3 levels to test our hypothesis that direct inhibition of Gal-3 may reduce subclinical cardiac fibrosis as assessed by biomarkers of collagen metabolism, echocardiographic measures of diastolic function, and vascular stiffness (**Visual Abstract**).

METHODS

Study Sample

We included individuals ages 21-70 years with physician-diagnosed hypertension, on stable therapy for at least 3 months, and elevated Gal-3 levels. Elevated Gal-3 was defined as ≥ 50^{th} sex-specific percentile (≥13.1 ng/mL in men, ≥14.3 ng/mL in women) as derived from normative values among participants of the Framingham Heart Study (9). Participants were screened from October 2013 to March 2018. Of this screening sample (n=275), we excluded participants with non-elevated Gal-3 (n=186), renal dysfunction defined as eGFR<45 mL/min/1.73m^2 (n=3), aldosterone antagonist use (n=1), abnormal laboratory values (i.e. hyperkalemia, anemia) (n=1), and those who withdrew participation after initial screening (n=16). The remaining participants (n=68) were eligible for randomization and subsequent follow-up as outlined below. The study was performed at the Massachusetts General Hospital and Boston University Medical Center. All participants provided written informed consent, and the study was approved by the appropriate Institutional Review Boards.

Study Procedures and Ascertainment of Outcomes

We randomly assigned 68 participants in double-blind fashion to receive either active MCP or matching placebo at a dose of 4.8 grams thrice daily for 6 months in a 1:1 ratio (clinicaltrials.gov NCT03349775). MCP and matching placebo were provided by EcoNugenics (Pectasol-C, Santa Rosa, CA) (**Figure 1**). Randomization was done separately for men and women and was performed in blocks of four to assure balanced sex representation and group sizes. Investigators and participants were blinded to study assignment.

Clinical Variables and Collagen Biomarkers

Medical history, physical examination, and fasting blood samples were obtained at baseline examination prior to randomization and again at final examination after 6 months of medication treatment. Blood serum was separated, aliquoted, and stored at -80C for further processing. Serum levels of PICP (EIA MicroVue CICP, Quidel Corporation, USA), PIIINP (RIA, Orion Diagnostica, Finland), CITP (RIA, Orion Diagnostica, Finland), and MMP-1 (alphaLISA, PerkinElmer, USA) were measured at a core laboratory (18). Assay performance characteristics are summarized in **Supplemental Table 1**. Measures of renal function (i.e. estimated glomerular filtration rate [eGFR] and urine albumin creatine ratio [UACR]) were obtained from fasting blood and urine samples at baseline and final examinations.

Arterial Tonometry Methods

Supine measurements of arterial stiffness were non-invasively assessed using applanation tonometry or volumetric displacement of the brachial artery using a cuff-based system (SphygmoCor XCEL or Sphygmocor CvMS, AtCor Medical, IL, USA) at baseline and final examinations (19,20). Central aortic pressure was derived using a generalized transfer function

applied to pressure tracings. Pulse wave analysis included ascertainment of augmentation pressure (AP; in mmHg) defined as the pressure the reflected wave contribution to systolic blood pressure, augmentation index (AIx) defined as ratio of AP to pulse pressure (PP), and AIx normalized to a heart rate of 75 bpm (Aix75). Carotid-femoral pulse-wave velocity (PWV; in meters/second) was obtained using ECG-gated pulse waveforms or cuff measurements (20). Pressure tracings and waveforms quality control metrics were applied including prespecified operator index and/or variability metrics. Intra-observer reproducibility measurements were performed on 16 randomly selected subjects, who underwent 2 separate pulse wave analysis measurements on the same day. The intra-class correlation coefficient for reported outcome variables was excellent and ranged between 93–94%.

Echocardiography

Participants underwent comprehensive transthoracic echocardiogram including two-dimensional, pulsed wave and tissue Doppler echocardiography using Vivid 7 and Vivid IQ ultrasonography systems (GE Healthcare, Milwaukee, WI) at baseline and final examinations. Standard measurements included cardiac dimensions, left ventricular ejection fraction (LVEF), fractional shortening, and diastolic function assessment as previously described (21). Left ventricular mass (LVM) was calculated as follows: 0.8(1.04[LVDD+LV posterior wall thickness + LV septal wall thickness]^3-LVEDD^3])+0.6 (22). Speckle tracking-based analyses of LV global longitudinal strain were performed offline with excellent reproducibility as previously described (2D Cardiac Performance Analysis v1.1; TomTec Imaging Systems; Unterschleissheim, Germany) (23,24). Primary measures obtained included global longitudinal strain of the apical 2- and 4-chamber views. Echocardiography images were analyzed by

observers blinded to participant treatment randomization, and measurements were made in triplicate and averaged whenever possible. The interobserver within-subject coefficients of variation for longitudinal strain measures ranged from 3.0 to 4.0% (24).

Statistical Analysis

Baseline clinical characteristics are presented by study group assignment as means ± standard deviations (SD) or percentages. Non-normally distributed variables including biomarkers were natural log-transformed due to right-skewed distributions. Galectin-3 values were standardized.

In cross-sectional analyses, we examined the association of Gal-3 with clinical characteristics, arterial stiffness, and renal function among all participants who attended the screening visit (n=275). We applied a log-normal generalized linear model for continuous outcomes and a logistic model for dichotomous outcomes. We adjusted for age, sex, body mass index (BMI), diabetes (DM), systolic blood pressure (SBP), and eGFR for cross-sectional analyses and further adjusted for heart rate in analyses of arterial tonometry measures.

