Original Investigation

Stress Cardiac Biomarkers, Cardiovascular and Kidney Outcomes, and Response to Canagliflozin in the CANVAS Program

Running Title: Stress Cardiac Biomarkers in the CANVAS Program

Muthiah Vaduganathan, MD, MPH, FACC[1]; Naveed Sattar, MD, PhD[2]; Jialin Xu, PhD[3]; Javed Butler, MD, MPH, MBA, FACC[4]; Kenneth W. Mahaffey, MD, FACC[5]; Bruce Neal, MB, ChB, PhD[6-8]; Wayne Shaw, DSL[9]; Norman Rosenthal, MD[9]; Michael Pfeifer, MD[10]; Michael K. Hansen, PhD[3]; James L. Januzzi Jr, MD, FACC[11]

[1]Division of Cardiovascular Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
[2]BHF Glasgow Cardiovascular Research Centre, University of Glasgow, UK
[3]Janssen Research & Development, LLC, Spring House, PA, USA
[4]University of Mississippi, Jackson, MS, USA
[5]Stanford Center for Clinical Research, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
[6]Department of Renal and Metabolic, The George Institute for Global Health, UNSW Sydney, Sydney, Australia
[7]The Charles Perkins Centre, University of Sydney, Sydney, Australia
[8]Imperial College London, London, UK
[9]Janssen Research & Development, LLC, Raritan, NJ, USA
[10]Janssen Scientific Affairs, LLC, Titusville, NJ, USA
[11]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Word Count: 3679

Funding: The CANVAS study and this substudy were funded by Janssen Research & Development, LLC. High-sensitivity cardiac troponin T and insulin-like growth factor binding protein 7 reagents were provided by Roche Diagnostics, Rotkreuz, Switzerland. Soluble suppression of tumorigenesis-2 reagents were provided by Critical Diagnostics, San Diego, California.

Disclosures: Dr. Vaduganathan has received research grant support or served on advisory boards for American Regent, Amgen, AstraZeneca, Bayer AG, Baxter Healthcare, Boehringer Ingelheim, Cytokinetics, Lexicon Pharmaceuticals, Relypsa, and Roche Diagnostics, speaker engagements with Novartis and Roche Diagnostics, and participates on clinical endpoint committees for studies sponsored by Galmed and Novartis. Dr. Sattar has consulted for Affimune, Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, MSD, Novartis, Novo Nordisk, Pfizer, and Sanofi; and has received grant support from Boehringer Ingelheim. Drs. Xu, Shaw, Rosenthal, and Hansen are employees of Janssen Research & Development, LLC. Dr. Butler reports relationships with Abbott, Adrenomed, Amgen, Array, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squib, CVRx, G3 Pharmaceuticals, Innolife, Janssen, LivaNova, American Regent, Medtronic, Merck, Novartis, Novo Nordisk, Relypsa, Roche,

Sanofi, V-Wave Ltd, and Vifor. Dr. Mahaffey's financial disclosures can be viewed at http://med.stanford.edu/profiles/kenneth-mahaffey. Dr. Neal has received research support and has served as a consultant and speaker for Janssen, with all fees paid to his employer, and interacts regularly on a nonfinancial basis with multiple large corporations in the pharmaceutical industry, the food processing industry, and the quick service restaurant industry in Australia and overseas. Dr. Pfeifer is an employee of Janssen Scientific Affairs, LLC. Dr. Januzzi is supported by the Hutter Family Professorship; is a Trustee of the American College of Cardiology; is a board member of Imbria Pharmaceuticals; has received grant support from Novartis Pharmaceuticals, Roche Diagnostics, Abbott, Singulex, and Prevencio; has received consulting income from Abbott, Janssen, Novartis, Pfizer, Merck, and Roche Diagnostics; and participates in clinical endpoint committees/data safety monitoring boards for Abbott, AbbVie, Amgen, Boehringer Ingelheim, Janssen, and Takeda. All other authors report no relevant disclosures.

Twitter Handles: @mvaduganathan @JavedButler1 @TheMahaf @JJheart_doc

Tweet: New data from the CANVAS Program: #SGLT2i canagliflozin delays rise in #troponin & #sST2 out to 6 years, highlighting favorable long-term cardioprotective benefits

Acknowledgements: The authors thank all participants, investigators, and trial teams for their participation in the trial. Canagliflozin was developed by Janssen Research & Development, LLC, in collaboration with Mitsubishi Tanabe Pharma Corporation.

Authorship Statement: The manuscript was drafted by the first author and revised based on input from all coauthors. Minimal technical editorial support was provided by Alaina Mitsch, PhD, of Cello Health Communications/MedErgy, and was funded by Janssen Research & Development, LLC.

Address for Correspondence
James L. Januzzi Jr, MD, FACC
Massachusetts General Hospital, Cardiology Division
55 Fruit Street Boston, MA 02114 USA
Telephone: 617-726-3443 Fax: 617-643-1620
E-mail: jjanuzzi@partners.org
Twitter: @JJheart_doc

Structured Abstract

Background: Circulating biomarkers reflecting different mechanistic pathways may identify at-risk individuals with diabetes who may benefit from sodium glucose co-transporter 2 (SGLT2) inhibitors.

Objective: To determine if measurement of hs-cTnT, sST2, and IGFBP7, either alone or in combination, may modify the treatment benefits of canagliflozin in the CANVAS trial.

Methods: In the CANVAS biomarker substudy, we evaluated the prognostic significance of baseline biomarker measurements, the long-term trajectory of each, and response to canagliflozin on key cardiovascular and kidney outcomes.

Results: Among the 4,330 study participants, baseline hs-cTnT, sST2, and IGFBP7 were available in 3,503 (81%), 3,084 (71%), and 3,577 (83%). 39% had elevated levels of hs-cTnT ≥14 pg/mL, 6% had sST2 >35 ng/mL, and 49% had IGFBP7 >96.5 ng/mL. Canagliflozin significantly slowed increases of hs-cTnT (P=0.027) and sST2 (P=0.033) through 6 years. Each biomarker was significantly associated with a range of cardiovascular and kidney outcomes, independent of clinical covariates. Canagliflozin reduced heart failure and kidney events regardless of baseline biomarker concentration. Patients with hs-cTnT ≥14 ng/L and those with sST2 >35 ng/mL appeared to derive greater relative benefit for MACE (both $P_{\text{interaction}} \leq 0.05$). A panel of all 3 biomarkers predicted each cardiac and kidney outcome evaluated; participants with an increasing number of abnormal circulating biomarkers appeared to have greater relative reductions in MACE from canagliflozin treatment ($P_{\text{interaction trend}}$=0.005).

Conclusions: Canagliflozin delays longitudinal rise in hs-cTnT and sST2 compared with placebo out to 6 years. Canagliflozin reduced heart failure and kidney events regardless of baseline biomarker concentration. Elevated cardiovascular biomarkers, either alone or in combination, may identify individuals who may derive greater MACE benefit from SGLT2 inhibition.

Trial Registration: CANVAS trial; ClinicalTrials.gov Identifier, NCT01032629

Condensed Abstract

In the CANVAS biomarker substudy, concentrations of high-sensitivity cardiac troponin T (hs-cTnT), soluble ST2, and insulin-like growth factor–binding protein 7 (IGFBP7) were measured serially. Canagliflozin slowed increases in hs-cTnT and soluble ST2 through 6 years. Biomarkers independently prognosticated cardiovascular and kidney outcomes. Canagliflozin improved heart failure and kidney outcomes regardless of baseline biomarker values. Participants with increasing numbers of abnormal circulating biomarkers appeared to have greater relative reductions in major adverse cardiovascular events from canagliflozin treatment ($P_{\text{interaction trend}}$=0.005), a finding that requires prospective validation.

