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## Self-sensing composite material based on piezoelectric nanofibers

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## **Mechanisms of action of the SGLT2 inhibitor canagliflozin on tubular inflammation and damage: a *post-hoc* mediation analysis of the CANVAS trial**

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## Abstract

**Aims:** Exposure of tubular cells to albumin stimulates pro-inflammatory pathways including the release of Monocyte Chemoattractant Protein-1 (MCP-1) which may result in interstitial fibrosis and tubular damage reflected by increased urinary kidney injury molecule-1 (KIM-1). SGLT2 inhibition reduces urine albumin-creatinine ratio (UACR) and small studies suggest it also reduces MCP-1 and KIM-1. We hypothesised that the reduction in KIM-1 observed with the SGLT2 inhibitor canagliflozin is mediated through its effect on UACR and MCP-1. To test this hypothesis, we assessed the proportion of effect of canagliflozin on KIM-1 mediated through its effects on MCP-1 and UACR in patients with type 2 diabetes and albuminuric kidney disease.

**Material and methods:** KIM-1 and MCP-1 were measured in urine samples of the CANVAS trial at baseline and week 52 with the Mesoscale QuickPlex SQ 120 platform. KIM-1 and MCP-1 were standardized by urinary creatinine. The proportion of mediated effect of canagliflozin through UACR and MCP-1/Cr on KIM-1/Cr was estimated with G-computation.

**Results:** In total, 763 (17.6% of total cohort) patients with micro- or macroalbuminuria were included. Baseline characteristics were well balanced between the canagliflozin and placebo group. At year 1, canagliflozin compared to placebo reduced UACR, MCP-1/Cr, and KIM-1/Cr by 40.4% (95%CI 31.0, 48.4), 18.1% (95%CI 8.9, 26.4), and 30.9% (95%CI 23.0, 38.0), respectively. The proportion of the effect of canagliflozin on KIM-1/Cr mediated by its effect on UACR and in turn on MCP-1/Cr was 15.2% (95%CI 9.4, 24.5).

**Conclusion:** Canagliflozin reduces urinary KIM-1 suggesting decreased tubular damage.

This effect was partly mediated through a reduction in MCP-1, indicative of reduced tubular inflammation, which was in turn mediated by a reduction in UACR. This post-hoc analysis suggest that urinary albumin leakage may lead to tubular inflammation and induction of injury, and provide mechanistic insight for how canagliflozin may ameliorate tubular damage, but further research is required to confirm these findings.

## Introduction

Clinical outcome trials have proven that sodium-glucose cotransporter type 2 (SGLT2) inhibitors have beneficial effects on kidney and heart failure outcomes in patients with and without diabetes and at varying stages of chronic kidney disease.(1,2) Although the mechanisms for kidney protection with SGLT2 inhibition are not completely understood, reductions in urinary albumin to creatinine ratio (UACR) contribute to the kidney protective effects as reported previously.(3,4) Some studies hypothesize that the reduction in intraglomerular pressure, which may precede a reduction in UACR, is a plausible pathway for the kidney protective effect of SGLT2 inhibitors.(5)

Patients with micro- or macroalbuminuria show higher degrees of tubular inflammation which may be due to increased exposure of tubular cells to albumin.(6-8) This stimulates pro-inflammatory cytokines, such as Monocyte Chemoattractant Protein-1 (MCP-1), a key inflammatory regulator in the kidney, which may result in interstitial fibrosis and tubular damage, reflected by an increased urinary kidney injury molecule-1 (KIM-1). Experimental studies have reported time and concentration-dependent increases in MCP-1 as a result of increased exposure to albumin.(9,10) Moreover, inhibition of MCP-1 reduces recruitment of monocytes in the tubules and thereby reduces tubular inflammation.(11) KIM-1, a well-established marker of tubular cell injury, shows similar time and concentration dependent relations to albumin as MCP-1.(12,13) KIM-1 is released in the setting of inflammation and inhibition of KIM-1 with specific pharmacological inhibitors ameliorates tubular fibrosis.(14) Previous studies have shown decreased urinary MCP-1 and KIM-1 in response to treatment with SGLT2 inhibitors.(15-17)

In this *post-hoc* analysis of the CANagliflozin cardioVascular Assessment Study (CANVAS) trial we hypothesised that the reduction in KIM-1 observed with SGLT2 inhibitors may be mediated, entirely or in part, through its effect on UACR and MCP-1. To test this hypothesis, we assessed the proportion of the effect of canagliflozin on KIM-1 explained by its effects on UACR and MCP-1 in participants with type 2 diabetes and micro- or macroalbuminuria.