A total of 52 individuals had complete biomarker data at baseline and 6-month follow-up and were included in primary analyses. We examined changes in log-transformed collagen metabolism biomarkers (PICP, PIIINP, CITP, MMP-1, and CITP:MMP-1 ratio) between baseline and final examination in both MCP and placebo treatment groups using paired t-tests. The change from baseline was compared between MCP and placebo groups using unpaired paired t-tests. In secondary analyses, we used paired t-tests to examine changes in Gal-3, renal function, vascular stiffness, and echocardiographic measures of strain, elastance, and diastolic

function in both treatment groups. Group differences were also assessed with using unpaired t-tests.

For primary analyses comparing between-group differences of collagen biomarkers between MCP and placebo treatment groups, a Bonferroni-corrected p-value threshold of P=0.0125 (0.05/4 collagen biomarkers). All other analyses were exploratory and considered statistically significant at a two-sided p-value <0.05. Data analyses were completed in SAS version 9.4 (SAS Institute Inc., Cary, NC), R version 3.6.0 and Rstudio (Boston, MA).

RESULTS

A total of 275 participants attended the screening visit (mean age 55±10, 59% women). In addition to hypertension, cardiovascular comorbidities included 24% with diabetes mellitus, 53% with obesity (BMI ≥30 kg/m^2), <1% with atrial fibrillation, and 8% current smokers (**Table 1** for clinical characteristics). The average Gal-3 level was 13.0±4.7 ng/mL in women, and 12.2±3.6 ng/mL in men, with 31% of both men and women meeting Gal-3 cut-point criteria for inclusion in the trial.

Clinical correlates of galectin-3 in screening sample

In cross-sectional analyses (n=275), we examined the association of Gal-3 with clinical characteristics, renal function, arterial stiffness, echocardiographic parameters, and collagen markers (**Supplemental Tables 2 & 3**). After multivariable adjustment, clinical predictors of circulating Gal-3 levels included presence of diabetes mellitus and eGFR. Specifically, the presence of diabetes was associated with a 0.45-SD increase in Gal-3 level (ß 0.445, SE 0.137, p=0.001) and a 1-SD increase in eGFR was associated with a 0.33-SD decrease in Gal-3 level (ß -0.326, SE 0.064, p=6.02 x 10^{-7}, **Supplemental Table 2**, **Figure 2**).

When examining the association of Gal-3 levels with arterial stiffness, echocardiographic parameters and collagen markers, we found no significant associations (**Supplemental Tables 2 & 3**).

Randomized trial of galectin-3 inhibitor modified citrus pectin (MCP) vs placebo

A total of 68 participants met enrollment criteria and were randomized to the Gal-3 inhibitor MCP vs matching placebo for a total duration of 6 months. Of randomized participants, 52 completed the final study visit: 22 in MCP treatment group and 30 in placebo group (**Figure 1**). Treatment groups were similar in age and sex (mean age 57±8, 50% women for MCP group, mean age 54±8, 53% women for placebo group), though the placebo group appeared to be heavier (BMI 33.5±6.5 for placebo group, BMI 30.0±5.9 for MCP group). Most common side effects included diarrhea (6 individuals [27%] of MCP treatment group vs. 7 individuals [23%] of placebo group), constipation (3 individuals [14%] of MCP treatment group vs. 8 individuals [27%] of placebo group), and flatulence (6 individuals [27%] of MCP treatment group vs. 6 individuals [20%] of placebo group). Less common side effects included nausea (1 individual in MCP treatment group) and asthma flare (1 individual in placebo group).

Effect of galectin-3 inhibition on collagen markers

In the primary trial analyses, levels of the collagen metabolism marker PIIINP increased by 0.15 log transformed units (p=0.02) after 6 months of treatment with MCP compared with no significant change in the placebo group (p=0.47) (**Table 2**; **Figure 3**). There was a borderline statistically significant between-group difference in the change in PIIINP levels (p=0.05). There

were no other differences comparing baseline to post-treatment collagen biomarkers (PICP, CITP, and MMP-1) after 6 months of treatment regardless of treatment assignment (**Table 2**).

Effect of galectin-3 inhibition on secondary endpoints

In secondary analyses, changes in Gal-3, renal function, as quantified by eGFR, creatinine, and UACR, and vascular stiffness, as quantified by AP, Aix, and PWV, did not meet statistical significance in either treatment group (**Table 2**). While measures of vascular stiffness in the MCP treatment group were lower at the end of the trial compared with unchanged or worse measures in the placebo arm, none of these differences were statistically significant. Echocardiographic parameters of cardiac structure and function are summarized in **Table 2**. LV mass index was lower after 6 months of MCP therapy (difference -5.47, SE 2.19 g/m^2, p=0.02) with no change in the placebo group. Similarly, mitral inflow deceleration time increased by 21 msec (SE 38.0, SE 8.71, p=0.02) in the MCP group without significant change in the placebo group. Between-group differences, however, were not significant. Other measures of diastolic function including mitral E/e' ratio as well as cardiac mechanics as measured by global longitudinal strain did not change in either treatment group.

DISCUSSION

We present the results of the first randomized trial of MCP, a direct Gal-3 inhibitor, in human subjects with HTN and elevated Gal-3 levels for the prevention of subclinical cardiac fibrosis. Our findings are three-fold. First, baseline Gal-3 levels were significantly higher in women compared with men. Second, in cross-sectional analyses, DM and reduced eGFR were independent predictors of Gal-3 level. Third, treatment with MCP compared with placebo did not significantly influence collagen biomarker levels, echocardiographic markers of diastolic function, or arterial stiffness. Taken together, our study confirms previous associations of Gal-3 with diabetes and eGFR, but did not find attenuation of subclinical measures of cardiovascular fibrosis with direct Gal-3 inhibition.

