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Brief title: Canagliflozin effects on CV biomarkers

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ABSTRACT

Background: Sodium glucose co-transporter 2 (SGLT2) inhibitors may reduce cardiovascular and heart failure risk in patients with type 2 diabetes mellitus (T2DM).

Objectives: To examine the effects of canagliflozin on cardiovascular biomarkers in older patients with T2DM.

Methods: In 666 T2DM patients randomized to receive canagliflozin 100 or 300 mg or placebo, we assessed median percent change in serum N-terminal pro-B type natriuretic peptide (NT-proBNP), high-sensitivity troponin I (hsTnI) , soluble (s)ST2, and galectin-3 from baseline to 26, 52, and 104 weeks.

Results: Both serum NT-proBNP and serum hsTnI levels increased in placebo recipients but remained largely unchanged in those randomized to canagliflozin. Hodges-Lehmann estimates of the difference in median percent change between pooled canagliflozin and placebo were –15.0%, –16.1%, and –26.8% for NT-proBNP, and –8.3%, –11.9%, and –10.0% for hsTnI at weeks 26, 52, and 104, respectively (all P <0.05). Serum sST2 was unchanged with canagliflozin and placebo over 104 weeks. Serum galectin-3 modestly increased from baseline with canagliflozin versus placebo, with significant differences observed at 26 and 52 weeks but not at 104 weeks. These results remained unchanged when only patients with complete samples were assessed.

Conclusions: Compared to placebo, treatment with canagliflozin delayed rise in serum NT-proBNP and hsTnI over 2 years in older T2DM patients. These cardiac biomarker data provide support for beneficial cardiovascular effect of SGLT2 inhibitors in T2DM.

CLINICAL TRIAL: ClinicalTrials.gov identifier: NCT01106651

KEY WORDS: sodium glucose co-transporter 2 inhibitor, cardiovascular stress, N-terminal pro-B type natriuretic peptide, high-sensitivity troponin, soluble ST2, galectin-3

CONDENSED ABSTRACT

Among 666 older patients with type 2 diabetes mellitus (T2DM) treated with canagliflozin or placebo, we measured serum concentrations of the prognostically important biomarkers NT-proBNP, hsTnI, sST2, and galectin-3 at baseline and 26, 52, and 104 weeks. Over time, NT-proBNP and hsTnI concentrations progressively increased with placebo but remained largely unchanged with canagliflozin. sST2 was unchanged with canagliflozin and placebo; galectin-3 concentrations modestly, but not persistently, increased with canagliflozin, possibly related to transient changes in kidney function. We conclude that canagliflozin delayed 2-year rise in NT-proBNP and hsTnI in T2DM patients, which may reflect beneficial cardiovascular effect of SGLT2 inhibitors.

ABBREVIATIONS LIST

ACE = angiotensin-converting enzyme
ARB = angiotensin receptor blocker
BMI = body mass index
BP = blood pressure
eGFR = estimated glomerular filtration rate
hsTnI = high-sensitivity troponin I
MACE = major adverse cardiovascular events

NT-proBNP = N-terminal pro-B type natriuretic peptide
SGLT2 = sodium glucose co-transporter 2
T2DM = type 2 diabetes mellitus

Sodium glucose co-transporter 2 (SGLT2) inhibitors are a new class of diabetes drugs that lower blood glucose in patients with type 2 diabetes mellitus (T2DM) through increased urinary excretion of glucose (1). SGLT2 inhibitors may have other cardiometabolic benefits; they cause natriuresis, a mild osmotic diuresis, and a net caloric loss that contribute to reductions in body weight and blood pressure (BP) (1). Additionally, increased delivery of sodium to the macula densa helps to restore normal glomerular pressure, which in turn results in improved renal function over the longer term (2).

SGLT2 inhibitors have recently been studied in large cardiovascular outcomes trials for evaluating the cardiovascular effects of newer T2DM agents (3). In the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME), treatment with empagliflozin resulted in reduction in the risk for major adverse cardiovascular events (3-point MACE; cardiovascular death, nonfatal stroke, and nonfatal myocardial infarction) compared with placebo, driven by a 38% reduction in cardiovascular death; empagliflozin also reduced the risk of hospitalization for heart failure by 35% relative to placebo (4). These effects were apparent early after initiating treatment with empagliflozin, suggesting that acute changes may be at least partially responsible for the observed outcomes (4). Hypotheses regarding the mechanism of cardiovascular benefit for SGLT2 inhibition observed in the EMPA-REG OUTCOME study have focused on the multiple effects beyond glucose lowering, such as diuresis and natriuresis, weight loss, BP lowering, metabolic effects on the myocardium, favorable hemodynamic changes and attenuation of cardiac remodeling (5-12); each may result in improved cardiovascular outcomes (11).

Biomarkers are useful in prognosis determination and informing the mechanism of benefit provided by therapeutic agents (13). N-terminal pro-B type natriuretic peptide (NT-

proBNP) is recommended for the diagnosis and management of heart failure, with potential utility in the prediction of coronary heart disease and stroke outcomes (14). Similarly, biomarkers of cardiomyocyte injury (e.g., high-sensitivity troponin I [hsTnI]) and those involved in cardiovascular stress/tissue fibrosis (e.g., soluble [s]ST2, galectin-3) may help elucidate prognosis and disease progression, with recent data in particular for hsTnI in T2DM (15).

There are very limited data on the effects of SGLT2 inhibitors on cardiovascular biomarkers (16-18). In this study, we sought to assess the longitudinal changes in the concentrations of NT-proBNP, hsTnI, sST2, and galectin-3 in older patients with T2DM randomized to receive canagliflozin or placebo in a 104-week study (19,20) to gain insights into the mechanisms of the potential beneficial cardiovascular effect of SGLT2 inhibitors.

METHODS

Patients

This post hoc, exploratory analysis was conducted using stored serum samples from a 104-week, randomized, double-blind, placebo-controlled study (ClinicalTrials.gov identifier: NCT01106651) that evaluated the efficacy and safety of canagliflozin 100 and 300 mg in older patients with T2DM. Full study design and key inclusion/exclusion criteria have previously been reported (19,20). Briefly, eligible patients were adults with T2DM who were 55 to 80 years of age, had HbA1c ≥7.0% and ≤10.0% and estimated glomerular filtration rate (eGFR) ≥50 mL/min/1.73 m^2, and were either not on any antihyperglycemic agent or were on a stable regimen of monotherapy or combination therapy. Patients with a history of myocardial infarction, unstable angina, previous coronary revascularization, cerebrovascular accident within 3 months before screening, history of New York Heart Association class III–IV symptoms, or uncontrolled hypertension were not eligible to participate. This study was conducted in

accordance with the ethical principles outlined in the Declaration of Helsinki and followed Good Clinical Practice and applicable regulatory requirements. Approval was obtained from institutional review boards and independent ethics committees for each participating center. Participants provided informed written consent prior to enrollment in the study.