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## Methods

### *Patients and study design*

This study was a *post-hoc* analysis of the CANVAS trial, which was a prospective, multi-centre, double-blind, placebo-controlled, randomised trial to assess the efficacy on primarily the cardiovascular and secondarily the kidney outcome and safety of the SGLT2 inhibitor canagliflozin. The trial was done in participants with type 2 diabetes at high risk for cardiovascular disease or who had a history of cardiovascular disease. The study design and main result of the CANVAS trial have been published previously.<sup>(1)</sup> In brief, a total of 4330 participants were enrolled and were randomly assigned using a web-based response system in a 1:1:1 ratio to canagliflozin 300 mg, canagliflozin 100 mg or matching placebo. The median follow-up duration during the trial was 6.1 years. During the study, all participants, care providers, trial staff and outcome assessors were blinded to treatment randomisation. The trial was approved by an ethics committee at each site and was conducted according to the principles of the Declaration of Helsinki. The trial is registered with clinicaltrials.gov (NCT01032629). All participants were given the opportunity to also provide informed consent for the collection of blood and urine samples for future exploratory biomarker research. This was optional and separate from the informed consent provided for the main trial.

Participants eligible for randomisation were diagnosed with type 2 diabetes with a glycated haemoglobin (HbA1c) of  $\geq 7.0\%$  and  $\leq 10.5\%$ , had an estimated glomerular filtration rate (eGFR) of  $>30$  mL/min/1.73m<sup>2</sup> and were either aged  $\geq 30$  years with a history of symptomatic atherosclerotic cardiovascular disease, or aged  $\geq 50$  years with  $\geq 2$  risk factors for cardiovascular disease. The risk factors were defined as a diabetes duration of at least 10 years, a systolic blood pressure  $>140$  mmHg, receiving  $>1$  antihypertensive agent, current smoking, micro- or macroalbuminuria, or a HDL-cholesterol level of  $<1$  mmol/L. Participants also needed to meet other criteria for inclusion as described previously.<sup>(1)</sup>

For this *post-hoc* exploratory biomarker study we included only patients in whom urine samples were collected (N=3475) and further excluded patients with normoalbuminuria at baseline (UACR $<30$  mg/g; N=2712), since canagliflozin does not decrease albuminuria in

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patients with normoalbuminuria precluding assessment of potential mediating effects in these patients.

### *Biomarker assessment*

Urine samples for exploratory biomarker research obtained at baseline and 52 weeks after randomisation were stored at -80°C. Urinary MCP-1 and KIM-1 at baseline and week 52 were measured as markers of inflammation and tubular cell injury, respectively. Urine biomarkers were measured using the Mesoscale QuickPlex SQ 120 platform (Meso Scale Diagnostics (MSD), Rockville, MD, USA), which is a high-performance electrochemiluminescence immunoassay. Samples were measured between April 2019 and February 2020. A random sample of 381 were measured in duplicates. The mean CV (SD) of these samples was 9% (11) and 6% (5) for urinary MCP-1 and KIM-1, respectively.

### *Statistical analysis*

Baseline characteristics with normal distributions were reported as means with standard deviations and skewed distributions were reported as median with interquartile ranges and were logarithmic transformed before analysis. Categorical baseline characteristics were reported as percentages.

Each urine biomarker was indexed to urine creatinine concentrations to adjust for hydration status. The effect of canagliflozin on MCP-1, UACR, and KIM-1, were assessed with analysis of covariance adjusted for the change in biomarker (MCP-1, UACR, and KIM-1 each indexed to urine creatinine) from baseline to week 52. The 1-year change in biomarker from baseline and the effect of canagliflozin on the biomarker adjusted for placebo were reported as percentages with 95% CI. The effect of canagliflozin on these biomarkers was also assessed in subgroups of UACR (micro- or macroalbuminuria) and eGFR (<60 or  $\geq 60$  ml/min/1.73m<sup>2</sup>).