In our sample of 275 eligible participants, we confirm previous observations including significantly higher baseline levels of Gal-3 in women compared with men, and significant cross-sectional association of Gal-3 with diabetes and an inverse association with eGFR We previously showed that in 2477 individuals in the community, women had greater increases in Gal-3 over time compared with men (10), and in a study of Gal-3 in 1650 participants with symptomatic HF, female sex was independently predictive of both baseline circulating Gal-3 levels and change in Gal-3 levels at 4 months (25). Interestingly, female sex was not independently associated with Gal-3 levels after multivariable adjustment in our study, highlighting the potential role of comorbidities, including DM and CKD, in modulating expression of Gal-3 in women preferentially compared with men. Indeed, previous studies have demonstrated that cardiovascular risk factors including age, DM and BMI are all associated with both Gal-3 levels and rise in Gal-3 over time in healthy individuals free of HF and in individuals with symptomatic HF (9,25). Renal dysfunction has been most strongly associated with Gal-3 in epidemiologic

studies of both healthy and HF individuals. In prior analyses examining the association of Gal-3 with incident HF events, adjustment for eGFR attenuated the association of Gal-3 with outcomes (9,10,25). We again show a strong inverse relationship between Gal-3 and eGFR, further validating the importance of Gal-3 with kidney function and highlighting the need to consider renal function when evaluating the association of Gal-3 with HF risk.

Beyond clinical risk factors, we further examined the associations of Gal-3 with vascular parameters, echocardiographic measures, and collagen turnover biomarkers, but did not find meaningful associations contrary to expectation. Two prior studies found increased arterial stiffness, as measured by PWV, with higher levels of Gal-3 (26,27) and a study of 115 patients presenting to the ED with acute dyspnea found that higher Gal-3 levels were significantly associated with echocardiographic markers of LV filling and diastolic function, valvular regurgitation, and RV function (28). We postulate that our neutral results may be related to the restricted sample size or the nature of our study sample, an ostensibly healthy sample with HTN that has not yet developed echocardiographic evidence of remodeling.

In our primary analysis, we compared differences in collagen turnover markers in 22 individuals randomized to treatment with MCP and 30 randomized to placebo. In contrast to our hypothesis that Gal-3 inhibition would reduce collagen turnover, we did not find significant differences in collagen markers between the active therapy and placebo arms. We observed a borderline statistically significant between-group difference in the change in PIIINP, although this was driven primarily by an increase in PIIINP in the MCP treatment group. While we did not find changes in markers of collagen metabolism, it is worth describing the motivation for this interventional trial. Circulating biomarkers of collagen metabolism have been well correlated with cardiac fibrosis in hypertensive heart disease and HF (29,30). Prior studies have

demonstrated a direct correlation between circulating markers of collagen turnover, histological myocardial fibrosis, and LV chamber stiffness (31). Gal-3 is expressed in activated macrophages with binding sites localized to the myocardial extracellular matrix and cardiac fibroblasts, and is thought to promote collagen deposition via activation of cardiac fibroblasts (7). As such, Gal-3 has been previously correlated with markers of collagen metabolism, specifically TIMP-1, in healthy and hypertensive subjects (32-35). Previous studies have found decreases in collagen markers with antihypertensive therapy in patients with HTN. Collectively, these observations were the driving rationale for this intervention study that interrogated the impact of direct Gal-3 inhibition on circulating markers of collagen metabolism, surrogate markers of cardiac fibrosis. The one borderline finding of increased PIIINP with MCP treatment is surprising. PIIINP, like Gal-3, is a biomarker of cardiac fibrosis and has been associated with HF prognosis. In a study of 63 patients with stable CAD, elevated serum levels of Gal-3 were associated with cardiac fibrosis assessed by late gadolinium enhancement (LGE) by cardiac magnetic resonance imaging as well as impaired LV diastolic function while PIIINP was associated with diastolic dysfunction but not LGE, suggesting that elevated levels of PIIINP may reflect earlier stages of disease progression compared with Gal-3 (36). While our findings should not be overinterpreted, it warrants further investigation into the relationship between Gal-3 inhibition and PIIINP. Finally, the present investigation sought to modulate the Gal-3 pathway in individuals with elevated Gal-3 levels, but had not yet developed irreversible cardiovascular remodeling or fibrosis. To that end, we enrolled individuals with modest elevations in Gal-3 levels ($\geq 50^{th}$ sex-specific percentile) based on prior population data (9). It is conceivable that treatment with MCP may have demonstrated greater clinical benefit in individuals with higher baseline Gal-3 levels or more advanced fibrosis.

Finally, we also report neutral results for our exploratory analyses examining the effect of Gal-3 inhibition on vascular function and cardiac diastolic function. Of interest, while the differences in vascular stiffness were not statistically different between the MCP therapy and placebo arms, the absolute reduction in vascular stiffness measures, in particular AP and AIx, were greater in the MCP group. Separately, there was a borderline but non-significant difference in LV mass index between the MCP and placebo groups. This result must be cautiously interpreted given its lack of significance but does argue for further exploration.