Key Words: biomarkers; canagliflozin; diabetes; heart failure; SGLT2 inhibitor

Abbreviations and Acronyms

CANVAS = CANagliflozin cardioVascular Assessment Study
eGFR = estimated glomerular filtration rate
HbA1c = glycated hemoglobin
HF = heart failure
hs-cTnT = high-sensitivity cardiac troponin T
IGFBP7 = insulin-like growth factor binding protein 7
MACE = major adverse cardiovascular events
NT-proBNP = amino-terminal pro-B type natriuretic peptide
SGLT2 = sodium glucose co-transporter 2
sST2 = soluble suppression of tumorigenesis-2

Introduction

The sodium glucose co-transporter 2 (SGLT2) inhibitors have emerged as a central cardio–renal–metabolic therapeutic class with demonstrated benefits in reducing cardiovascular events, delaying kidney progression, and, in select cases, extending survival (1–3). These putative benefits have been demonstrated in primary prevention of incident cardiovascular disease and secondary prevention of recurrent events. As such, current multi-specialty clinical practice guidelines endorse early consideration of SGLT2 inhibitors among individuals with type 2 diabetes facing high cardiovascular risk, especially among those with established atherosclerotic cardiovascular disease (ASCVD) (4). However, most individuals with diabetes do not have clinically overt cardiovascular disease and less guidance is available for optimal risk stratification and treatment allocation of SGLT2 inhibitors among this larger cohort.

Cardiovascular biomarkers assaying different pathophysiological processes have been shown to efficiently risk stratify individuals with diabetes for future cardiovascular events (5). These biomarkers, when measured alone or in combination, have been projected to identify at-risk persons who may derive greater absolute treatment benefits from SGLT2 inhibitors (5), however this hypothesis has not been well tested in the context of randomized clinical trials. Combination biomarkers have an established role in treatment algorithms to guide therapeutic decision-making for other common preventive therapies (such as low density lipoprotein-cholesterol and high-sensitivity C-reactive protein in lipid-lowering management), and may similarly have a role in the cost-effective allocation of SGLT2 inhibitors in broad risk populations. The CANVAS (CANagliflozin cardioVascular Assessment Study) trial is one of 2 cardiovascular outcomes trials that evaluated SGLT2 inhibitors in patients with diabetes with and without established cardiovascular disease. A dedicated biomarker substudy of CANVAS

measured a diverse panel of biomarkers informing different axes of risk and pathophysiological abnormalities, and provides a unique opportunity to explore whether the treatment benefits of SGLT2 inhibitors are indeed modified by assessment of these cardiovascular biomarkers.

In this post hoc analysis leveraging the CANVAS biomarker substudy, we evaluated high-sensitivity cardiac troponin T (hs-cTnT; a marker of myocardial injury), soluble ST2 (sST2; a marker reflecting vascular congestion, tissue inflammation, and fibrosis), and insulin-like growth factor–binding protein 7 (IGFBP7; a marker of tissue senescence and remodeling) at baseline and out to 6 months. We had 3 primary aims: 1) examine the effects of canagliflozin on the trajectory of these biomarkers out to 6 years; 2) confirm the associations of these biomarkers (either alone or collectively) with cardiovascular and kidney outcomes; and 3) determine whether the treatment benefits of canagliflozin are modified by measurement of these biomarkers.

Methods

Data Sharing. Clinical data from the CANVAS Program are available in the public domain via the Yale University Open Data Access Project (http://yoda.yale.edu/).

The CANVAS Program. The design (6) and primary results (7) of the CANVAS Program have been previously published. In brief, the program consisted of 2 trials, CANVAS and CANVAS-R. Data from these analyses are from the CANVAS trial, which randomized participants with type 2 diabetes (glycated hemoglobin [HbA1c] ≥7.0% and ≤10.5%), estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m^2, and high risk for cardiovascular events to placebo or canagliflozin 100 mg or 300 mg (1:1:1) and followed them for up to 6 years. High cardiovascular risk was defined as age ≥30 years and presence of established atherosclerotic cardiovascular

disease or age ≥50 years with ≥2 cardiovascular risk factors. The study was conducted according to the ethical principles put forth in the Declaration of Helsinki. All participants provided written informed consent, and the study protocol was approved by the institutional review boards and ethics committees of each participating site.

Biomarker Substudy and Biomarker Measurement. We included all participants who separately consented for inclusion in the biomarker substudy of the CANVAS trial. Plasma samples were drawn and stored at –80 ºC. Concentrations of hs-cTnT and IGFBP7 (Roche Diagnostics, Penzberg, Germany) and sST2 (Critical Diagnostics, San Diego, California) were measured at baseline, Year 1, and for hs-cTnT and sST2, Year 6.

All samples were run on first freeze-thaw cycles in a blinded fashion (to treatment arm or visit) and during the same period, thereby minimizing inter-assay variations. hs-cTnT was tested using the Roche Elecsys®e411 electrochemiluminescence analyzer, while sST2 was tested using a Roche c501 instrument (immunoturbidimetric measurements). sST2 was measured using a sandwich monoclonal enzyme-linked immunosorbent assay (Critical Diagnostics). IGFBP7 was measured using a sandwich immunoassay developed on the Elecsys® platform for electrochemiluminescence; the measurements were done on a Roche cobas e601 system and the coefficient of variation within runs were ≤2% and between runs ≤5%.

Clinical Outcomes. The primary endpoint of the CANVAS trial was MACE, a composite of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke. Secondary endpoints included all-cause mortality, cardiovascular death, and a composite cardiovascular death or HF hospitalization. A renal composite endpoint comprised a 40% decrease in eGFR (sustained for

≥2 consecutive measures), the need for kidney replacement therapy (dialysis or transplantation), or renal death. An additional renal composite endpoint employed in CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation; ClinicalTrials.gov Identifier: NCT02065791) was evaluated, which included all of these components plus cardiovascular death. Independent clinical endpoints committees adjudicated all major cardiovascular, kidney, and mortality outcomes.

Statistical Analyses. We first evaluated the effect of treatment allocation to canagliflozin or placebo on biomarker trajectory. Given skewed distributions, each biomarker was log-transformed. We constructed linear mixed models to assess the effects of treatment on follow-up biomarker levels at Years 1 and 6, adjusting for baseline biomarker values and key covariates selected using Bayesian Information Criterion in the placebo arm. Baseline candidate covariates included age, eGFR, body mass index, systolic blood pressure, HbA1c, duration of diabetes, urine albumin:creatinine ratio (UACR), low-density lipoprotein-cholesterol, history of HF, history of atherosclerotic cardiovascular disease, statin use, and diuretic use. Treatment effects on biomarker changes were summarized as the geometric mean ratio (GMR) and 95% confidence interval (CI) and the nominal *P* value was reported. Serial biomarker changes were assessed among individuals with available samples at any timepoint. However, to address the potential for attrition (intervention deaths or loss to follow-up out to 6 years), an additional complete case analysis was conducted in which biomarker trends were assessed among participants with available samples at baseline, 1 year, and 6 years.

We then evaluated the association between baseline measurement of each of the biomarkers with cardiovascular and kidney outcomes. Biomarkers were modeled continuously

and as dichotomous variables and separately assessed associations with subsequent outcomes. For the continuous analyses, given variable units and skewed distributions, biomarkers were log-transformed and risk associations were expressed per standardized unit change. For the dichotomous analysis, we applied thresholds to define elevated levels of the 3 biomarkers, selected based on clinical relevance and previously established cut-points: hs-cTnT >14 ng/L, sST2 >35 ng/mL, and IGFBP7 >96.5 ng/mL (8–10). A base model was first built for each endpoint from the same covariates listed above. Cox proportional hazards regression models on time to event endpoints were used to evaluate the association between biomarkers and cardiovascular and kidney outcomes, after adjusting for the covariates included in the base model and treatment allocation. Associations were summarized as hazard ratios (HRs) and 95% CIs, with a nominal *P* value reported. Concordance probability (C-index) was calculated for each outcome to assess the incremental improvement in discrimination when adding the biomarker as a continuous value to the base clinical model.