Endpoints/Assessments

Serum samples were collected at baseline and at weeks 26, 52, and 104 and stored at −80°C. NT-proBNP was measured on the cobas e601 immunoanalyzer using the proBNP II electrochemiluminescent immunoassay (Roche Diagnostics, Indianapolis, IN), with interassay coefficients of variation (CVs) of 2.5% at 137.2 pg/mL (low quality control concentration) and 2.3% at 4,830 pg/mL (high quality control concentration). hsTnI and galectin-3 were measured on the Architect i2000SR immunoanalyzer using chemiluminescent microparticle immunoassays (Abbott Laboratories, Abbott Park, IL). CVs were 4.0% at 20.4 ng/L and 3.7% at 15,050 ng/L for hsTnI, and 4.0% at 9.3 ng/mL and 2.9% at 74.4 ng/mL for galectin-3. sST2 was measured using a sandwich monoclonal enzyme-linked immunosorbent assay (Critical Diagnostics, San Diego, CA) and the CVs were 7.6% at 28.2 ng/mL and 7.5% at 60.0 ng/mL. For each assay, all samples were run in a blinded fashion and at the same period, thereby minimizing interassay variations.

To understand secular trends in biomarkers as a function of treatment allocation, absolute and percent change from baseline in serum levels of NT-proBNP, hsTnI, sST2, galectin-3, eGFR, and hematocrit were analyzed at each time point for patients with data at baseline and at any follow-up time point thereafter. Given the non-normality of these biomarker data including change and percent change from visit to visit, the medians of the change and percent change were analyzed. Data for the 2 canagliflozin doses were pooled after it was determined that there

was no dose response observed on any of the biomarkers. A sensitivity analysis was also performed to evaluate absolute and percent change from baseline in biomarkers in the cohort of patients with complete sets of samples (i.e., data available at all visits, including baseline and weeks 26, 52, and 104).

Statistical Analyses

Nonparametric Hodges-Lehmann estimates of the difference between canagliflozin and placebo in median change and median percent changes from baseline were calculated for each biomarker at each time point. The distribution-free confidence intervals (CIs) and nominal *P* values for the differences in the median and median percent changes were based on the Wilcoxon rank sum test (21). Standard error (SE) for the median and median percent change at each time point was estimated using the bootstrap technique by simulated repeated samples for each biomarker and treatment group. Spearman correlation coefficients between change from baseline in the specific biomarker and change from baseline in selected clinical parameters (ie, HbA1c, body weight, systolic BP, hemoglobin, hematocrit, eGFR) were determined within each treatment group at each time point.

RESULTS

Patients

Of 714 patients in the overall study population, 666 patients (93.3%) had serum samples at baseline and ≥1 post-baseline follow-up time point and were included in this analysis. Among patients included in the biomarker assessments, baseline characteristics were balanced between groups and were generally consistent with the overall study population (**Table 1**); 77% had a history of hypertension and 30% had a history of microvascular disease (ie, neuropathy, retinopathy, or nephropathy). The majority of patients (74%) were taking an angiotensin-

converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB); 25%, 23%, and 34% of patients were on beta-blockers, calcium channel blockers, and diuretics, respectively (**Table 1**). Of those taking diuretics, the majority took thiazides (27.8% in the placebo arm and 27.1% in the canagliflozin arm), while loop diuretics (4.6% and 3.5%) or mineralocorticoid receptor antagonists (0.5% and 3.1%) were less commonly used. During the course of the study, no changes in electrocardiographic parameters, such as PR interval, QRS interval, QT/QTc, or RR intervals, were noted between treatment groups (data not shown).

Biomarker Changes

Table 2 summarizes the observed changes in serum NT-proBNP, hsTnI, sST2, galectin-3, eGFR, and hematocrit at all time points. From a baseline median of approximately 45 pg/mL, serum NT-proBNP concentrations increased with placebo but changed only minimally with canagliflozin over the 2-year study period (**Figure 1A**). Hodges-Lehmann estimates (95% CI) of the difference in median percent change between canagliflozin and placebo at weeks 26, 52, and 104 were –15.0% (–27.4, –3.3), –16.1% (–28.8, –3.8), and –26.8% (–42.3, –10.7), respectively. A between-group treatment effect was observed at 26 weeks and persisted over 104 weeks (nominal P <0.05 at weeks 26 and 52, nominal P <0.01 at week 104). Considering the relationship between baseline and 104-week concentrations of NT-proBNP (**Online Figure 1A**), a lower slope from baseline to final measurement was observed in those treated with canagliflozin.

From a baseline median of approximately 3.3 pg/mL, serum hsTnI also gradually increased with placebo at each time point, but was reduced or unchanged with canagliflozin over 104 weeks (**Figure 1B**). Hodges-Lehmann estimates (95% CI) of the difference in median percent change between canagliflozin and placebo at weeks 26, 52, and 104 were –8.3% (–14.0,

–2.5), –11.9% (–18.0, –5.6), and –10.0% (–17.3, –2.6), respectively. Differences between canagliflozin and placebo were significant at each time point (nominal *P* <0.01 for each between-group difference). Considering the correlation between baseline and 104-week concentrations of hsTnI (**Online Figure 1B**), a lower slope from baseline to final measurement was observed in those treated with canagliflozin.

Baseline serum sST2 concentrations were approximately 29 ng/mL. In contrast to NT-proBNP and hsTnI, median sST2 levels were unchanged in both the canagliflozin and placebo groups at each time point (Hodges-Lehmann estimates [95% CI] of the difference in median percent change of –0.8% [–3.3, 1.7], 0.2% [–2.6, 3.0], and –0.4% [–3.5, 2.7] at weeks 26, 52, and 104, respectively; nominal *P* >0.05 at each time point; **Figure 1C**).

Baseline serum galectin-3 concentrations were approximately 17 ng/mL. Small increases from baseline in serum galectin-3 were observed with canagliflozin relative to placebo at 26 weeks (6.6% [95% CI: 3.7, 9.6]; nominal *P* <0.01) and 52 weeks (5.1% [95% CI: 2.0, 8.3]; nominal *P* <0.01); by 104 weeks, the difference in galectin-3 was still numerically higher in the canagliflozin arm but not statistically significant (3.0% [95% CI: –0.7, 6.6]; nominal *P* = 0.11; **Figure 1D**). It is of note that similar trends in eGFR were seen as in the galectin-3 data; modest decreases in eGFR were seen at 26 and 52 weeks with canagliflozin compared to placebo, but by 104 weeks, no difference in change in eGFR was observed between treatment groups.

With the exception of a negative correlation between galectin-3 concentrations and eGFR, there were generally no clinically meaningful correlations between change in biomarkers and change in selected physiologic parameters at any time point (**Supplemental Table 1**).

In a sensitivity analysis among patients who had biomarker data at baseline and all three time points, changes in cardiovascular biomarkers were consistent with those seen in the primary analysis (**Online Figure 2A-D**).

DISCUSSION

In this randomized trial of older patients with T2DM with biomarker profiles consistent with generally higher risk for cardiovascular events, we found that serum concentrations of NT-proBNP and hsTnI, biomarkers with proven prognostic value for cardiovascular risk in T2DM (22), rose over a 2-year period in patients allocated to placebo, while canagliflozin treatment attenuated their rise. In contrast, we found no obvious effect of treatment with canagliflozin on concentrations of sST2, with a modest, nonpersistent rise in galectin-3. The effects on NT-proBNP and hsTnI seen with canagliflozin versus placebo in this post hoc analysis are compatible with attenuation of cardiovascular risk in those treated with SGLT2 inhibitors (**Central Illustration**). To the extent it is unclear if benefits seen in the EMPA-REG OUTCOME study could be expected from treatment with all SGLT2 inhibitors, our results provide novel data regarding possible cardiovascular benefits from canagliflozin treatment.