We estimated the effect of canagliflozin on KIM-1/Cr explained by MCP-1/Cr and UACR using g-computation. This consisted of first specifying linear models for the main

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effects of 1) canagliflozin on UACR, 2) canagliflozin and UACR on MCP-1/Cr, and 3) canagliflozin, UACR, and MCP-1/CR on KIM-1/Cr. These models enabled us to produce counterfactual scenarios in the g-formula to estimate firstly the percentage of the effect of canagliflozin on KIM-1/Cr not explained by its effect on MCP-1/Cr and UACR; secondly, the percentage of the effect of canagliflozin on KIM-1/Cr, which was explained by MCP-1/Cr or UACR alone; and thirdly, the percentage of the effect of canagliflozin on KIM-1 explained by its effect on MCP-1/Cr, which was in turn explained by its effect on UACR. Standard errors were calculated using a non-parametric bootstrap. Additional details about the g-computation are provided in Supplementary File 1. All biomarkers were logarithmic transformed before entering them in the analyses.

All analysis were performed in Stata/SE Version 17.0 (StataCorp LCC, College Station, TX, USA) or R Version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). P values <0.05 were considered statistically significant for all analyses.

## Results

### *Study population*

Out of the 4330 participants in the CANVAS trial, 763 (17.6%) had micro- or macroalbuminuria and urine samples at both baseline and week 52 to measure MCP-1 and KIM-1. Baseline characteristics of these 763 subjects generally were similar to the overall CANVAS population with the exception that systolic blood pressure was slightly higher and a history of heart failure was more frequently present (Supplement table 1). The baseline characteristics of the 763 included participants were well balanced between the canagliflozin and placebo groups. The mean age of the participants was 62.9 (7.7) years, 560 (73.4%) were male, mean duration of type 2 diabetes was 14.5 (7.4) years, mean HbA1c was 8.4% (0.9), mean eGFR was 77.2 ml/min/1.73m<sup>2</sup> (17.7) and median UACR was 8.9 mg/mmol (IQR 4.8, 25.6). Median MCP-1/Cr and KIM-1/Cr were 26.3 ng/mmol (IQR 17.8, 42.0) and 110.5 ng/mmol (IQR 61.8, 186.0), respectively (Table 1).

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### *Effect of canagliflozin on urinary MCP-1/Cr and urinary KIM-1/Cr*

Canagliflozin reduced UACR by 40.4% (95% CI 31.0, 48.4) compared to placebo in this study sample, similar to observations reported in the entire CANVAS trial.<sup>(18)</sup> Canagliflozin significantly reduced both MCP-1/Cr and KIM-1/Cr compared to placebo by 18.1% (95% CI 8.9, 26.4) and 30.9% (95% CI 23.0, 38.0), respectively (Figure 1). The reduction in MCP-1/Cr and KIM-1/Cr with canagliflozin compared to placebo was also observed in subgroups by UACR ( $\geq 3$ ,  $< 30$  mg/mmol and  $\geq 30$  mg/mmol) and eGFR ( $< 60$  ml/min/1.73m<sup>2</sup> and  $\geq 60$  ml/min/1.73m<sup>2</sup>), although the proportional effect of canagliflozin on MCP-1/Cr was more pronounced in patients with baseline UACR  $\geq 30$  mg/mmol (p for interaction 0.02) or eGFR  $< 60$  ml/min/1.73m<sup>2</sup> (p for interaction 0.01; Table 2).

### *Effect of canagliflozin on KIM-1 mediated through effect on MCP-1/Cr and UACR*

For each biomarker (UACR, MCP-1/Cr, and KIM-1/Cr) we calculated the 1-year change from baseline and modelled these to estimate the direct and indirect effects of canagliflozin on UACR, MCP-1/Cr, and KIM-1/Cr. The direct effect of canagliflozin compared to placebo on KIM-1/Cr was 53.4% (95% CI 39.2, 68.4; Figure 2). The effect of canagliflozin on KIM-1/Cr explained by its effect on UACR or MCP-1/Cr alone was 8.2% (95% CI 3.1, 15.3) and 23.2% (95% CI 5.6, 37.1), respectively (Figure 2). The percentage of the effect of canagliflozin on KIM-1/Cr explained by its effect through UACR and subsequently MCP-1/Cr was 15.2% (95% CI 9.4, 24.5; Figure 2).