To add clinical relevance and after confirming that each biomarker displayed limited correlation with one another, among patients with available baseline data for all 3 biomarkers of interest, a multi-marker risk panel was created, assigning 1 point for each abnormally elevated biomarker (total range, 0-3). The association between the biomarker risk panel and cardiovascular and kidney outcomes was similarly assessed as an ordinal variable (with patients with 0 abnormal biomarkers serving as a reference cohort). Modification of the treatment effects of canagliflozin versus placebo by baseline biomarker measurement (dichotomized based on clinically relevant cut-points) or the multi-marker panel (assessed as an interaction test of trend across the ordinal variable) was assessed.

Given similar treatment effects with both doses of canagliflozin, these arms were pooled for analytic purposes. No adjustment was made for multiplicity of statistical testing in this exploratory study. All statistics were performed using R version 3.6.1, with 2-sided *P* values.

Results

Among the 4,330 participants in the CANVAS trial, baseline hs-cTnT, sST2, and IGFB7 were available in 3,503 (81%), 3,084 (71%), and 3,577 patients (83%), respectively. Patient disposition and availability of individual biomarkers at each follow-up time point is summarized in **Supplemental Figure 1**. Representative baseline characteristics are displayed in **Table 1** summarizing the clinical profiles and background therapies of participants with available hs-cTnT at baseline. Mean age was 63 ± 8 years, 34% were women, and 82% were White. There were no differences in baseline characteristics between participants allocated to placebo and the 2 canagliflozin arms. Participants who did and did not have available biomarker measurements had similar clinical characteristics and treatment patterns, except participants with missing data were less often White and more often Asian (representative data for hs-cTnT presented in **Supplemental Table 1**).

Effects of Canagliflozin on Biomarker Trajectory. Baseline geometric mean of hs-cTnT among placebo-treated participants was 12.2 pg/mL, and 38% had hs-cTnT ≥14 pg/mL (**Figure 1**). Summary statistics of log-transformed baseline values of each biomarker is presented in **Supplemental Table 2**. During follow-up, hs-cTnT increased in the placebo and canagliflozin arms up to 1 year; among the 1,000 patients with available data out to 6 years, treatment with canagliflozin slowed the increase in hs-cTnT. In adjusted models, canagliflozin was associated

with significantly lower hs-cTnT concentrations at Year 6 compared with placebo (GMR, 93.8% [95% CI: 88.6-99.3]; *P* = 0.027).

Baseline geometric mean of sST2 among placebo-treated participants was 16.3 ng/mL, and 7% had sST2 >35 ng/mL. sST2 levels remained relatively stable in both arms through 1 year. During follow-up through 6 years, sST2 increased in the placebo arm, while sST2 levels remained stable in canagliflozin-treated participants. In adjusted models, canagliflozin was associated with significantly lower sST2 concentrations at Year 6 compared with placebo (GMR, 87.9% [95% CI: 78.0-99.0]; *P* = 0.033).

Baseline geometric mean of IGFBP7 was 96.9 ng/mL, with 49% of patients with levels >96.5 ng/mL. IGFBP7 levels similarly increased in both placebo and canagliflozin arms through 1 year; measurements were not available at Year 6.

For all 3 biomarkers, consistent trends were observed in complete case analyses among participants with available samples at all time points. In complete case analyses, canagliflozin remained associated with significantly lower hs-cTnT concentrations (GMR, 93.7% [95% CI: 88.4%-99.2%]; *P* = 0.025) and sST2 concentrations (GMR, 87.3% [95% CI: 77.4%-98.6%]; *P* = 0.029) at Year 6 compared with placebo (**Supplemental Figure 2**).

Association of Baseline Biomarker Levels With Cardiovascular and Kidney Outcomes. Each biomarker was robustly associated with a range of cardiovascular and kidney outcomes, independent of clinical covariates, either when assessed as a continuous, log-transformed marker (**Supplemental Table 3**) or based on previously established cut-offs (**Table 3**). IGFBP7 was particularly associated with subsequent risks of hospitalization for HF, with an adjusted HR of 14.2 (95% CI 7.51-26.8; *P* <0.001) per unit increase in the log-transformed biomarker. The

addition of each biomarker to a base clinical model (without this biomarker) yielded improvements in model discrimination that were especially noteworthy for hs-cTnT (C-index increase by +0.01 to +0.06 across outcomes) and IGFBP7 (C-index increase by 0 to +0.05 across outcomes), but less evident for sST2 (C-index change by 0 to +0.02 across outcomes).

Multi-marker Risk Analysis. Baseline measures of each biomarker displayed weak correlations with one another (all r<0.30; **Supplemental Figure 3**). Among 3,040 participants who had available data for all 3 biomarkers at baseline, 1,102 (36%), 1,051 (35%), 819 (27%), and 68 (2%) had 0, 1, 2, and 3 elevated biomarkers, respectively. The hazard of MACE, HF events, and kidney outcomes increased in a graded fashion with increasing numbers of elevated biomarkers. Estimation of the multi-marker risk panel improved discrimination compared with a base clinical model by +0.04 to +0.06 across outcomes (P≤0.002 for all C-statistic changes; **Figure 3**).

Treatment Modification by Baseline Biomarkers. Treatment with canagliflozin had similar effects on HF and kidney outcomes regardless of hs-cTnT, sST2, or IGFBP7 elevations (all $P_{\text{interaction}}$ >0.10). However, hs-cTnT may have modified treatment effects of canagliflozin on MACE, such that patients with elevated hs-cTnT ≥14 pg/mL (HR, 0.71; 95% CI 0.56-0.91) appeared to derive greater relative treatment benefits compared with patients with normal hs-cTnT<14 pg/mL (HR, 1.10 [95% CI: 0.81-1.49]; $P_{\text{interaction}}$ = 0.05). A treatment interaction was also observed between abnormal levels of hs-cTnT and treatment effects of canagliflozin on non-fatal myocardial infarction ($P_{\text{interaction}}$ = 0.04; **Table 2**). Similarly, relative benefits on MACE events appeared greater among individuals with sST2≥35ng/mL (HR, 0.53 [95% CI: 0.27-1.05]) compared with patients with normal sST2<35ng/mL (HR, 0.92 [95% CI: 0.75-1.12]; $P_{\text{interaction}}$ =

0.04). IGFBP7 levels may have modified treatment responses to canagliflozin for the stroke endpoint ($P_{interaction}$ = 0.05 **Table 2**). When assessed together, treatment effects with canagliflozin were consistent for HF and kidney outcomes across the multi-marker panel, but the multi-marker panel may identify participants who would derive differential treatment benefits with canagliflozin for MACE (**Figure 4**). Treatment effects ranged from patients with 0 abnormal biomarkers (HR, 0.99 [95% CI: 0.66-1.49), 1 abnormal biomarker (HR, 1.34 [95% CI 0.94-1.89]), 2 abnormal biomarkers (HR, 0.61 [95% CI: 0.45-0.82]), and 3 abnormal biomarkers (HR, 0.46 [95% CI: 0.18-1.17]); $P_{interaction\ trend}$ = 0.005. Absolute risk reductions ranged from 0.1 (per 100 patient-year) in those with no abnormal biomarkers to 5.4 (per 100 patient-years) in those with all 3 abnormal circulating biomarkers (**Figure 4**). The number of abnormal biomarkers appears to consistently modify treatment effects on MACE events in those with established cardiovascular disease and those with multiple cardiovascular risk factors (but without known cardiovascular disease); **Supplemental Table 4**.