The present study has several limitations worth mentioning. First, there was significant attrition bias, as premature discontinuation of study drug was significantly higher in the MCP arm compared with placebo due to gastrointestinal side effects. As such, adherence to treatment at 6 months was 65% in the MCP arm compared with 88% in the placebo group. Second, owing to significant attrition, the sample size was inherently reduced, potentially limiting power to detect meaningful differences between the MCP and placebo groups. Moreover, after premature discontinuation of study drug, baseline characteristics were no longer balanced between intervention and placebo arms, potentially confounding interpretation of trial results. Finally, due to variable gastrointestinal MCP absorption related to oral administration, participants were instructed to take MCP a half hour before or two hours after food for optimal absorption.

In summary, we present the results of the first proof-of-concept interventional trial of Gal-3 inhibition for the attenuation of cardiac fibrosis in human subjects. We demonstrate higher baseline Gal-3 levels in women compared with men, and further validate previous associations of Gal-3 with clinical risk factors including DM and reduced eGFR. Gal-3 inhibition via MCP did not influence surrogate measures of cardiac fibrosis including collagen turnover markers, echocardiographic measures, and vascular function in human subjects at risk for HF. Whether

Gal-3 inhibition among individuals with existing HF may meaningfully attenuate cardiac fibrosis and ultimately, clinical CV events remains to be studied.

CLINICAL PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE

In the first proof-of-concept interventional trial of Gal-3 inhibition in human subjects with HTN, Gal-3 inhibition with MCP did not influence collagen markers, echocardiographic measures, or vascular function. However, we showed that baseline Gal-3 levels were higher in women compared with men, and confirmed previous associations of Gal-3 with diabetes and reduced eGFR.

TRANSLATIONAL OUTLOOK

Future studies are needed to examine whether Gal-3 inhibition can attenuate cardiac fibrosis and cardiovascular events in individuals with HF.

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Figure Legends

Visual Abstract. Randomized placebo-controlled trial of Galectin-3 inhibition with modified citrus pectin in patients with HTN and elevated Gal-3. Abbreviations: GAL=3 = galectin-3.

Figure 1. Consort Diagram. Flow chart shows the number of patients who were screened, randomized into the treatment groups, completed the study, and included in the final analysis.

Figure 2. Cross-sectional correlation of galectin-3 with eGFR. Analyses are adjusted for age and sex. Abbreviations: eGFR = estimated glomerular filtration rate.

Figure 3. Difference in collagen metabolism biomarkers pre- and post-MCP. Boxes show interquartile ranges and bars represent 25th and 75^{th} percentile values. Dots represent outliers. Squares represent the means. * indicates $p<0.05$.

Table 1. Baseline Demographic and Clinical Characteristics

	Screening Sample	Randomized Controlled Trial Sample	
	N=275	MCP, N=22	Placebo, N=30
Age, years	55 (10)	57 (8)	54 (8)
Men, n (%)	112 (41%)	11 (50%)	14 (47%)
Race: White, n (%)	128 (48%)	8 (36%)	18 (60%)
Black, n (%)	108 (41%)	13 (59%)	11 (37%)
Body mass index, kg/m^2	31 (14)	30.0 (5.9)	33.5 (6.5)
Obesity, n (%)	147 (53%)	9 (41%)	21 (70%)
Systolic blood pressure, mmHg	128 (14)	126 (12)	129 (10)
Diastolic blood pressure, mmHg	80 (9)	78 (11)	81 (9)
Heart rate, bpm	70 (3)	73 (18)	74 (11)
Hypertensive treatment, n (%)	373 (99%)	22 (100%)	30 (100%)
Diabetes mellitus, n (%)	65 (24%)	5 (23%)	12 (40%)
Current smokers, n (%)	21 (8%)	6 (27%)	0 (0%)
Laboratory Parameters			
Galectin-3, mg/dL	12.7 (4.3)	17.9 (3.5)	17.5 (4.1)
Estimated GFR, $mL/min/1.73m^2$	94.1 (18.2)	92.2 (12.9)	88.3 (16.4)
Creatinine, mg/dL	0.87 (0.25)	0.89 (0.14)	0.91 (0.17)
Hematocrit, %	41.3 (3.4)	42.5 (3.5)	41.9 (3.4)
Arterial Tonometry Parameters			
Augmentation pressure, mmHg	11.6 (7.1)	11.7 (2.1)	8.9 (2.0)
Augmentation index, %	28.2 (11.8)	29.1 (10.8)	23.8 (10.3)
Pulse-wave velocity, m/sec	11.5 (39)	9.3 (2.1)	8.9 (2.0)

Values are means (standard deviations) or counts (percentages) unless otherwise noted. Abbreviations: GFR = glomerular filtration rate