## **Discussion**

The underlying mechanisms for how SGLT2 inhibitors exert kidney protection is not completely understood. Still, there are many theories proposed regarding the mechanistic effects including a reduction in intra-glomerular pressure and intra-renal inflammation.<sup>(19)</sup> In this study we aimed to connect some of these pathways and demonstrated that the reduction in KIM-1/Cr, indicative of kidney damage, is to some extent explained by a reduction in MCP-

1/Cr, indicative of intra-renal inflammation, which in turn was partly explained by the reduction in UACR.

Several small clinical studies have previously demonstrated that SGLT2 inhibitors decrease urinary MCP-1 and KIM-1 levels. Dapagliflozin compared to placebo reduced KIM-1/Cr in patients with type 2 diabetes after 6 weeks treatment by approximately 23% relative to placebo.(17) Similar findings were observed in two other small placebo controlled studies with dapagliflozin in patients with type 2 diabetes.(15,16) However, previous studies included only a small number of participants and were of short duration. In the current study we extend the previous findings to a large cohort of patients with type 2 diabetes and provide robust evidence that in this setting canagliflozin compared to placebo reduces markers of kidney inflammation and injury over at least 52 weeks. These effects appeared particularly pronounced in patients with macroalbuminuria compared to microalbuminuria.

How canagliflozin reduces urinary KIM-1, a well-established marker of tubule cell injury, is not completely understood. Based on a previous study that demonstrated that the degree of albuminuria reduction was associated with the degree of urinary KIM-1 reduction during treatment with dapagliflozin(17), and because experimental studies have demonstrated that enhanced exposure of tubular cells to albumin stimulates pro-inflammatory responses, including the release of MCP-1, NFK- $\beta$  and endothelin-1(20), we hypothesised that the reduction in urinary KIM-1 is explained by reduced intra-renal inflammation and albuminuria. Our results support our hypothesis, suggesting that at least a part of the effect of canagliflozin on tubular cell injury was partly mediated by a reduction in intra-renal inflammation, which in turn was mediated by a reduction in albuminuria. In this respect, it is of interest to note that early reduction in albuminuria has been associated with the acute decline in eGFR on initiation of SGLT2 inhibition.(5) The acute decline in eGFR is a clinical manifestation of the reduction in intra-glomerular pressure and glomerular hyperfiltration observed with all SGLT2 inhibitors. We therefore speculate that by reducing glomerular hyperfiltration, albumin leakage across the glomerular membrane decreases which results in decreased tubular exposure, inflammation, and damage.

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Previous studies have shown that KIM-1 is associated with fibrosis in tubular tissues. Experimental and clinical studies have suggested that SGLT2 inhibitors exert anti-fibrotic effects which may contribute to the observed reduction in urinary KIM-1 in our study.<sup>(13)</sup> Further clinical studies are needed collecting preferably kidney tissue biopsies to delineate the potential anti-fibrotic effects of SGLT2 inhibitors

Key strengths of this study are its sample size relative to prior studies evaluating changes in biomarkers in response to SGLT2 inhibitors, the randomised controlled trial setting, and the 52-week study observation period. This study also has limitations. Firstly, an inherent limitation to any mediation analyses is that we cannot be certain that the identified mediators are truly on the causal pathway and therefore our results are at best viewed as hypothesis generating and the proportion of the total effect of canagliflozin on KIM-1 explained by MCP-1 and in turn UACR may be overestimated. Second, there may have been other pro-inflammatory substances involved which we did not measure. Third, UACR and MCP-1 were measured concurrently which makes it impossible to be sure that effects on MCP-1 were attributable to reductions in UACR rather than the reverse, that effects on UACR were mediated by MCP-1. Each biomarker evaluated here was measured only once at baseline and after 52 weeks, and there is inherent measurement error with any biomarker. Thus, the measurements may not fully capture the biology of albuminuria, inflammation, and damage which would underestimate the degree of mediation observed here. Moreover, SGLT2 inhibitors increase urinary glucose excretion which may reduce inflammation independent of reductions in albuminuria. This, or other as yet unexplored pathways, may explain why the proportion of the total effect explained was only 15%, when a higher proportion might have been anticipated based on our broader knowledge of the pathophysiology. Finally, our mediation analyses were limited by their capacity to control for interaction between variables.