Numerous theories have emerged to explain how SGLT2 inhibitors may reduce cardiovascular risk; however, no consensus exists as to the mechanism of such risk reduction. The early divergence of survival curves seen in the EMPA-REG OUTCOME study suggests an acute effect in particular on heart failure outcomes (4). It has been proposed that sodium and fluid loss, reduction in BP and body weight, attenuation of inflammation and oxidative stress, improvement in arterial stiffness, as well as preservation of renal function may contribute to the observed cardiac benefits (7,10,11,23). Interest has also focused on metabolic effects in the myocardium, including changes in glucagon handling, mitigation of glucotoxicity, and shift to

fatty acid metabolism, as well as attenuation of cardiac remodeling (5-9,11). Treatment with SGLT2 inhibitors has been shown to increase levels of ketone bodies, which may be a more favorable energetic substrate for the heart compared with glucose or fatty acids (5,6). Additionally, SGLT2 inhibitors may inhibit the sodium-hydrogen exchanger, leading to reduction of intracellular sodium and calcium in a cariporide-dependent fashion (24), which may foster a cardioprotective effect. Finally, in a basic science model of heart failure, empagliflozin treatment or knockdown of the *slc5A2* gene (simulating SGLT2 inhibition) created a phenotype with improved cardiac function and reduced BNP expression (25). Our biomarker results help to further the understanding of how SGLT2 inhibition might exert a favorable impact on cardiovascular events.

We lack data on biomarker concentrations during the first 26 weeks of treatment with canagliflozin, making it impossible to determine whether the biomarker changes observed in this analysis are somewhat related to diuretic effects from SGLT2 inhibition; studies suggest there is a 10% reduction in plasma volume after 1 week of treatment with canagliflozin, but the plasma volume nearly returns to baseline by week 12 (26). An alternative or linked possibility is to consider that our findings indicate prevention of rise in NT-proBNP or hsTnI.

Biomarker measurements may help inform the mechanism of benefit in patients treated with novel therapies (13), with change over time frequently imparting greater prognostic information than a single measurement or knowledge of absolute concentration. Our results represent the first larger scale, placebo-controlled data regarding cardiac biomarkers in patients treated with SGLT2 inhibition. In a recent study of 66 patients treated with empagliflozin but without placebo control, serum NT-proBNP concentrations were unchanged after 4 weeks in patients with or without T2DM (16). In another small study of 75 patients with T2DM

randomized to dapagliflozin, hydrochlorothiazide, or placebo, no differences in NT-proBNP were seen over 12 weeks of follow-up (17). Thus, our results gathered in much larger numbers and for a much longer period of time substantially extend the understanding of how novel drugs for T2DM may exert favorable cardiovascular effects.

Concentrations of each biomarker measured in this exploratory analysis are consistent with those expected for an older patient population who are at least at moderate risk for cardiovascular events (27). Furthermore, over time, placebo-treated patients demonstrated increases in both NT-proBNP and hsTnI; such changes, though modest, may be indicative of increasing risk for cardiovascular events and heart failure (14,27). Our findings indicate that treatment with canagliflozin was associated with a blunting of the rise in NT-proBNP and hsTnI over time. Taken together, these results are compatible with the early and sustained cardiovascular benefits as seen in the EMPA-REG OUTCOME study.

Baseline sST2 concentrations in our study participants indicate a generally higher-risk patient population with a median value near the 90th percentile for a normal healthy population (28). We did not observe any effect on sST2 concentrations with canagliflozin. In contrast, relatively smaller but significant increases in galectin-3 concentrations were observed at 26 and 52 weeks in patients treated with canagliflozin; by 104 weeks, galectin-3 concentrations were still numerically, but not significantly, higher in the canagliflozin arm. Renal function is a known confounder of galectin-3, and canagliflozin treatment is associated with initial reductions in eGFR that trend back toward baseline with continued treatment (29). Indeed, modest reductions in eGFR paralleled increase in galectin-3 and there was a correlation between change in galectin-3 and change in eGFR over time: thus, change in renal function may account for the

declining between-group difference across time points. It is unknown whether a small early increase in galectin-3 with canagliflozin is clinically relevant.

Though the current results are the first larger scale, placebo-controlled assessment of multiple cardiovascular biomarkers in patients with T2DM treated with canagliflozin, there are a few limitations of this study. First, not all patients had samples at every time point; however, a sensitivity analysis using data from patients with samples at all 3 time points showed consistent results. Also, exclusion of patients with eGFR <50 mL/min/1.73 m^2 might render our data less generalizable to those with worse renal function; this exclusion criterion was due to use of metformin in an older patient population. On the other hand, this exclusion criterion minimizes confounding effects of worse renal function on biomarker concentrations. Differences in the concentrations of NT-proBNP and hsTnI between placebo and canagliflozin-treated patients were relatively modest. However, small changes in both biomarkers may be substantially prognostic, and consistency across multiple time points suggests that these changes for NT-proBNP and hsTnI are more likely to be robust. Lastly, we lack data on other novel biomarkers with prognostic value such as mid-regional pro-adrenomedullin or growth differentiation factor-15. Larger studies should confirm our findings, and ideally future outcomes trials should examine links between biomarker changes and long-term cardiovascular disease outcomes.

In summary, our findings suggest that canagliflozin treatment was associated with attenuation of biomarkers associated with adverse cardiovascular outcomes in this population of older patients with T2DM. As it is difficult to know for sure if the benefits seen in the EMPA-REG OUTCOME study related to treatment with empagliflozin can be extrapolated to treatment with canagliflozin, our results are important, and might predict similar risk reduction from canagliflozin treatment. Results from the CANVAS Program, including the CANagliflozin

cardioVascular Assessment Study (CANVAS; NCT01032629) and CANVAS-R (NCT01989754), will provide direct evidence on the effects of canagliflozin on cardiovascular outcomes in patients with a history or high risk of cardiovascular disease (30-32).

PERSPECTIVES

Competency in medical knowledge: Treatment with SGLT2 inhibitors has been shown to reduce cardiovascular risk in patients with T2DM, but the mechanism of this benefit remains unknown. Biomarkers such as NT-proBNP, hsTnI, sST2, and galectin-3 are associated with myocardial stress, myocardial necrosis, and cardiovascular fibrosis. These biomarkers are not only prognostic for the onset of cardiovascular events in patients with T2DM, but their measurement may be helpful to inform possible mechanism of benefit from treatment with SGLT2 inhibitors.

Translational outlook #1: Increases in NT-proBNP and hsTnI among older placebo-treated T2DM patients may indicate increasing risk for cardiovascular disease events, cardiovascular mortality, and heart failure.

Translational outlook #2: Although relatively modest in size, our study suggests that treatment with canagliflozin prevented myocardial wall stress reflected in favorable impact on NT-proBNP and hsTnI concentrations.

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FIGURE LEGENDS

Central Illustration. Proposed mechanisms of benefit of canagliflozin and effect on cardiac biomarkers. Through its beneficial effects on the heart, canagliflozin prevented rise in NT-proBNP and hsTnI. Possibly through transient reduction in eGFR, galectin-3 increased modestly. NT-proBNP, N-terminal pro-B type natriuretic peptide; hsTnI, high-sensitivity troponin I; eGFR, estimated glomerular filtration rate.