*P-value for comparison of MCP vs placebo groups

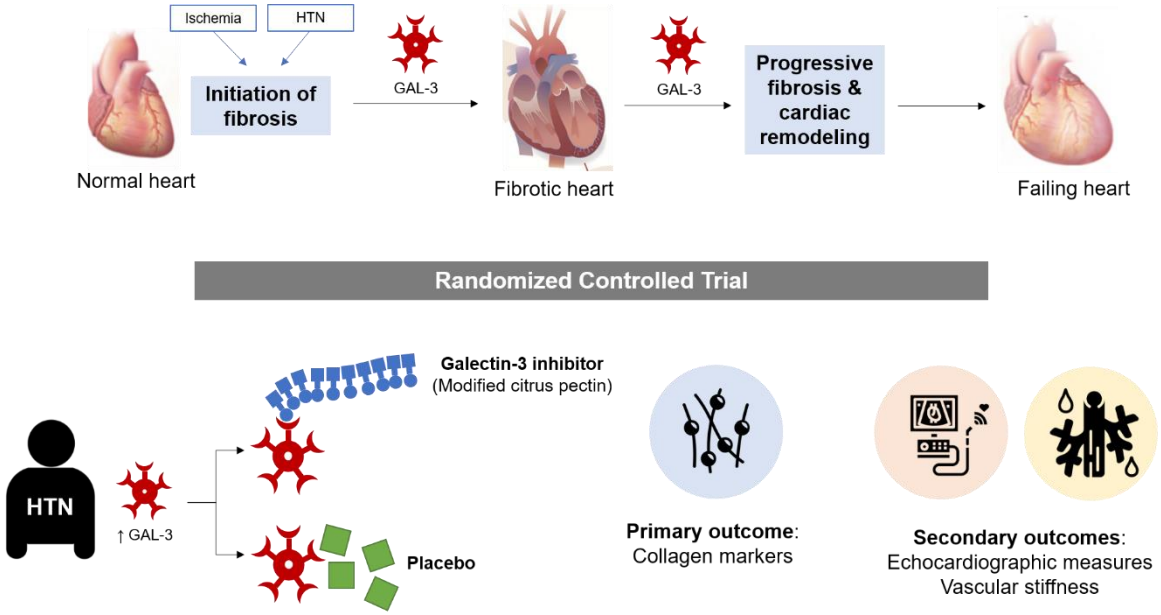
Table 2. Effect of galectin-3 inhibition on clinical and laboratory parameters, arterial tonometry measures, collagen markers, and echocardiographic measures

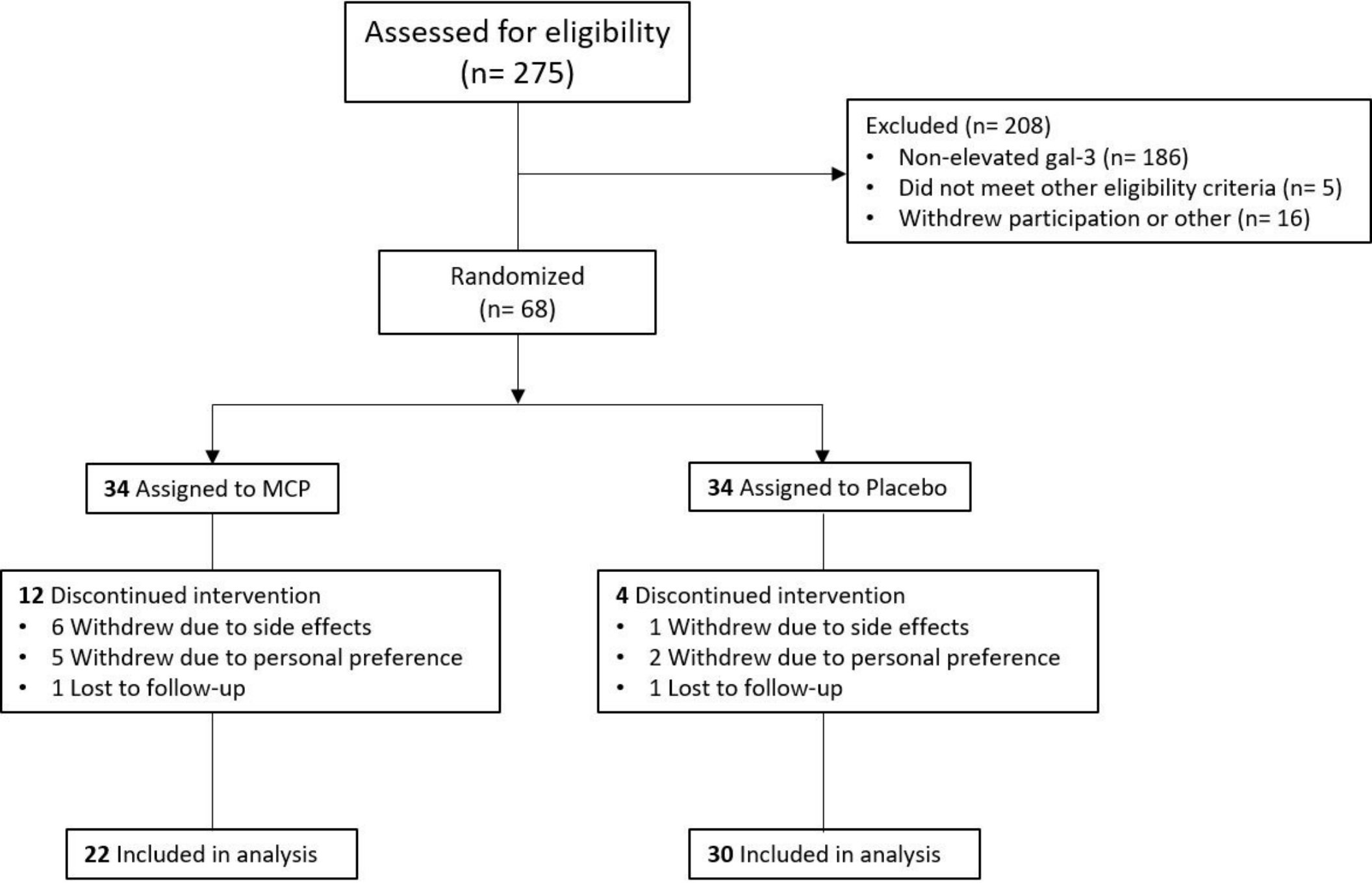
	Placebo			MCP			Between-group difference p-value
	Pre	**Post**	**Difference**	**Pre**	**Post**	**Difference**	
Clinical Parameters							
BMI, kg/m^2	33.5 (6.5)	33.6 (6.6)	0.13	30.0 (5.9)	30.3 (5.5)	0.06	0.88
Systolic BP, mmHg	129 (10)	127 (15)	-1.50	126 (12)	128 (15)	1.83	0.50
Diastolic BP, mmHg	81 (9)	78 (9)	-2.86	78 (11)	81 (11)	1.60	0.14
Heart rate, bpm	74 (11)	76 (12)	1.21	73 (18)	74 (11)	2.70	0.74
Laboratory Values							
Galectin-3, mg/dL	17.6 (4.1)	17.1 (10.3)	-0.48	17.9 (3.5)	17.9 (6.8)	0.01	0.81
eGFR, mL/min/1.73m^2	89.2 (16.0)	89.4 (15.1)	0.29	91.7 (13.0)	89.9 (14.6)	-1.88	0.51
Creatinine, mg/dL	0.91 (0.17)	0.90 (0.20)	-0.01	0.89 (0.14)	0.90 (0.10)	0.02	0.61
UACR, mg/g	24.7 (44.6)	18.1 (32.2)	-7.84	17.7 (26.0)	19.8 (32.7)	-0.49	0.48
Arterial Tonometry Measures							
AP, mmHg	8.9 (2.0)	10.0 (7.3)	1.07	11.7 (2.1)	10.6 (5.9)	-1.09	0.13
AIx, %	23.8 (10.3)	23.3 (14.8)	-0.59	29.1 (10.8)	25.9 (11.7)	-3.27	0.36
PWV, m/s	8.9 (2.0)	8.8 (2.2)	0.00	9.3 (2.1)	8.7 (1.9)	-0.50	0.32
Collagen Markers*							
PiCP, ng/mL	4.37 (0.39)	4.30 (0.30)	-0.07	4.29 (0.52)	4.35 (0.54)	0.06	0.10
CITP, ng/mL	1.20 (0.34)	1.23 (0.40)	0.03	1.15 (0.35)	1.16 (0.41)	0.01	0.73
PIIINP, ng/mL	1.38 (0.40)	1.32 (0.47)	-0.06	1.40 (0.37)	1.53 (0.33)	0.15	0.054
MMP, ng/mL	2.09 (0.72)	2.03 (0.81)	-0.06	2.08 (0.60)	2.04 (0.52)	-0.04	0.76