In summary, we demonstrate that, compared to placebo, canagliflozin reduces KIM-1/Cr, indicative of decreased tubule cell injury, in a large cohort of participants with type 2 diabetes, established cardiovascular disease or multiple cardiovascular risk factors and

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micro- or macroalbuminuria. This effect was partly mediated through a reduction in MCP-1/Cr, indicative of reduced tubular inflammation, which was in turn mediated by a reduction in UACR. These data suggest that urinary albumin leakage may lead to tubular inflammation and induction of tubule cell injury, and provide new mechanistic insight for how canagliflozin may ameliorate tubular damage.

### **Acknowledgments and funding**

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### **Conflicts of interest**

T. Sen, A. Koshino, M.J. Bijlsma and J. Li have nothing to disclose.

B. Neal is an employee of the George Institute for Global Health and is supported by a National Health and Medical Research Council Investigator Grant. His institution has received fees for his roles in advisory boards, steering committees, or scientific presentations from AstraZeneca, Janssen, Merck, and Mundipharma.

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M.K. Hansen is an employee of Janssen Research & Development, LLC

J.H. Ix is serving on the National Academy of Medicine panel “Review of the Dietary Reference Intakes for Sodium and Potassium” and is receiving research grant support from the National Institute of Diabetes and Digestive and Kidney Diseases; the National Heart, Lung, and Blood Institute; and the American Heart Association.

H.J.L. Heerspink has served as a consultant for AbbVie, AstraZeneca, Bayer, Boehringer Ingelheim, Chinook, CSL Behring, Dimerix, Eli-Lilly, Gilead, Goldfinch, Janssen, Merck, Novo Nordisk, and Travele Pharmaceuticals; and has received grant support from AbbVie, AstraZeneca, Boehringer Ingelheim, Janssen, and Novo Nordisk.

### **Author contributions**

TS, AK, BN contributed to collection of the data. TS and MB performed statistical analysis. All authors contributed to the interpretation of the data. TS and HJLH wrote the first draft of the manuscript. BN, CA, MKH and HJLH were involved in the design. All authors provided critical revision for important intellectual content and approved the final version of the manuscript for submission. The corresponding author (HJLH) takes full responsibility for the work and/or the conduct of the study, had access to the data and controlled the decision to publish.

### **Data sharing statement**

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Deidentified participant data will be made available on reasonable request 2 years after the date of publication. Requests should be directed to the senior author (Hiddo JL Heerspink). Requestors will be required to send a protocol, statistical analysis plan and sign a data access agreement to ensure the appropriate use of the study data.

### Legend to figures

**Figure 1.** Geometric means (95% CI) of MCP-1/Cr and KIM-1/Cr at baseline and week 52 in the placebo and canagliflozin group.

**Figure 2.** Mediation of canagliflozin through direct and other pathways (green), UACR (red), MCP-1/Cr (marker of inflammation; blue) and both MCP-1/Cr and UACR (yellow) on KIM-1/Cr (marker of tubular damage).

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**Table 1.** Baseline characteristics of the total, placebo and canagliflozin-treated group.