Figure 1. Median percent change from baseline in (A) NT-proBNP, (B) hsTnI, (C) sST2, and (D) galectin-3 over 104 weeks. Treatment with canagliflozin prevented rise of NT-proBNP and hsTnI over a 104-week period, compared to placebo. Galectin-3 concentrations increased modestly, while sST2 concentrations were unchanged. *Nominal P <0.05 vs placebo. **Nominal P <0.01 vs placebo. hsTnI, high-sensitivity troponin I; NT-proBNP, N-terminal pro-B type natriuretic peptide; SE, standard error; sST2, soluble ST2.

Table 1. Baseline demographic and disease characteristics among patients with biomarker assessments. The population reflects a generally higher-risk cohort of patients with T2DM.

Characteristic*	**Placebo (N = 216)**	**Canagliflozin (N = 450)**
Male, n (%)	133 (62)	248 (55)
Age, y	63.2 (6.3)	64.0 (6.3)
55 to <65 y, n (%)	136 (63)	269 (60)
≥65 y, n (%)	80 (37)	181 (40)
Race, n (%)		
White	170 (79)	349 (78)
Black or African American	16 (7)	34 (8)
Asian	19 (9)	37 (8)
Other†	11 (5)	30 (7)
HbA1c, %	7.8±0.8	7.7±0.8
BMI, kg/m^2	31.9±4.8	31.4±4.5
Median (IQR) T2DM duration, y	10.0 (6.0, 15.0)	10.3 (6.1, 16.0)
eGFR, mL/min/1.73 m^2	76.1±16.5	78.2±16.9
Systolic BP, mmHg	131.2±12.3	130.8±14.0
History of microvascular disease, n (%)	55 (25)	145 (32)

History of hypertension, n (%)	169 (78)	346 (77)
Concomitant medications, n (%)		
ACE inhibitor/ARB	163 (76)	327 (73)
Beta-blockers	60 (28)	109 (24)
Calcium channel blockers	48 (22)	103 (23)
Diuretics	73 (34)	151 (34)

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SD, standard deviation; T2DM, type 2 diabetes mellitus.

*Data are mean±SD unless otherwise indicated.

†Includes American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, multiple, other, and not reported.

Table 2. Summary of changes in serum concentrations of cardiovascular biomarkers, eGFR, and hematocrit. Treatment with canagliflozin resulted in prevention of rise in NT-proBNP and hsTnI over a 2-year period.

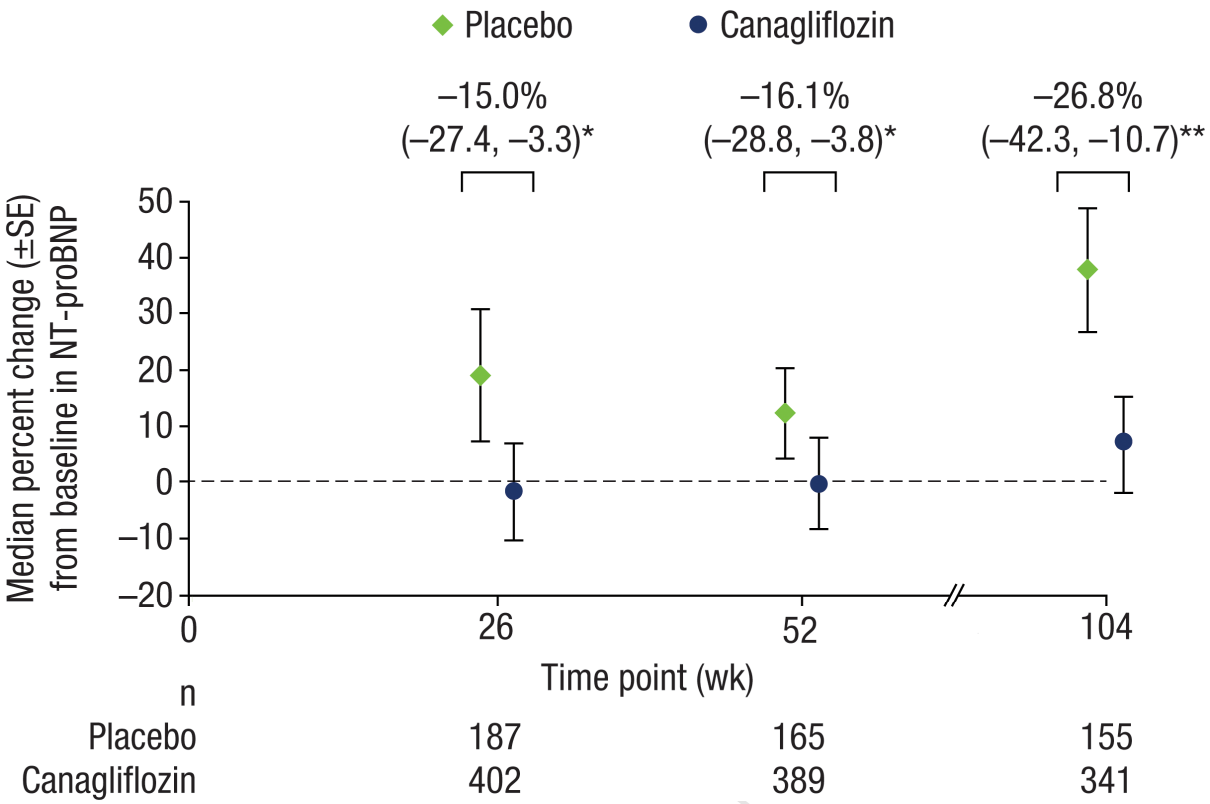
	Week 26		Week 52		Week 104	
Parameter	**Placebo**	**Canagliflozin**	**Placebo**	**Canagliflozin**	**Placebo**	**Canagliflozin**
Serum NT-proBNP, n	187	402	165	389	155	341
Median (IQR) baseline, pg/mL	48.3 (23.3, 112.8)	48.6 (25.9, 108.4)	43.6 (23.8, 105.8)	48.1 (26.1, 107.1)	43.4 (23.1, 96.1)	47.4 (25.8, 103.1)
Median change (SE)[*] from baseline, pg/mL	3.6 (3.6)	–0.8 (4.0)	4.3 (3.6)	–0.3 (3.0)	12.5 (4.5)	2.4 (3.2)
Difference (95% CI) vs placebo[†]		–7.2 (–13.5, –1.0)[‡]		–8.9 (–16.2, –2.4)[§]		–11.8 (–19.9, –4.3)[§]
Serum hsTnI, n	172	344	145	329	140	294
Median (IQR) baseline, pg/mL	3.4 (2.2, 5.6)	3.3 (2.2, 5.0)	3.3 (2.2, 5.1)	3.1 (2.2, 5.0)	3.3 (2.2, 5.4)	3.2 (2.2, 5.0)
Median change (SE)[*] from baseline, pg/mL	0.2 (0.1)	–0.2 (0.1)	0.2 (0.1)	–0.2 (0.1)	0.3 (0.1)	0.0 (0.1)
Difference (95% CI) vs placebo[†]		–0.3 (–0.5, –0.1)[§]		–0.4 (–0.6, –0.1)[§]		–0.4 (–0.6, –0.1)[§]