Discussion

In this post hoc biomarker substudy of the CANVAS trial, serial measurement of markers of myocardial injury, inflammation/fibrosis, and remodeling provided several key findings (**Central Illustration**): 1) a substantial proportion of seemingly stable patients with type 2 diabetes in CANVAS had evidence of abnormal circulating biomarkers, suggesting subclinical disease activity; 2) these biomarkers alone and in combination predicted subsequent clinical outcomes, independent of and incremental to common clinical parameters; 3) while hs-cTnT and sST2 showed similar trajectories in both arms through 1 year, longitudinal increases through 6 years were slowed with canagliflozin compared with placebo; 4) participants similarly benefited

from canagliflozin with respect to HF and kidney outcomes, irrespective of baseline elevations in biomarkers; and 5) presence of multiple abnormal biomarkers appeared to identify patients with greater relative benefits of canagliflozin on MACE events, including among those without apparent cardiovascular disease.

Patients with type 2 diabetes face broad systemic risks of end-organ injury and failure (11). Circulating biomarkers measured longitudinally may track disease activity even prior to a clinical event. Despite seemingly "stable" clinical status at study entry, nearly 40% and 50% of participants had evidence of abnormal hs-cTnT and IGFBP7 values, respectively, indicating evidence of chronic myocardial injury and tissue senescence/fibrosis. Adding to a growing body of evidence (9,12–14), in this substudy of CANVAS, these biomarkers were consistently independently associated with cardiovascular and kidney outcomes and improved discrimination beyond other commonly collected clinical information in this cohort with type 2 diabetes at high cardiovascular risk. In the CANVAS trial, we recently showed that plasma IGFBP7 was strongly linked with endpoints signaling progression of diabetic kidney disease (10). We extend these findings by establishing the association between plasma IGFBP7 and multiple cardiovascular events, especially HF, independent of UACR and eGFR.

We previously showed that treatment with canagliflozin resulted in lower NT-proBNP concentrations during follow-up in the CANVAS trial (15). We now extend that finding, showing that SGLT2 inhibitors delay late biomarker rises in hs-cTnT and sST2, suggesting potential chronic effects in attenuating myocardial injury, vascular congestion, tissue inflammation, and fibrosis. Importantly, rather than reducing biomarkers below baseline measurements, canagliflozin prevented longitudinal rises in biomarkers. A prior experience of older adults with type 2 diabetes similarly showed that canagliflozin delayed the rise in high-

sensitivity troponin I over 2 years, but did not observe meaningful between-arm differences in serum sST2 during this time frame (16). Modest effects on intra-cardiac filling pressures (17), together with limited short-term systemic hemodynamic effects (18), may explain the lack of dynamic early biomarker responses. While the clinical effects of SGLT2 inhibition may be detected as early as days or weeks after treatment initiation (19–21), these biomarker data reinforce the disease-stabilizing effects of these therapies with long-term treatment.

Despite their well-established role of biomarkers in prognostication, routine measurement is not recommended for cardiovascular risk stratification in diabetes (class III) (22) in part due to the lack of established linked clinical action in response to an abnormal value. Given the large population of patients with type 2 diabetes and the wide-ranging forecasted risk based on clinical factors alone, there is a potential future role of biomarkers in more accurate risk stratification and identification of patients who stand to benefit from cardioprotective therapies. In the present analysis, we found biomarker elevations, either alone or in combination, did not identify patients who may differentially respond to canagliflozin with respect to HF and kidney outcomes. These results are congruent with a secondary analysis of DECLARE-TIMI 58 (Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58) trial, which similarly showed consistent treatment effects with dapagliflozin on HF events irrespective of baseline measurement of natriuretic peptides or hs-cTnT (14). Nonetheless, as participants with elevated biomarker concentrations face the highest absolute clinical risks, this cohort would be expected to derive similar relative but higher absolute benefits with respect to HF and kidney outcomes compared with those without elevated levels.

In contrast to the consistency of SGLT-2 inhibitor benefit in preventing HF and kidney events across a spectrum of risk (2,23–25), risk reductions with respect to MACE appear more

heterogeneous (1,26). In our study, patients with elevated hs-cTnT or sST2 and those with multiple abnormal circulating biomarkers appeared to derive greater relative treatment benefits with canagliflozin for MACE events. These data are consistent with prior class-wide observations that SGLT-2 inhibitors modestly reduce the risk of MACE events with benefits most evident in those with established cardiovascular disease (1). Interestingly, preliminary findings from DELCARE-TIMI 58 consistently found more pronounced MACE benefits among participants with elevated hs-TnT levels (27).

We also identified potential heterogeneity in canagliflozin's effects on stroke by concentrations of IGFBP7 levels. As IGFBP7 appears to be a particularly strong marker of prognosis in diabetic kidney disease (10), it is of interest that heterogeneity in canagliflozin's effect on the stroke endpoint was observed in CANVAS by baseline eGFR levels, either dichotomized at 60 mL/min/1.73 m^2 or assessed as a continuous function (28). Notably, in a recent trial of type 2 diabetes and chronic kidney disease, the investigational SGLT1/2 inhibitor sotagliflozin significantly reduced the risk of total fatal or nonfatal strokes by 34% (29).

Individuals with type 2 diabetes without clinically overt cardiovascular disease face ostensibly lower cardiovascular risks. Quality improvement efforts to close gaps in cardioprotective therapies are often targeted to those with established cardiovascular disease (for secondary prevention). Our data suggest that patients without known cardiovascular disease but with 2 or more abnormal circulating biomarkers face absolute risks of MACE that are similar or higher than those of experienced by patients with established cardiovascular disease. Although MACE was the primary endpoint in CANVAS and while some of this heterogeneity has been corroborated in biomarker assessments in other trials (27), these data should be cautiously

interpreted given multiple comparisons and post hoc biomarker measurement; future prospective studies would be required to confirm these findings.

Study Limitations. This study is subject to certain limitations. This was a post hoc investigation of a cardiovascular outcomes trial intended to be exploratory. Given this exploratory framework with preplanned outcomes, we did not adjust for multiple comparisons as this may not be appropriate in this setting (30). As such, these data remain hypothesis-generating and require independent validation. Generalizability of these findings may be limited in populations less represented in clinical trial settings. This CANVAS biomarker substudy required separate patient consent from the main trial, and thus is subject to response bias. Measurement of sST2 was available in ~70% of CANVAS participants due to sample volume limitation. It is reassuring that clinical profiles and treatment patterns were largely comparable between participants who did and did not have available biomarker samples. Differences in racial composition of those with available or missing data may suggest that biomarkers were not available from certain sites or countries, especially in Asia. Although the treatment groups remained well balanced in this biomarker substudy, we additionally adjusted for selected covariates to understand independent treatment effects on biomarker trajectories. Given the limited available follow-up samples at 1 year and 6 years, we focused on prognostic assessments of baseline measurements and were unable to examine the prognostic value of serial measurement of these biomarkers or perform biomarker-based mediation analyses. IGFBP7 concentrations were unavailable at 6 years, and thus we cannot comment on long-term effects of canagliflozin on its levels. While intervening deaths or loss-to-follow-up between baseline and year 6 may potentially exert a conservative bias on the trajectories of biomarkers, we observed similar biomarker patterns in individuals with all

available samples at each time-point. Finally, we attempted to account for potential confounding by adjusting risk associations for a broad set of covariates but the list was not exhaustive. These variables were selected a priori and aimed to reflect established risk factors of the cardiovascular and kidney outcomes of interest.

Conclusions. In conclusion, patients with type 2 diabetes at high cardiovascular risk have evidence of abnormal circulating levels of multiple biomarkers that appear to longitudinally worsen with time. Canagliflozin slows the long-term rise of markers of myocardial injury and inflammation/fibrosis. Canagliflozin reduced HF and kidney events regardless of baseline biomarker concentration. Elevated cardiovascular biomarkers, either alone or in combination, may identify individuals who may derive greater MACE benefit from SGLT2 inhibition. Further data are required to rigorously evaluate a biomarker-based strategy for risk-appropriate selection of cardioprotective therapies such as SGLT2 inhibitors in type 2 diabetes, especially among the large proportion of individuals with clinically evident cardiovascular disease.