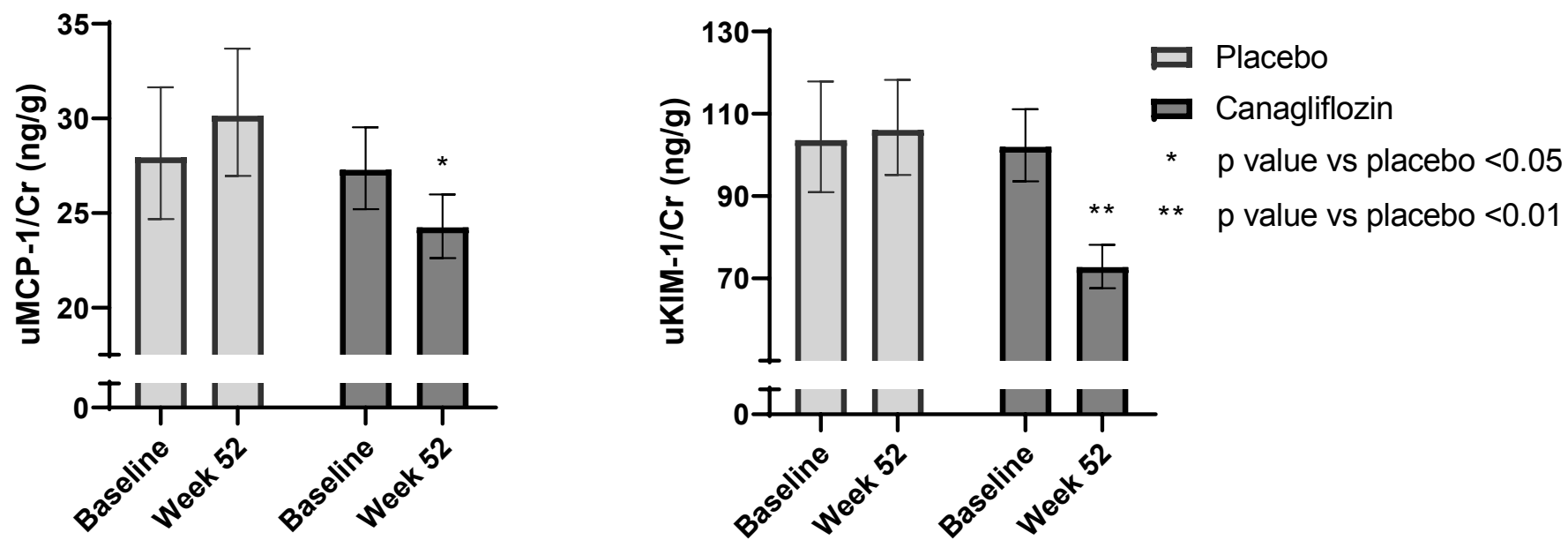
Characteristic	Total N=763	Placebo N=239	Canagliflozin N=524
Age, years	62.9 (7.7)	62.9 (7.7)	62.9 (7.7)
Male sex, <i>n</i> (%)	560 (73.4)	171 (71.6)	389 (74.2)
Current smoker, <i>n</i> (%)	142 (18.6)	53 (22.2)	89 (17.0)
Race, <i>n</i> (%)			
White	618 (81.0)	193 (80.8)	425 (81.1)
Asian	92 (12.1)	27 (11.3)	65 (12.4)
Other	53 (6.9)	19 (7.9)	34 (6.5)
History of HF, <i>n</i> (%)	114 (15.0)	37 (15.5)	77 (14.7)
Duration of diabetes, years	14.5 (7.4)	13.9 (7.8)	14.7 (7.2)
History of CVD, <i>n</i> (%)	435 (57.0)	128 (53.6)	307 (58.6)
BMI, kg/m <sup>2</sup>	32.6 (6.0)	32.4 (5.4)	32.7 (6.3)
Systolic BP, mmHg	141.2 (16.2)	142.1 (16.0)	140.8 (16.2)
Diastolic BP, mmHg	78.3 (9.9)	79.3 (9.7)	77.9 (10.0)
HbA <sub>1c</sub>			
mmol/mol	67.9 (9.9)	67.4 (9.6)	68.2 (10.0)
%	8.4 (0.9)	8.3 (0.9)	8.4 (0.9)
eGFR, ml min <sup>-1</sup> [1.73 m] <sup>-2</sup>	77.2 (17.7)	78.8 (16.0)	76.5 (18.3)
eGFR <60, <i>n</i> (%)	130 (17.0)	30 (12.6)	100 (19.1)
eGFR ≥60, <i>n</i> (%)	633 (83.0)	209 (87.4)	424 (80.9)
UACR, mg/mmol (IQR)	8.9 (4.8, 25.6)	9.0 (5.0, 29.9)	8.8 (4.8, 23.6)
Microalbuminuria, <i>n</i> (%)	603 (79.0)	180 (75.3)	423 (80.7)
Macroalbuminuria, <i>n</i> (%)	160 (21.0)	59 (24.7)	101 (19.3)
uMCP-1, pg/ml (IQR)	210 (123, 348)	224 (123, 371)	206 (123, 335)
uKIM-1, pg/ml (IQR)	805 (423, 1505)	790 (390, 1492)	817 (437, 1526)
MCP-1/Cr, ng/mmol (IQR)	26.3 (17.8, 42.0)	27.4 (18.6, 44.6)	25.7 (17.3, 41.8)
KIM-1/Cr, ng/mmol (IQR)	110.5 (61.8, 186.0)	105.3 (62.1, 188.4)	111.6 (61.2, 183.8)

Abbreviations: HF: heart failure; CVD: cardiovascular disease; BMI: body mass index; BP: blood pressure.