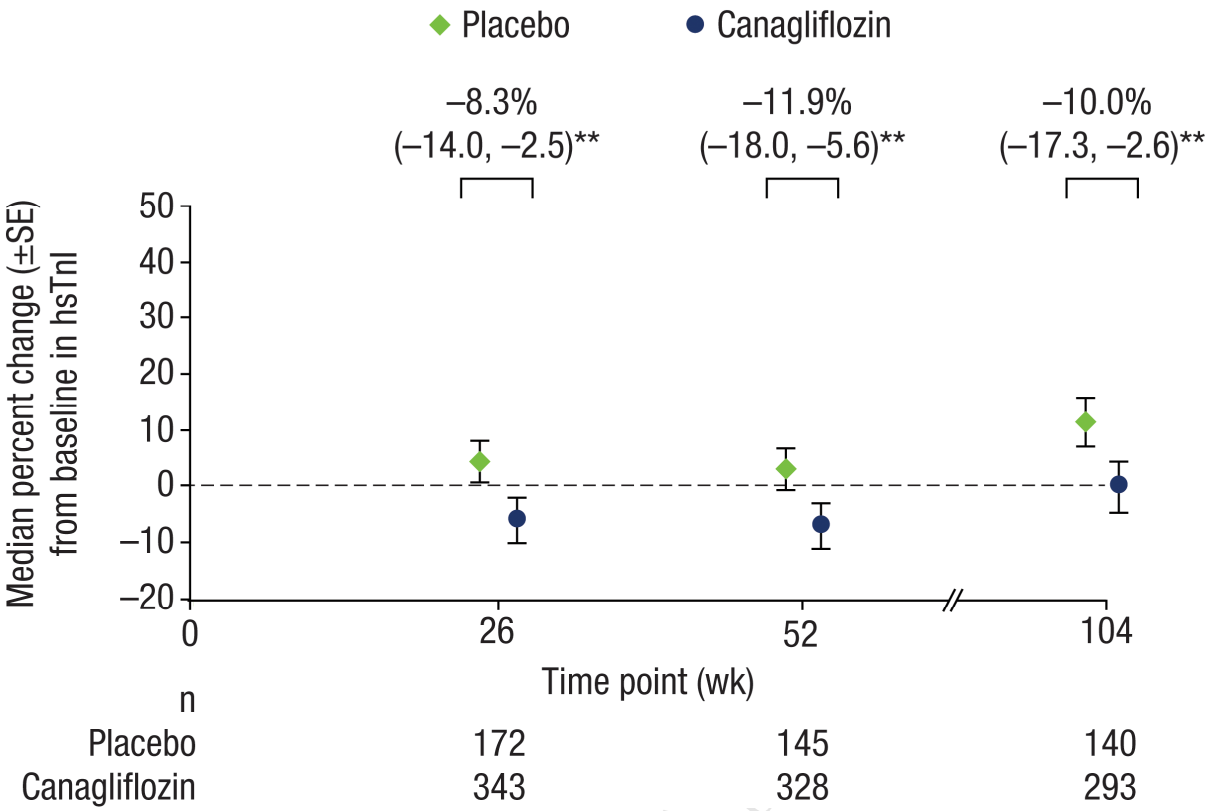
Serum sST2, n	187	409	165	392	155	343
Median (IQR) baseline, ng/mL	28.8 (25.0, 35.8)	29.0 (23.9, 34.3)	28.8 (25.0, 35.8)	29.0 (24.2, 34.4)	28.4 (24.7, 36.7)	28.9 (23.8, 34.2)
Median change (SE)* from baseline, ng/mL	–0.7 (0.5)	–1.1 (0.4)	–0.5 (0.5)	–0.4 (0.5)	0.2 (0.5)	0.3 (0.4)
Difference (95% CI) vs placebo†		–0.3 (–1.0, 0.5)		0.1 (–0.8, 0.9)		–0.1 (–1.0, 0.8)
Serum galectin-3, n	172	343	145	330	140	294
Median (IQR) baseline, ng/mL	17.3 (14.8, 20.1)	17.1 (13.7, 20.8)	17.4 (15.1, 20.4)	16.9 (13.7, 20.8)	17.2 (14.6, 20.2)	17.0 (13.7, 20.8)
Median change (SE)* from baseline, ng/mL	0.2 (0.3)	1.1 (0.4)	–0.1 (0.3)	0.8 (0.3)	0.3 (0.4)	0.8 (0.4)
Difference (95% CI) vs placebo†		1.2 (0.7, 1.7)§		0.9 (0.3, 1.4)§		0.6 (–0.0, 1.2)
eGFR, n	216	450	216	450	216	450
Median (IQR) baseline, mL/min/1.73 m^2	74.0 (64.0, 86.0)	77.0 (66.0, 89.0)	74.0 (64.0, 86.0)	77.0 (66.0, 89.0)	74.0 (64.0, 86.0)	77.0 (66.0, 89.0)
Median change (SE)* from baseline,	–1.0 (0.9)	–3.0 (1.0)	–1.0 (1.1)	–3.0 (1.0)	–3.0 (1.4)	–3.0 (1.1)

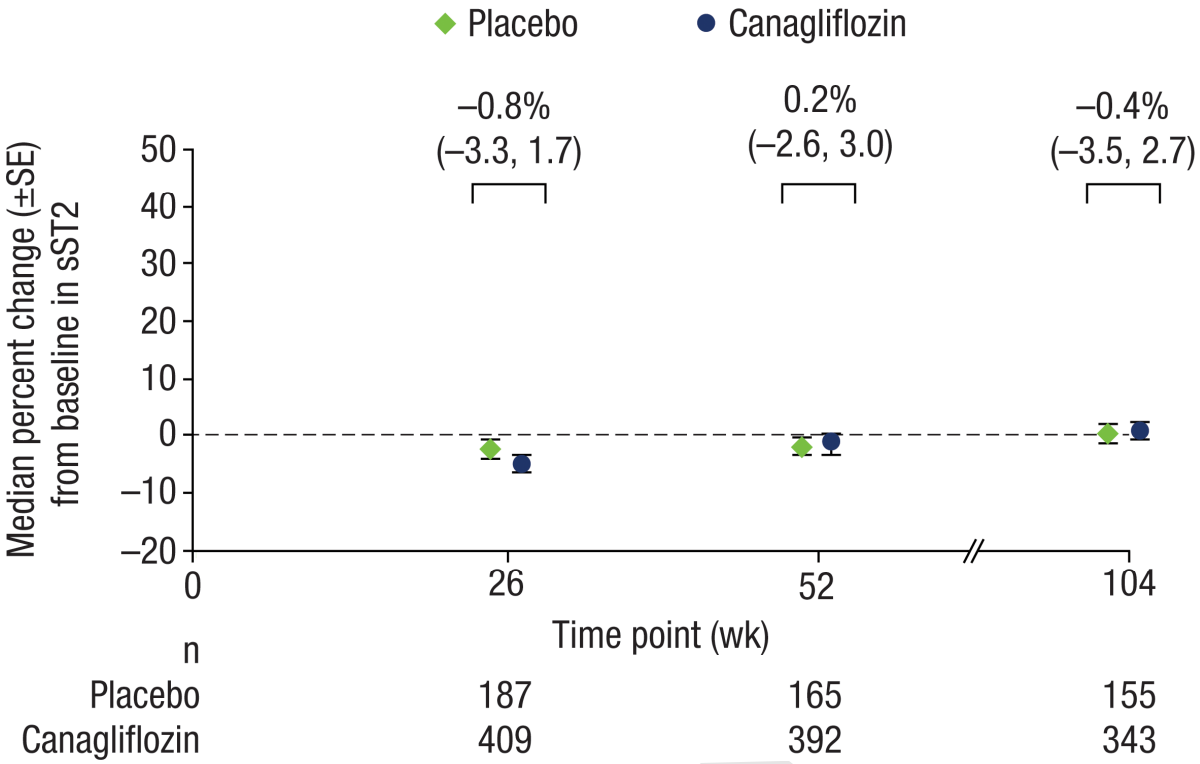
mL/min/1.73 m^2						
Difference (95% CI) vs placebo[†]		–2.0 (–3.0, 0.0)[‡]		–1.0 (–2.0, 1.0)		0.0 (–1.0, 2.0)
Hematocrit, n	215	450	215	450	215	450
Median (IQR) baseline, fraction	0.41 (0.39, 0.43)	0.41 (0.39, 0.43)	0.41 (0.39, 0.43)	0.41 (0.39, 0.43)	0.41 (0.39, 0.43)	0.41 (0.39, 0.43)
Median change (SE)[*] from baseline, fraction	0.000 (0.002)	0.020 (0.005)	0.000 (0.002)	0.020 (0.004)	–0.010 (0.004)	0.020 (0.004)
Difference (95% CI) vs placebo[†]		0.02 (0.02, 0.03)[§]		0.02 (0.02, 0.03)[§]		0.02 (0.02, 0.03)[§]