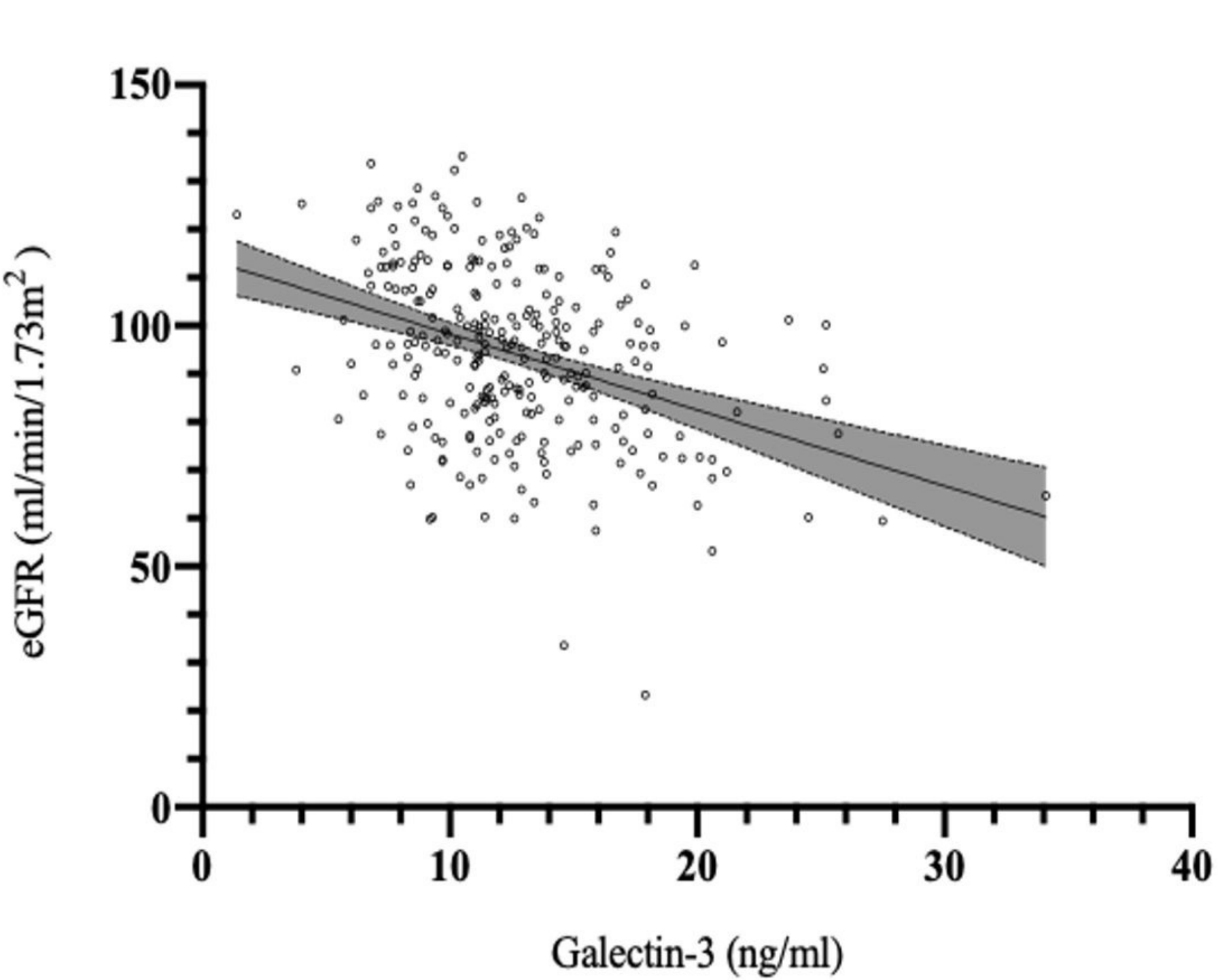
CITP:MMP Ratio	0.36 (0.82)	0.46 (0.88)	-0.09	0.32 (0.64)	0.37 (0.68)	-0.05	0.70
Echocardiography Measures							
LV end diastolic dimension, cm	4.66 (0.44)	4.62 (0.48)	-0.04	4.52 (0.36)	4.42 (0.42)	-0.10	0.57
LV wall thickness, cm	2.03 (0.27)	2.06 (0.25)	0.04	1.97 (0.26)	1.93 (0.25)	-0.04	0.23
LA diameter, cm	3.79 (0.83)	3.74 (0.39)	-0.05	3.50 (0.49)	3.62 (0.33)	0.12	0.12
LV mass index, g/m^2	78.8 (16.60)	79.7 (17.1)	0.91	76.7 (18.1)	71.2 (16.1)	-5.47	0.08
Fractional Shortening, %	42.5 (7.50)	41.5 (6.17)	-0.93	41.80 (6.55)	38.3 (6.51)	-3.50	0.26
DT, ms	215.1 (34.6)	223.4 (37.6)	8.27	207.9 (29.1)	229.3 (32.4)	21.38	0.28
E/a Ratio	1.04 (0.29)	0.99 (0.30)	-0.05	1.20 (0.68)	0.97 (0.27)	-0.24	0.20
E Prime Ratio	8.95 (3.54)	8.86 (2.77)	-0.09	8.54 (2.10)	8.23 (2.70)	-0.31	0.74
EndoGLS, % ^	-17.5 (3.20)	-17.4 (3.35)	-0.16	-18.5 (5.65)	-17.8 (3.67)	0.80	0.23