Clinical Perspectives

Competency in Medical Knowledge: A substantial proportion of patients with type 2 diabetes at high cardiovascular risk have evidence of abnormal circulating markers of myocardial injury and remodeling.

Translational Outlook: The SGLT2 inhibitor, canagliflozin, attenuates longitudinal rises in cardiovascular biomarkers through 6 years of follow-up underscoring the long-term disease-stabilizing effects of these therapies.

Translational Outlook: Canagliflozin reduced risks of HF and kidney events irrespective of cardiovascular biomarker levels, but appeared to have more pronounced benefits with respect to MACE among those with multiple abnormal biomarkers.

References

1. Zelniker TA, Wiviott SD, Raz I, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet 2019;393:31–9.
2. Neuen BL, Young T, Heerspink HJL, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol 2019;7:845–54.
3. Zannad F, Ferreira JP, Pocock SJ, et al. SGLT2 inhibitors in patients with heart failure with reduced ejection fraction: a meta-analysis of the EMPEROR-Reduced and DAPA-HF trials. Lancet 2020;396:819–29.
4. Pharmacologic approaches to glycemic treatment: Standards of medical care in diabetesd2021. Diabetes Care 2021;44:S111–24.
5. Pandey A, Vaduganathan M, Patel K V., et al. Biomarker-Based Risk Prediction of Incident Heart Failure in Pre-Diabetes and Diabetes. JACC Heart Fail 2021;9:215–23.
6. Neal B, Perkovic V, De Zeeuw D, et al. Rationale, design, and baseline characteristics of the Canagliflozin Cardiovascular Assessment Study (CANVAS) - A randomized placebo-controlled trial. Am Heart J 2013;166.
7. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 2017;377:644–57.
8. Wu AHB, Wians F, Jaffe A. Biological variation of galectin-3 and soluble ST2 for chronic heart failure: Implication on interpretation of test results. Am Heart J 2013;165:995–9.
9. Everett BM, Brooks MM, Vlachos HEA, Chaitman BR, Frye RL, Bhatt DL. Troponin and Cardiac Events in Stable Ischemic Heart Disease and Diabetes. N Engl J Med

2015;373:610–20.

10. Januzzi JL, Butler J, Sattar N, et al. Insulin-Like Growth Factor Binding Protein 7 Predicts Renal and Cardiovascular Outcomes in the Canagliflozin Cardiovascular Assessment Study. Diabetes Care 2021;44:210–6.
11. Gregg EW, Hora I, Benoit SR. Resurgence in Diabetes-Related Complications. JAMA 2019:1867–8.
12. Cavender MA, White WB, Jarolim P, et al. Serial measurement of high-sensitivity troponin i and cardiovascular outcomes in patients with type 2 diabetes mellitus in the EXAMINE trial. Circulation 2017;135:1911–21.
13. Scirica BM, Bhatt DL, Braunwald E, et al. Prognostic implications of biomarker assessments in patients with type 2 diabetes at high cardiovascular risk: A secondary analysis of a randomized clinical trial. JAMA Cardiol 2016;1:989–98.
14. Zelniker TA, Morrow DA, Mosenzon O, et al. Relationship between baseline cardiac biomarkers and cardiovascular death or hospitalization for heart failure with and without sodium–glucose co-transporter 2 inhibitor therapy in DECLARE-TIMI 58. Eur J Heart Fail 2021;23:1026–36.
15. Januzzi JL, Xu J, Li JW, et al. Effects of Canagliflozin on Amino-Terminal Pro–B-Type Natriuretic Peptide: Implications for Cardiovascular Risk Reduction. J Am Coll Cardiol 2020;76:2076–85.
16. Januzzi JL, Butler J, Jarolim P, et al. Effects of Canagliflozin on Cardiovascular Biomarkers in Older Adults With Type 2 Diabetes. J Am Coll Cardiol 2017;70:704–12.
17. Nassif ME, Qintar M, Windsor SL, et al. Empagliflozin Effects on Pulmonary Artery Pressure in Patients with Heart Failure: Results from the EMBRACE-HF Trial.

Circulation 2021:1673–86.

18. Serenelli M, Böhm M, Inzucchi SE, et al. Effect of dapagliflozin according to baseline systolic blood pressure in the Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure trial (DAPA-HF). Eur Heart J 2020;41:3402–18.
19. Packer M, Anker SD, Butler J, et al. Effect of Empagliflozin on the Clinical Stability of Patients with Heart Failure and a Reduced Ejection Fraction: The EMPEROR-Reduced Trial. Circulation 2021:326–36.
20. Bhatt DL, Szarek M, Steg PG, et al. Sotagliflozin in Patients with Diabetes and Recent Worsening Heart Failure. N Engl J Med 2021;384:117–28.
21. Berg DD, Jhund PS, Docherty KF, et al. Time to Clinical Benefit of Dapagliflozin and Significance of Prior Heart Failure Hospitalization in Patients with Heart Failure with Reduced Ejection Fraction. JAMA Cardiol 2021;6:499–507.
22. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J 2020;41:255–323.
23. Fitchett D, Butler J, Van De Borne P, et al. Effects of empagliflozin on risk for cardiovascular death and heart failure hospitalization across the spectrum of heart failure risk in the EMPA-REG OUTCOME trial. Eur Heart J 2018;39:363–70.
24. Fitchett D, Inzucchi SE, Cannon CP, et al. Empagliflozin Reduced Mortality and Hospitalization for Heart Failure across the Spectrum of Cardiovascular Risk in the EMPA-REG OUTCOME Trial. Circulation 2019;139:1384–95.
25. Bhatia K, Jain V, Gupta K, et al. Prevention of heart failure events with sodium–glucose co-transporter 2 inhibitors across a spectrum of cardio-renal-metabolic risk. Eur J Heart

Fail 2021;23:1002–8.

26. McGuire DK, Shih WJ, Cosentino F, et al. Association of SGLT2 inhibitors with cardiovascular and kidney outcomes in patients with type 2 diabetes: A Meta-analysis. JAMA Cardiol 2021;6:148–58.

27. Zelniker TA, Morrow DA, Mosenzon O, et al. Relationship Between Cardiac Biomarkers and Major Adverse Cardiovascular Events in DECLARE-TIMI 58. Circulation. 2020;142:A15701. Presented at the American Heart Association Scientific Sessions 2020.

28. Neuen BL, Ohkuma T, Neal B, et al. Cardiovascular and renal outcomes with canagliflozin according to baseline kidney function data from the CANVAS program. Circulation 2018;138:1537–50.

29. Bhatt DL, Szarek M, Pitt B, et al. Sotagliflozin in Patients with Diabetes and Chronic Kidney Disease. N Engl J Med 2021;384:129–39.

30. Althouse AD. Adjust for Multiple Comparisons? It's Not That Simple. Ann Thorac Surg 2016;101:1644–5.

FIGURE LEGENDS

Figure 1. Baseline Distributions of Biomarkers. Histograms of high-sensitivity cardiac troponin T (hs-cTnT) (Panel A), soluble ST2 (sST2) (Panel B), and insulin-like growth factor–binding protein 7 (IGFBP7) (Panel C) in log scale. The vertical dotted line reflects previously applied thresholds to identify elevated biomarker levels. hs-cTnT = high-sensitivity cardiac troponin T; IGFBP7 = insulin-like growth factor–binding protein 7; sST2 = soluble suppression of tumorigenesis-2.

Figure 2. Stress Cardiac Biomarkers at Baseline and in Follow-up. Given skewed distributions, each biomarker is summarized as geometric means and the proportion of participants with abnormal elevated biomarker levels based on previously established cut-points (8–10). CANVAS = CANagliflozin cardioVascular Assessment Study; CI = confidence interval; hs-cTnT = high-sensitivity cardiac troponin T; IGFBP7 = insulin-like growth factor–binding protein 7; sST2 = soluble suppression of tumorigenesis-2.