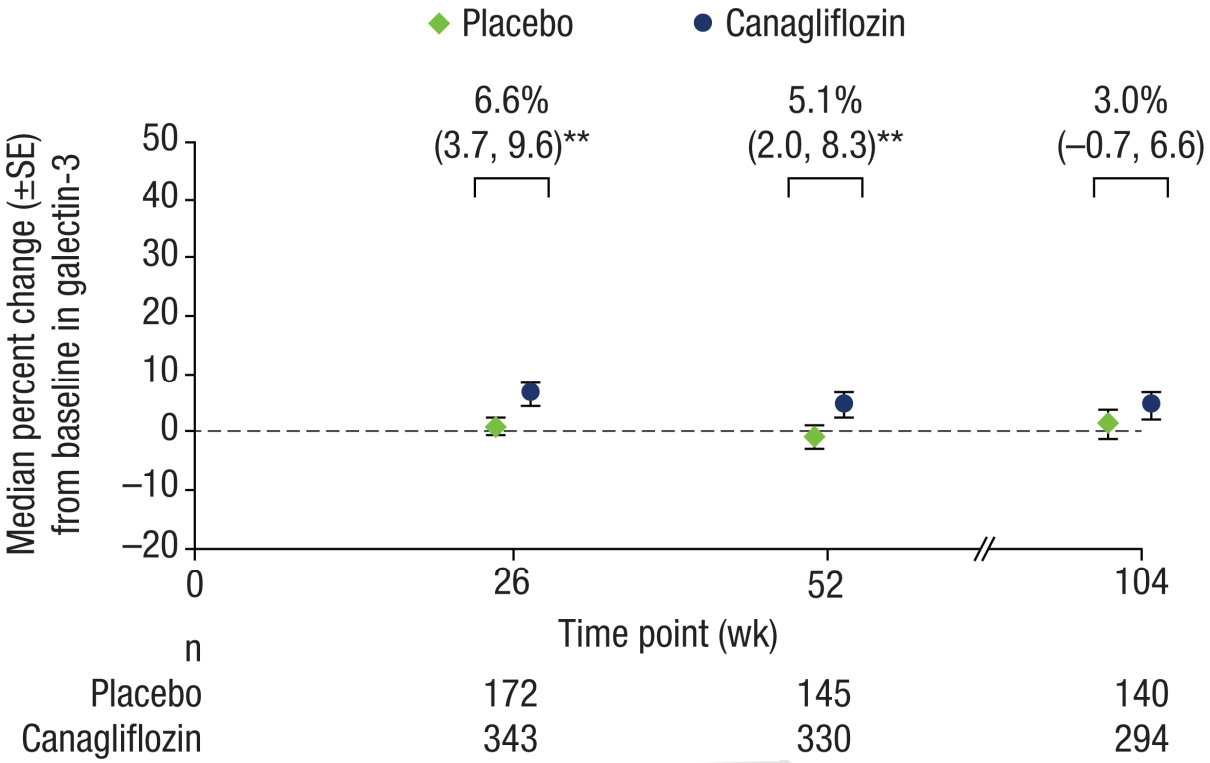
CI, confidence interval; hsTnI, high sensitivity troponin I; IQR, interquartile range; NT-proBNP, N-terminal pro-B type natriuretic peptide; SE, standard error. [*]The SE for the median was estimated using the bootstrap technique by simulated repeated samples for each biomarker and treatment group. [†]Data are non-parametric Hodges-Lehmann estimate; 95% CIs were estimated based on the Wilcoxon rank sum test. [‡]Nominal P <0.05 vs placebo. [§]Nominal P <0.01 vs placebo.

Proposed effect of SGLT2 inhibition	Effect on biomarkers
• Diuresis, natriuresis • Blood pressure lowering • Loss of body weight • Reduced inflammation/oxidative stress • Improved vascular compliance • Long-term preservation of renal function • Metabolic effects on myocardium, improving energetics • Inhibition of Na/H co-transporter • Improvement in myocardial remodeling	↓ NT-proBNP ↓ hsTnI
• Transient decrease in eGFR	↑ Galectin-3









ONLINE MATERIAL

Online Figure Legends

Online Figure 1. Scatterplots of (A) NT-proBNP and (B) hsTnI concentrations at week 104 versus baseline.

Online Figure 2. Sensitivity analysis results: median percent change from baseline in (A) NT-proBNP, (B) hsTnI, (C) sST2, and (D) galectin-3 over 104 weeks among patients with complete data (baseline and weeks 26, 52, and 104).

*Nominal P <0.01 vs placebo. **Nominal P <0.05 vs placebo. hsTnI, high sensitivity troponin I; NT-proBNP, N-terminal pro-B type natriuretic peptide; SE, standard error; sST2, soluble ST2.