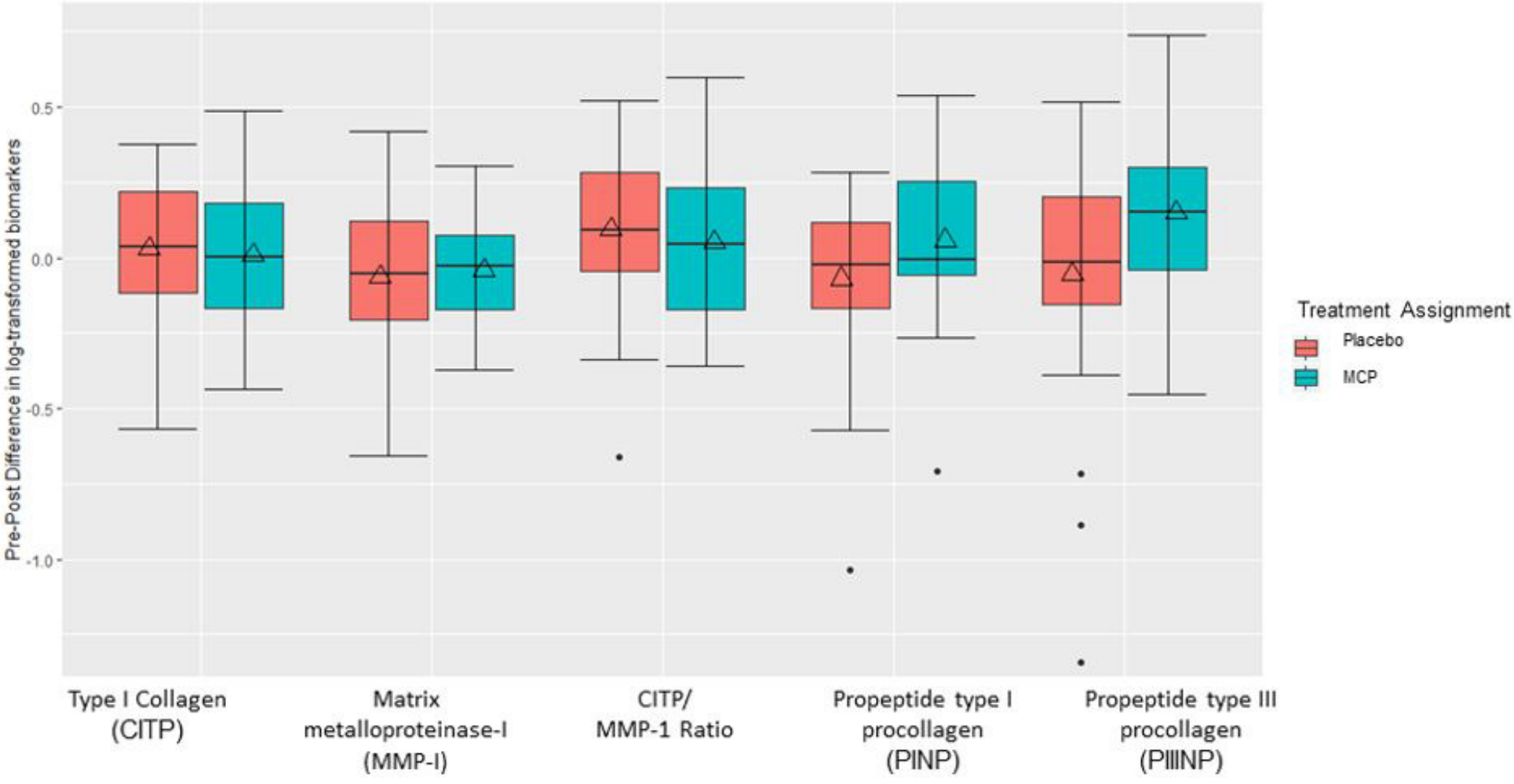
Values are means (standard deviations). *Collagen markers are log-transformed. ^Average of Apical 2 and Apical 4 global longitudinal strain. Abbreviations: AIx = augmentation index, AP = augmentation pressure, BMI = body mass index, CITP = C-terminal telopeptide of type I collagen, DT = deceleration time, EndoGLS = endocardial global longitudinal strain, eGFR = estimated glomerular filtration rate, PICP= C-terminal propeptide of type I procollagen, PIIINP = C-terminal propeptide of type III procollagen, PWV = pulse-wave velocity, TIMP-1/MMP-1 = (tissue inhibitor of) matrix metalloproteinase type 1, UACR = urine albumin to creatinine ratio