Figure 3. Multi-marker Risk Panel and Cardiovascular and Kidney Outcomes. Among 3,040 patients with available baseline data for all 3 biomarkers of interest, a multi-marker panel was created, assigning 1 point for each abnormally elevated biomarker (total score range, 0–3). Major adverse cardiovascular events (MACE) include cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. CANVAS = CANagliflozin cardioVascular Assessment Study; CI = confidence interval; eGFR = estimated glomerular filtration rate; HF = heart failure; HRadj = adjusted hazard ratio; Ref = reference.

*Covariates for adjustment were selected using the Bayesian Information Criterion in the modified intention-to-treat population of patients randomized to placebo in the main study along with all 3 baseline biomarker levels and treatment allocation.

†The C-index presented incorporates both the multi-marker score and a base model of covariates. In parentheses, the improvement in the C-index is shown as the change above a base clinical model excluding the multi-marker score.

‡Renal composite endpoint from CANVAS comprised a 40% decrease in eGFR (sustained for ≥2 consecutive measures), the need for kidney replacement therapy (dialysis or transplantation), or renal death.

Figure 4. Treatment Effects of Canagliflozin on Major Adverse Cardiovascular Events based on Single or Multiple Abnormal Circulating Biomarkers

Relative treatment effects are displayed as hazard ratios (HR) with 95% confidence intervals in brackets. Absolute risk reductions (ARR) represent the absolute risk difference in incidence rates (per 100 patient-years).

hs-cTnT = high-sensitivity cardiac troponin T; IGFBP7 = insulin-like growth factor–binding protein 7; sST2 = soluble suppression of tumorigenesis-2.

Central Illustration. Stress Cardiac Biomarkers, Adverse Outcomes, and Response to Canagliflozin. CANVAS = CANagliflozin cardioVascular Assessment Study; hs-cTnT = high-sensitivity cardiac troponin T; IGFBP7 = insulin-like growth factor–binding protein 7; sST2 = soluble suppression of tumorigenesis-2; T2DM, type 2 diabetes mellitus.

Table 1. Representative Characteristics of CANVAS Study Participants with Available Baseline hs-cTnT Samples

Characteristic	Canagliflozin (n = 2,339)	Placebo (n = 1,164)
Age, years	62.8 (7.9)	62.5 (7.8)
Women, n (%)	780 (33)	391 (34)
Race, n (%)		
White	1,917 (82)	957 (82)
Asian	214 (9)	106 (9)
Black	55 (2)	31 (3)
eGFR, mL/min/1.73 m^2	77.1 (18.7)	76.8 (18.9)
Body mass index, kg/m^2	32.8 (6.1)	32.6 (6.2)
Systolic blood pressure, mmHg	136.4 (15.9)	137.4 (15.7)
Glycated hemoglobin, %	8.2 (0.9)	8.1 (0.9)
Diabetes duration, years	13.6 (7.5)	13.3 (7.5)
UACR, mg/g	85.3 (328.0)	78.5 (311.8)
History of heart failure, n (%)	300 (13)	170 (15)
Diuretic use, n (%)	1,088 (47)	538 (46)
Insulin use, n (%)	1,230 (53)	602 (52)
Metformin use, n (%)	1,701 (73)	838 (72)
Sulfonylurea use, n (%)	1,018 (44)	506 (43)
Glucagon-like peptide-1 receptor agonists use, n (%)	60 (3)	25 (2)
Renin-angiotensin-aldosterone system inhibitor use, n (%)	1,910 (82)	953 (82)
Statin use, n (%)	1,744 (75)	843 (72)
β-blocker use, n (%)	1,193 (51)	590 (51)

CANVAS = CANagliflozin cardioVascular Assessment Study; eGFR = estimated glomerular filtration rate; hs-cTnT = high-sensitivity cardiac troponin T; UACR = urine albumin:creatinine ratio.

All continuous variables are expressed as mean (standard deviation).

Table 2. Elevated Biomarkers, Cardiovascular and Kidney Outcomes, and Treatment Effect Modification With Canagliflozin in the CANVAS Trial

A) hs-cTnT	hs-cTnT ≥14 pg/mL vs <14 pg/mL		
Event	**HR_{adj} (95% CI)***	***P* value**	**Canagliflozin-Biomarker $P_{interaction}$**
MACE†	2.30 (1.71–3.11)	<0.001	0.05
Hospitalization for HF	3.90 (2.01–7.56)	<0.001	0.73
Hospitalization for HF or cardiovascular death	2.96 (2.01–4.36)	<0.001	0.44
All-cause death	2.35 (1.65–3.34)	<0.001	0.92
Cardiovascular death	2.85 (1.81–4.49)	<0.001	0.48
Nonfatal stroke	1.54 (0.87–2.74)	0.14	0.55
Nonfatal myocardial infarction	2.44 (1.49–3.99)	<0.001	0.04
Renal composite‡	2.06 (1.21–3.51)	0.008	0.71
Renal composite‡ **or cardiovascular death**	2.40 (1.69–3.41)	<0.001	0.63

B) sST2	sST2 ≥35 ng/mL vs <35 ng/mL		
Event	**HR_{adj} (95% CI)***	***P* value**	**Canagliflozin-Biomarker $P_{interaction}$**
MACE†	1.95 (1.22–3.11)	0.005	0.04
Hospitalization for HF	1.98 (0.77–5.06)	0.154	0.47
Hospitalization for HF or cardiovascular death	2.08 (1.19–3.65)	0.01	0.32
All-cause death	2.06 (1.20–3.53)	0.009	0.22

Cardiovascular death	2.43 (1.28–4.58)	0.006	0.08
Nonfatal stroke	1.57 (0.56–4.40)	0.39	0.88
Nonfatal myocardial infarction	1.94 (0.88–4.26)	0.10	0.13
Renal composite‡	1.52 (0.65–3.58)	0.34	0.45
Renal composite‡ or cardiovascular death	2.00 (1.18,3.38)	0.010	0.38

C) IGFBP7	**IGFBP7 ≥96.5 ng/mL vs <96.5 ng/mL**		
Event	**HR_{adj} (95% CI)***	***P* value**	**Canagliflozin-Biomarker $P_{interaction}$**
MACE†	1.51 (1.12–2.02)	0.007	0.15
Hospitalization for HF	2.49 (1.29–4.83)	0.007	0.58
Hospitalization for HF or cardiovascular death	2.07 (1.41–3.05)	<0.001	0.28
All-cause death	1.72 (1.21–2.44)	0.003	0.96
Cardiovascular death	1.99 (1.26–3.14)	0.003	0.57
Nonfatal stroke	1.53 (0.86–2.72)	0.15	0.05
Nonfatal myocardial infarction	1.28 (0.80–2.05)	0.30	0.52
Renal composite‡	2.68 (1.53–4.68)	<0.001	0.25
Renal composite‡ or cardiovascular death	2.19 (1.53–3.14)	<0.001	0.18

CANVAS = CANagliflozin cardioVascular Assessment Study; CI = confidence interval; eGFR = estimated glomerular filtration rate; hs-cTnT = high-sensitivity cardiac troponin T; HF = heart

failure; HR_{adj} = adjusted hazard ratio; IGFBP7 = insulin-like growth factor–binding protein 7; MACE = major adverse cardiovascular events; sST2 = soluble suppression of tumorigenesis-2.
*Covariates for adjustment were selected using the Bayesian Information Criterion in the modified intention-to-treat population of patients randomized to placebo in the main study along with baseline biomarker level and treatment allocation.
†Major adverse cardiovascular events include cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke.
‡Renal composite endpoint from CANVAS comprised a 40% decrease in eGFR (sustained for ≥2 consecutive measures), the need for kidney replacement therapy (dialysis or transplantation), or renal death.

Figure 1. Baseline Distributions of Biomarkers.