Supplemental Table 1. Spearman correlation coefficients between change in biomarkers and change in selected parameters

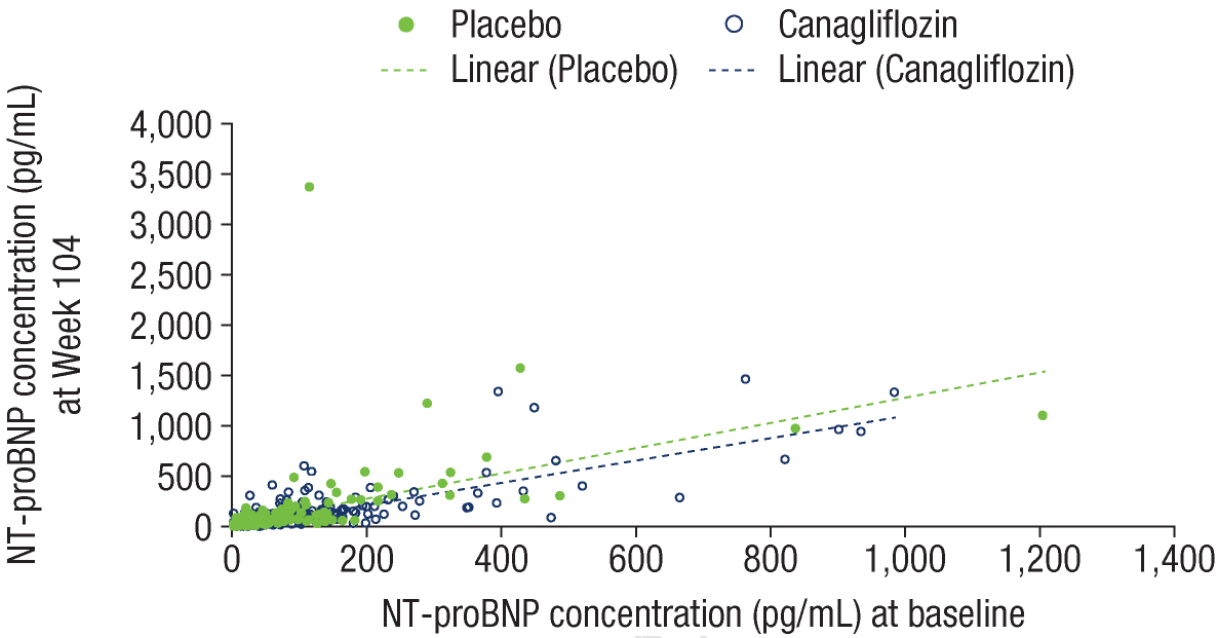
Parameter	NT-proBNP, pg/mL		hsTnI, pg/mL		sST2, ng/mL		Galectin-3, ng/mL	
	Placebo	**Canagliflozin**	**Placebo**	**Canagliflozin**	**Placebo**	**Canagliflozin**	**Placebo**	**Canagliflozin**
Week 26								
HbA1c, %	–0.0950	–0.0650	0.1128	–0.0047	–0.0396	0.1549*	–0.0493	–0.0646
Body weight, kg	0.0267	–0.0459	0.0232	0.0728	0.0661	0.0603	–0.1094	–0.2016*
Systolic BP, mmHg	0.1175	0.0577	0.0424	0.0472	0.0215	0.0228	–0.0547	–0.1765*
Hemoglobin, g/L	–0.2038*	–0.1211*	–0.0049	–0.0945	–0.0331	0.0360	0.0159	0.0313
Hematocrit, fraction	–0.0967	–0.0645	–0.0521	–0.1195*	0.0321	0.0451	0.0192	0.0856
eGFR, mL/min/1.73 m^2	0.1782*	0.0923	–0.0144	–0.0688	0.0267	0.0922	–0.1206	–0.2515*
Week 52								
HbA1c, %	–0.1955	–0.0612	0.1222	–0.0091	–0.0097	0.0723	–0.1242	–0.0055
Body weight, kg	–0.0894	–0.0331	0.0756	0.0463	0.0388	0.0708	0.0408	–0.0862
Systolic BP, mmHg	0.0651	0.1680*	0.0297	0.1198	–0.1467	0.1030*	–0.0357	–0.1686*
Hemoglobin, g/L	–0.3000*	–0.1599*	–0.0303	0.0051	–0.1373	0.0360	0.0241	0.1618*
Hematocrit, fraction	–0.1772	–0.0940	–0.0073	–0.0068	–0.1289	–0.0309	0.0436	0.1384*

eGFR, mL/min/1.73 m^2	–0.0387	0.2068*	0.0374	0.0221	–0.1686*	0.0422	–0.1000	–0.1882*
Week 104								
HbA1c, %	–0.1584	–0.0463	0.0770	0.0013	0.0987	0.0569	–0.0793	–0.0858
Body weight, kg	–0.0787	–0.0322	0.0184	0.0801	0.0871	0.0530	0.0274	–0.1137
Systolic BP, mmHg	0.0391	0.0980	0.0593	0.1751*	0.1084	0.0986	–0.0160	–0.1181
Hemoglobin, g/L	–0.2721*	–0.1903*	0.0240	–0.0120	0.0067	0.0423	0.0915	0.0496
Hematocrit, fraction	–0.2427*	–0.1392*	–0.0418	–0.0811	–0.0459	0.0060	0.0029	0.0896
eGFR, mL/min/1.73 m^2	0.0246	0.1097	–0.1128	0.0138	–0.0614	–0.0449	–0.1597	–0.2355*

BP, blood pressure; eGFR, estimated glomerular filtration rate; hsTnI, high sensitivity troponin I; NT-proBNP, N-terminal pro-B type natriuretic peptide;sST2, soluble ST2. *Nominal P <0.05.

Online Figure 1

A.



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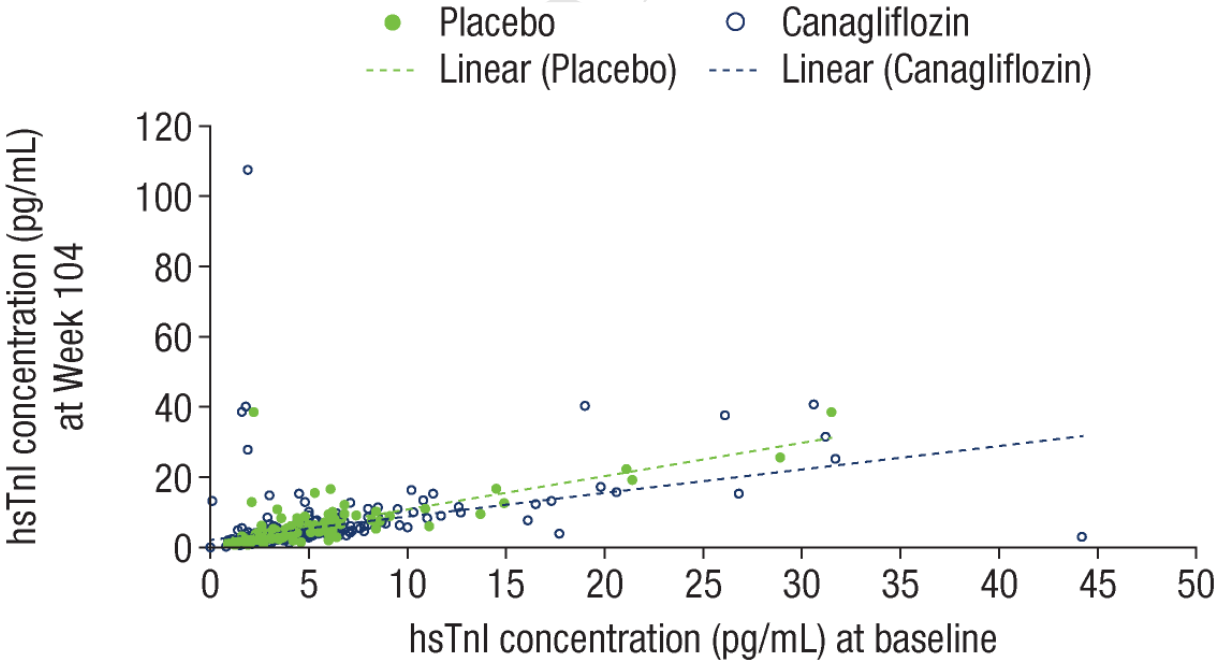
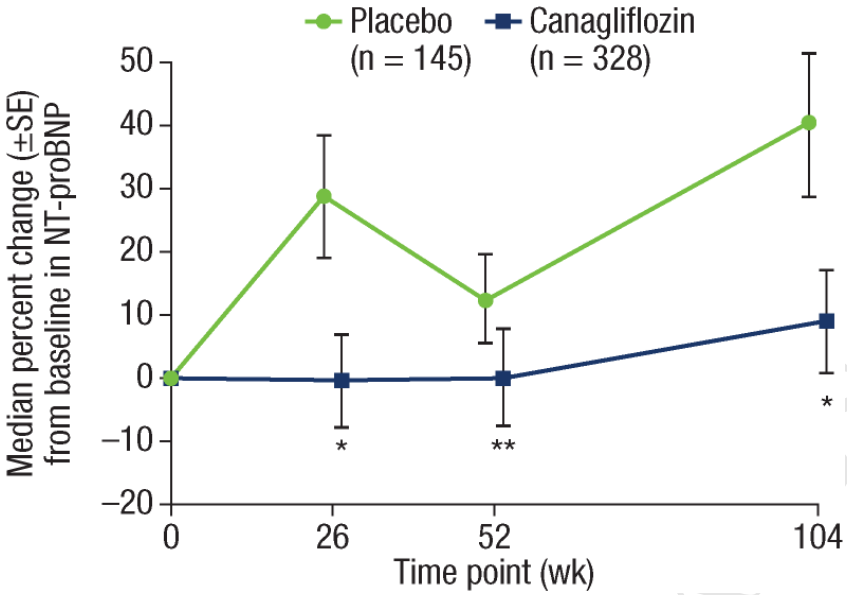