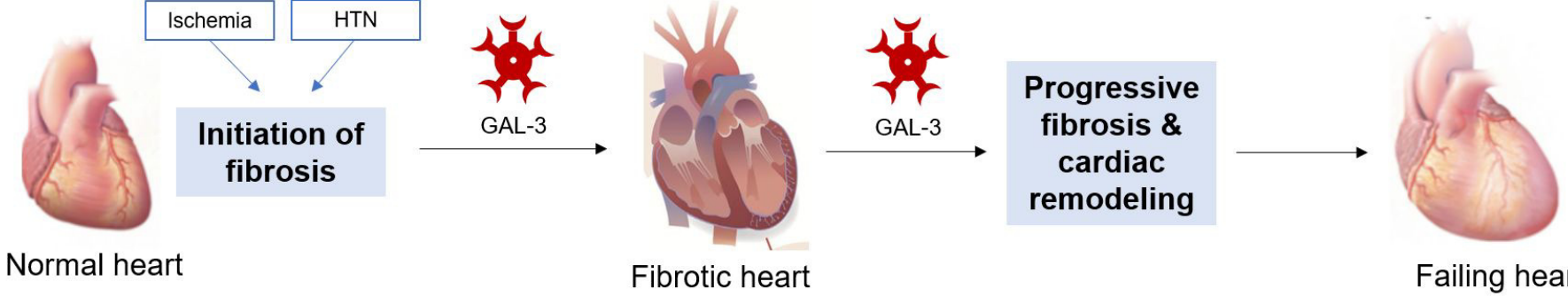
Visual Abstract



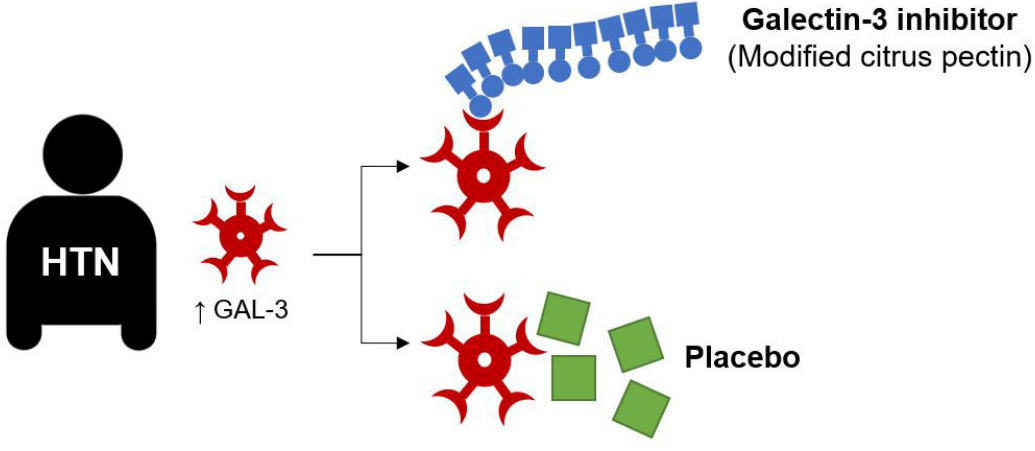

Assessed for eligibility
(n= 275)
Excluded (n= 208)
• Non-elevated gal-3 (n= 186)
• Did not meet other eligibility criteria (n= 5)
• Withdrew participation or other (n= 16)
Randomized
(n= 68)
34 Assigned to MCP
34 Assigned to Placebo
12 Discontinued intervention
• 6 Withdrew due to side effects
• 5 Withdrew due to personal preference
• 1 Lost to follow-up
4 Discontinued intervention
• 1 Withdrew due to side effects
• 2 Withdrew due to personal preference
• 1 Lost to follow-up
22 Included in analysis
30 Included in analysis


Galectin-3 and eGFR
150
100
50
0
eGFR (ml/min/1.73m2)
0
10
20
30
40
Galectin-3 (ng/ml)


Pre-Post Difference in log-transformed biomarkers
0.5
0.0
-0.5
-1.0
Type I Collagen (CITP)
Matrix metalloproteinase-I (MMP-I)
CITP/ MMP-1 Ratio
Propeptide type I procollagen (PINP)
Propeptide type III procollagen (PIIINP)
Treatment Assignment
Placebo
MCP



Randomized Controlled Trial



Primary outcome:
Collagen markers

Secondary outcomes:
Echocardiographic measures
Vascular stiffness