**Table 2.** Changes in UACR, MCP-1/Cr and KIM-1/Cr in the canagliflozin and placebo group from baseline to week 52 overall and in participant subgroups defined by baseline UACR and eGFR.

Biomarker	Baseline biomarker in canagliflozin	Baseline biomarker in placebo	Canagliflozin change, % (95% CI)	Placebo change, % (95% CI)	Placebo corrected effect canagliflozin, % (95% CI)	<i>p</i> interaction
UACR, mg/mmol	2.2	2.2	-43.4 (-47.8, -38.5)	-5.0 (-15.8, 7.1)	-40.4 (-48.4, -31.0)	
UACR, mg/mmol						0.01
≥3, <30	1.9	2.0	-39.7 (-45.0, -34.0)	-9.8 (-21.4, 3.6)	-33.2 (-43.4, -21.2)	
≥30	4.2	4.3	-55.0 (-62.3, -46.3)	6.1 (-16.0, 33.9)	-57.6 (-68.4, -43.1)	
eGFR, ml min <sup>-1</sup> [1.73 m] <sup>-2</sup>						0.83
<60	2.6	2.4	-38.9 (-50.6, -24.2)	8.2 (-26.4, 59.9)	-43.5 (-63.6, -12.2)	
≥60	2.1	2.2	-44.5 (-49.2, -39.4)	-6.3 (-17.3, 6.2)	-40.8 (-49.2, -31.0)	
MCP-1/Cr, ng/mmol	25.7	27.4	-11.8 (-16.9, -6.3)	7.7 (-1.4, 17.6)	-18.1 (-26.4, -8.9)	
UACR, mg/mmol						0.02
≥3, <30	24.7	24.8	-7.7 (-13.9, -1.2)	5.0 (-5.4, 16.6)	-12.1 (-22.5, -0.4)	
≥30	31.0	36.3	-25.3 (-33.4, -16.3)	12.2 (-3.4, 30.4)	-33.5 (-44.9, -19.6)	
eGFR, ml min <sup>-1</sup> [1.73 m] <sup>-2</sup>						0.01
<60	22.9	34.8	-7.7 (-20.7, 7.4)	63.1 (24.0, 114.5)	-43.4 (-58.7, -22.5)	
≥60	26.8	27.0	-12.7 (-18.2, -6.9)	1.4 (-7.5, 11.2)	-14.0 (-23.1, -3.7)	
KIM-1/Cr, ng/mmol	111.6	105.3	-28.9 (-33.0, -24.4)	3.0 (-5.8, 12.7)	-30.9 (-38.0, -23.0)	
UACR, mg/mmol						0.20
≥3, <30	109.4	96.5	-26.6 (-31.5, -21.3)	1.5 (-8.7, 12.9)	-27.7 (-36.3, -17.9)	
≥30	129.8	145.9	-36.0 (-43.2, -27.8)	2.8 (-12.2, 20.4)	-37.7 (-48.9, -24.0)	
eGFR, ml min <sup>-1</sup> [1.73 m] <sup>-2</sup>						0.18
<60	90.2	97.7	-5.5 (-18.3, 9.3)	15.2 (-11.7, 50.3)	-18.0 (-39.4, 11.1)	
≥60	115.1	105.3	-33.2 (-37.5, -28.6)	0.7 (-8.5, 10.7)	-33.7 (-40.9, -25.5)	

**Figure 1.** Geometric means (95% CI) of MCP-1/Cr and KIM-1/Cr at baseline and week 52 in the placebo and canagliflozin group.



Median (IQR) uMCP/Cr at week 52 was 28.1 ng/g (17.8, 50.8) and 22.9 ng/g (15.4, 35.1) for the placebo and canagliflozin treated group, respectively.

Median (IQR) uKIM/Cr at week 52 was 107.5 ng/g (62.5, 176.3) and 75.0 ng/g (45.3, 125.1) for the placebo and canagliflozin treated group, respectively.

**Figure 2.** Mediation of canagliflozin through direct and other pathways (green), UACR (red), MCP-1/Cr (marker of inflammation; blue) and both MCP-1/Cr and UACR (yellow) on KIM-1/Cr (marker of tubular damage).