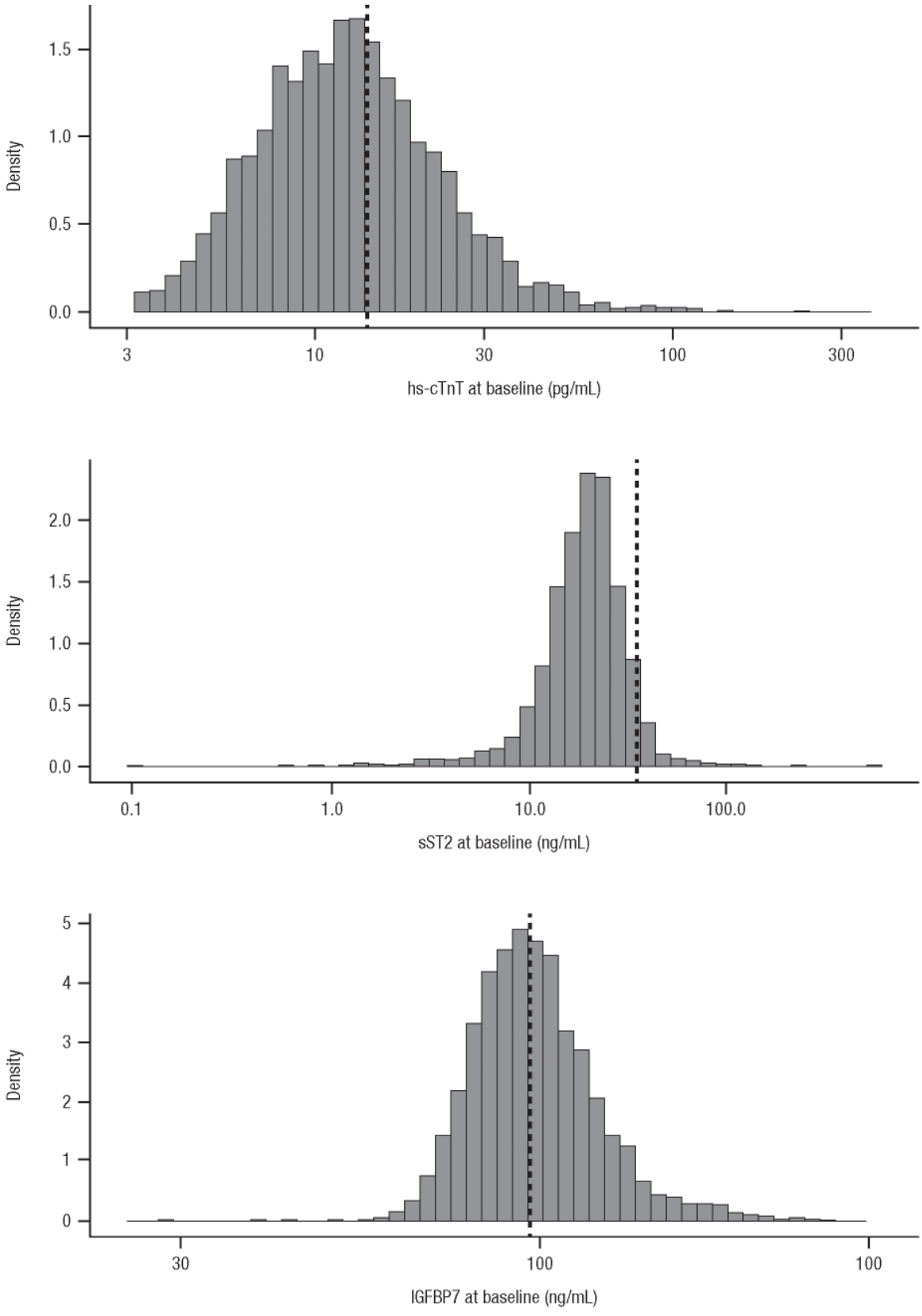
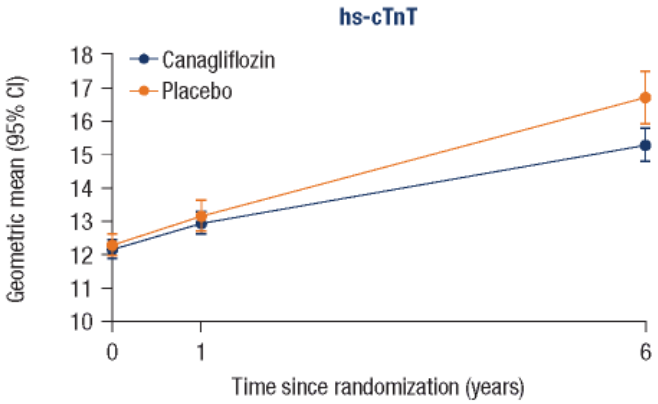
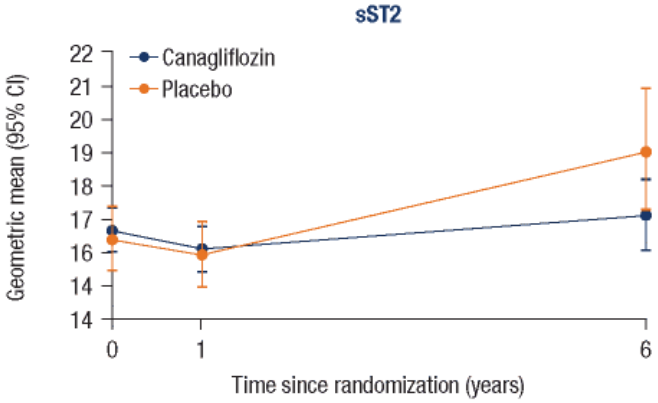


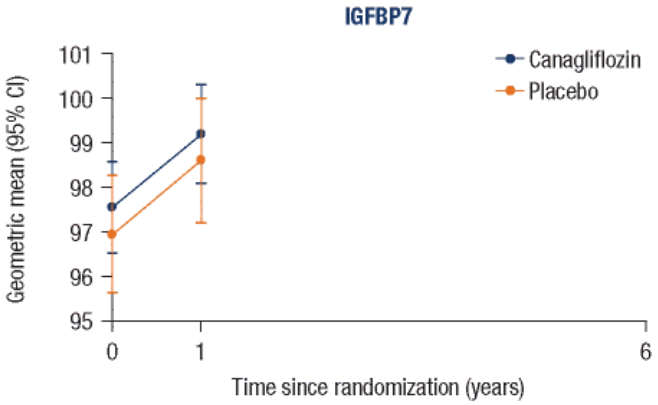
Figure 2. Stress Cardiac Biomarkers at Baseline and in Follow-up.



Treatment assignment	Visit	hs-cTnT n	hs-cTnT Geometric mean	hs-cTnT >14 pg/mL, n (%)
Placebo	Baseline	1,164	12.2	439 (38)
	Year 1	964	13.0	420 (44)
	Year 6	297	16.0	174 (59)
Canagliflozin	Baseline	2,339	12.1	913 (39)
	Year 1	2,034	12.9	871 (43)
	Year 6	703	15.0*	380 (54)



Treatment assignment	Visit	sST2 n	sST2 Geometric mean	sST2 >35 ng/mL, n (%)
Placebo	Baseline	1,022	16.3	70 (7)
	Year 1	885	15.7	50 (6)
	Year 6	297	19.0	24 (8)
Canagliflozin	Baseline	2,062	16.6	110 (5)
	Year 1	1,875	16.0	95 (5)
	Year 6	703	16.9*	48 (7)



Treatment assignment	Visit	IGFBP7 n	IGFBP7 Geometric mean	IGFBP7 >96.5 ng/mL, n (%)
Placebo	Baseline	1,193	96.9	581 (49)
	Year 1	963	98.6	480 (50)
Canagliflozin	Baseline	2,384	97.5	1,164 (49)
	Year 1	2,031	99.1	1,054 (52)

Figure 3. Multi-marker Risk Panel and Cardiovascular and Kidney Outcomes.