Online Figure 2

A.



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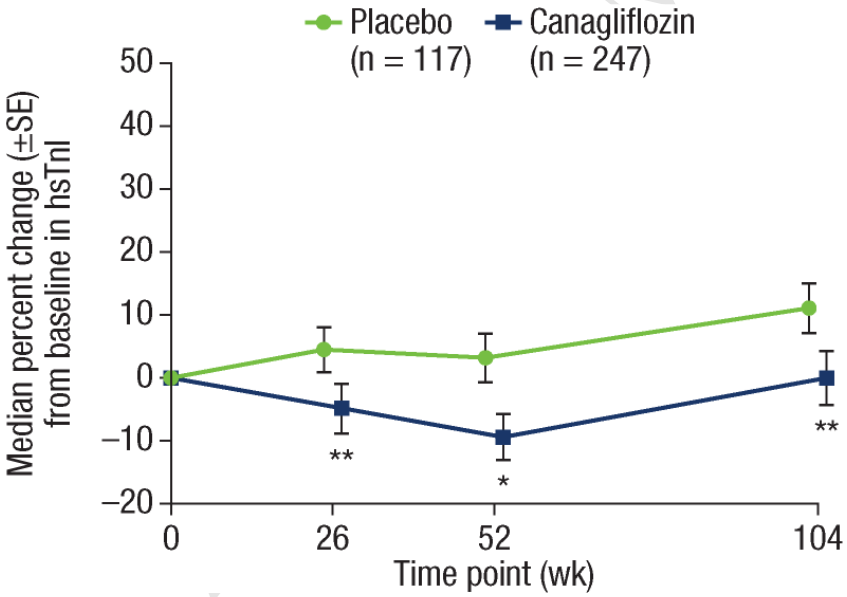

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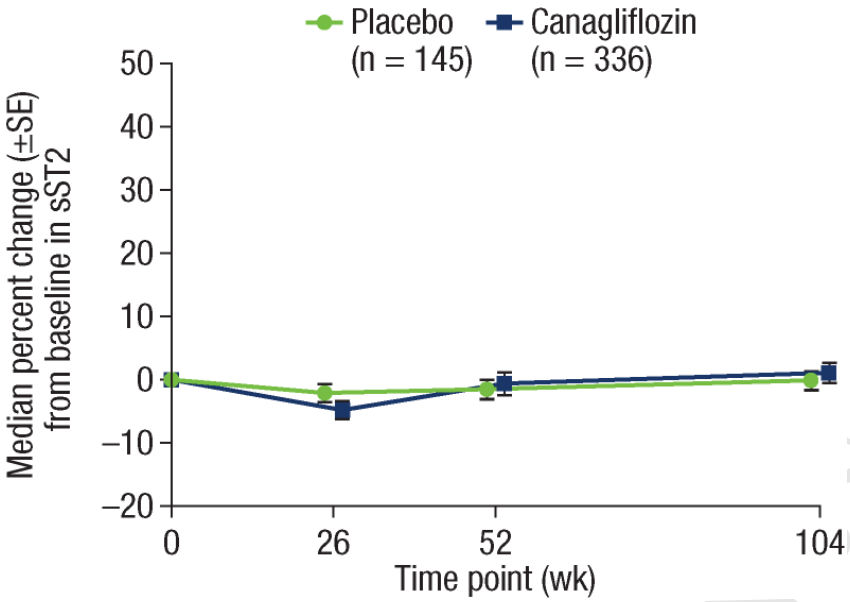
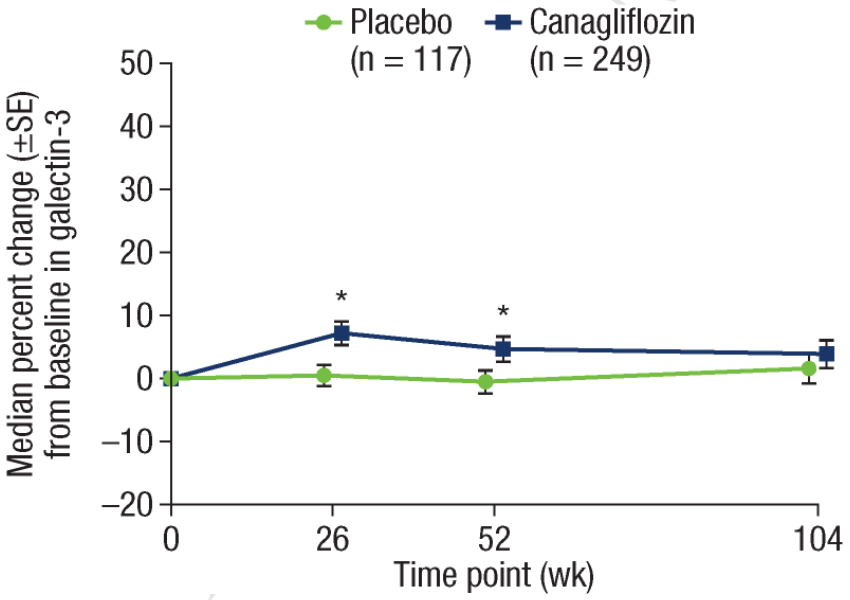

Placebo
(n = 145)
Canagliflozin
(n = 336)
Median percent change (±SE) from baseline in sST2
50
40
30
20
10
0
–10
–20
0
26
52
104
Time point (wk)

D.


Placebo
(n = 117)
Canagliflozin
(n = 249)
Median percent change (±SE) from baseline in galectin-3
50
40
30
20
10
0
–10
–20
*
*
0
26
52
104
Time point (wk)