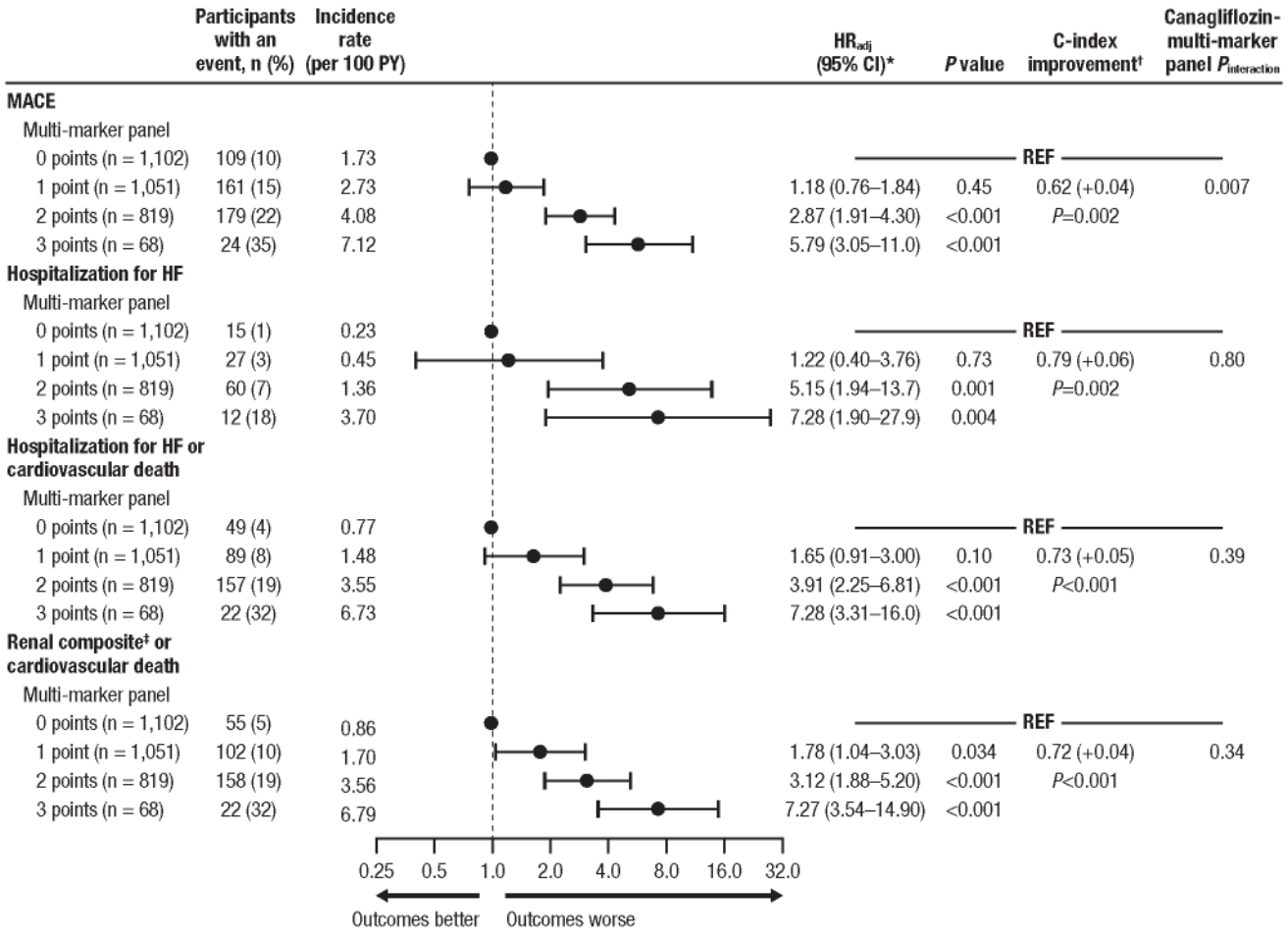
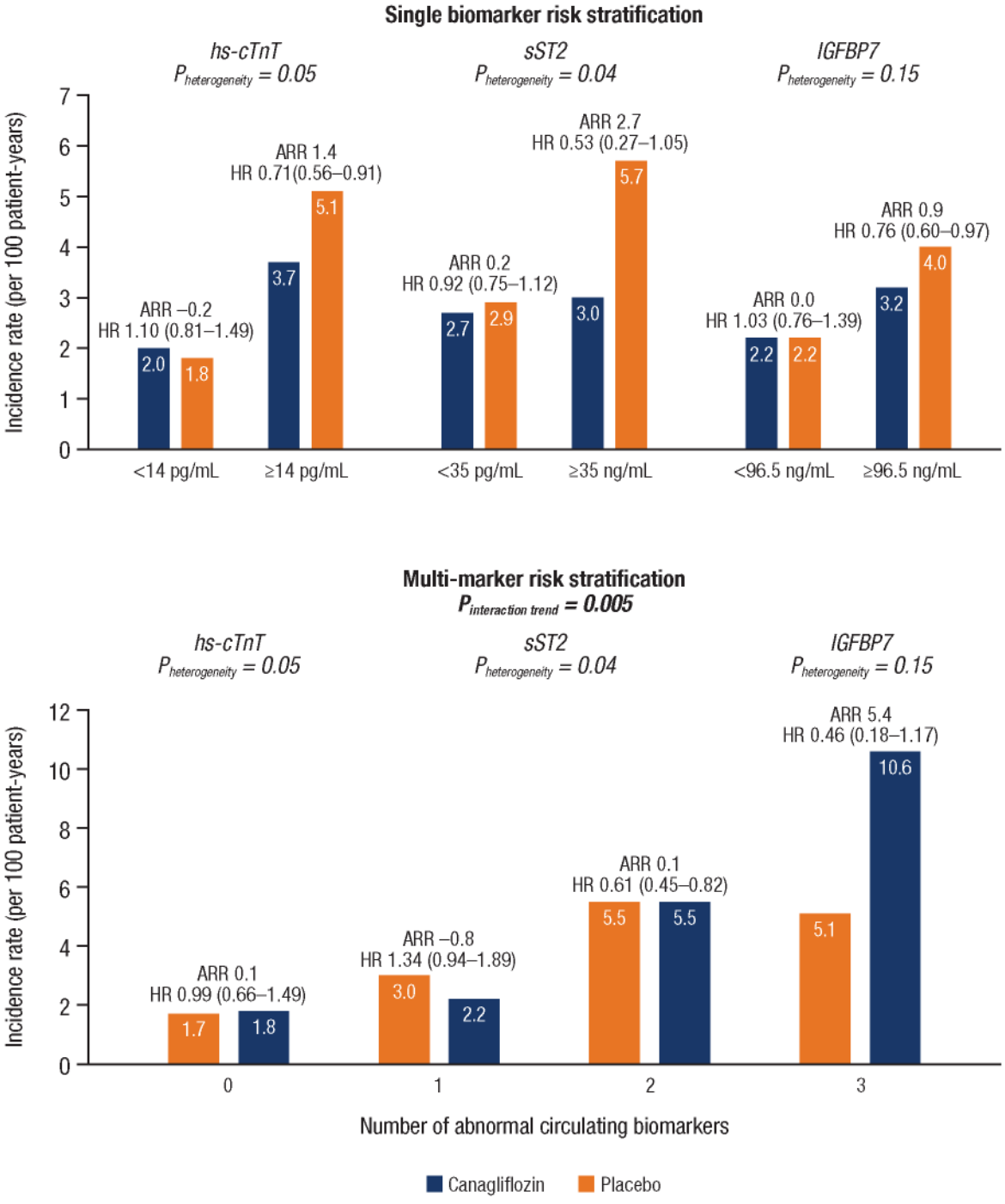


Figure 4. Treatment Effects of Canagliflozin on Major Adverse Cardiovascular Events based on Single or Multiple Abnormal Circulating Biomarkers



Central Illustration. Stress Cardiac Biomarkers, Adverse Outcomes, and Response to Canagliflozin.

