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Markers of Kidney Injury, Inflammation, and Fibrosis Associated With Ertugliflozin in Patients With CKD and Diabetes

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Introduction: Sodium-glucose cotransporter-2 (SGLT2) inhibitors improve cardiovascular and kidney outcomes through mechanisms that are incompletely understood. In this exploratory post-hoc analysis of the VERTIS RENAL trial, we report the association between the SGLT2 inhibitor, ertugliflozin, and markers of kidney injury, inflammation, and fibrosis in participants with type 2 diabetes (T2D) and stage 3 chronic kidney disease (CKD).

Methods: Participants were randomized to ertugliflozin (5 or 15 mg/d) or placebo, and plasma samples for biomarker analysis were collected at baseline, 26 weeks, and 52 weeks.

Results: Ertugliflozin-treated participants had lower plasma levels of kidney injury molecule-1 (KIM-1) at 26 weeks ($P = 0.044$) and 52 weeks ($P = 0.007$) and higher eotaxin-1 at 52 weeks ($P = 0.007$) post-randomization compared with placebo. The change in KIM-1 was not associated with the baseline urine albumin to creatinine ratio (UACR) or the estimated glomerular filtration rate (eGFR, P interaction > 0.05). Additionally, the change in KIM-1 was positively correlated with the change in UACR in participants treated with ertugliflozin ($P = 0.0071$). No other significant associations between ertugliflozin and changes in the markers of tubular injury, inflammation, fibrosis, oxidative stress, and endothelial dysfunction were observed.

Conclusion: In conclusion, in participants with T2D and stage 3 CKD, ertugliflozin was associated with a sustained lowering of the tubular injury marker KIM-1 regardless of baseline kidney function.

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See Commentary on Page 2022

Type 2 diabetes (T2D) is a leading cause of CKD, with >30% of patients with T2D developing diabetic kidney disease (DKD) at some point in their lifetime.[1] SGLT2 inhibitors reduce glycated hemoglobin (HbA1c) and body weight by increasing glycosuria via the inhibition of SGLT2 in the proximal tubule of the kidney.[2] Importantly, these agents slow DKD progression, independent of their glycemic effects through mechanisms that are incompletely understood.[3–5]

One potential contributing factor leading to kidney protection is an increase in proximal tubular natriuresis and restoration of tubuloglomerular feedback, leading to plasma volume contraction and reductions in systemic and glomerular hypertension.[6,7] The restoration of tubuloglomerular feedback mediated by SGLT2 inhibition may reduce albuminuria, glomerular shear

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stress, and associated endothelial injury. SGLT2 inhibition also alters energy dynamics within the proximal tubule. In the setting of diabetes, energy-intensive sodium and glucose reabsorption in the proximal tubule is elevated, leading to renal hypoxia, generation of reactive oxygen species, and a proinflammatory milieu, which have been implicated in the progression of DKD and cardiovascular disease.[8] Accordingly, the reduction of solute transport mediated by SGLT2 inhibitors may reduce oxygen requirements and ameliorate renal hypoxia, preventing tubular cell injury and DKD progression. Both animal models and exploratory analyses in humans have demonstrated that SGLT2 inhibition may also help attenuate oxidative stress and inflammation in the setting of DKD.[9–12] Importantly, although these mechanisms have been demonstrated in experimental models, it is not fully understood if these mechanisms translate to patients with DKD.

A variety of plasma biomarkers have been used to elucidate potential cardiovascular and kidney protective mechanisms with novel therapies and to better understand disease pathogenesis, including factors linked with tubular injury, inflammation, and fibrosis.[13–19] The aim of this exploratory, post-hoc analysis was to examine the association of the SGLT2 inhibitor ertugliflozin with circulating biomarkers of tubular injury, inflammation, fibrosis, and oxidative stress in participants with stage 3 CKD and T2D. We hypothesized that ertugliflozin treatment would be associated with lower levels of these biomarkers when compared with placebo and that potential on-treatment changes in these pathway markers would correlate with improvements in clinical markers of kidney function. Second, we assessed whether potential changes in biomarkers varied across differing levels of baseline kidney function (as assessed with estimated glomerular filtration rate [eGFR] and urine albumin to creatinine ratio [UACR]).

METHODS

Study Participants

We performed an exploratory post-hoc biomarker analysis in a subset (231) of participants of the previously published VERTIS RENAL trial (Study of the Efficacy and Safety of Ertugliflozin in Participants With Type 2 Diabetes Mellitus With Stage 3 Chronic Kidney Disease Who Have Inadequate Glycemic Control on Antihyperglycemic Therapy) (NCT01986855).[20] Briefly, participants in this double-blind randomized control study had HbA1c of 7.0% to 10.5% and stage 3 CKD (eGFR $\geq$30 to $<$60 ml/min per 1.73 m^2) at baseline. Participants had to be taking standard T2D therapy (or therapies) including insulin and/or sulfonylureas and were randomized to once daily ertugliflozin 5 mg, 15 mg, or placebo. Participants on metformin underwent a $\geq$10-week washout period before randomization as per protocol. The VERTIS RENAL trial had a primary end point of change from baseline in HbA1c at week 26.[20] In the current exploratory analysis, changes in plasma biomarkers and clinical trends were assessed over time in participants who (i) consented to the collection of samples for exploratory analyses at the time of the trial and (ii) had at minimum a baseline sample in addition to 1 follow-up sample at (or close to) postrandomization week 26 and/or week 52 (Figure 1). Off-cycle visit information was used when available and occurred within 2 months of a missing standard schedule study visit. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. It was conducted in accordance with the principles of good clinical practice and approved by the appropriate institutional review boards and regulatory agencies. All participating patients provided written, informed consent.

Biomarkers

In this study, biomarkers were quantified using Luminex xMAP technology or enzyme-linked immunosorbent assays. The multiplexing analysis for biomarkers was performed using the Luminex 100 System (Luminex, Austin, TX) by Eve Technologies Corp. (Calgary, Alberta, Canada). Platelet-derived growth factor AA; platelet-derived growth factor AB/BB; and regulated on activation, T cell expressed, and secreted were measured in the samples using the MILLIPLEX Human Cytokine/Chemokine 3-plex kit (Millipore, St. Charles, MO) according to the manufacturer's protocol, with an assay sensitivity ranging from 0.4 to 2.2 pg/ml. Individual analyte values are available in the MILLIPLEX protocol. Eotaxin-1, fibroblast growth factor 2, granulocyte-macrophage colony-stimulating factor, interferon alpha-2, monocyte chemoattractant protein-3, interleukin-12p40, macrophage-derived chemokine, interleukin-12p70, soluble cluster of differentiation 40-ligand, interleukin-2, interleukin-6, interleukin-8, monocyte chemoattractant protein-1, macrophage inflammatory protein 1α, and tumor necrosis factor-α were quantified using the MILLIPLEX Human Cytokine/Chemokine 15-plex kit (Millipore) according to the manufacturer's protocol, with an assay sensitivity ranging from 0.4 to 7.6 pg/ml. Soluble tumor necrosis factor receptor 1 and 2 were measured using the MILLIPLEX Human Soluble Cytokine Receptor 2-plex kit (Millipore) according to the manufacturer's

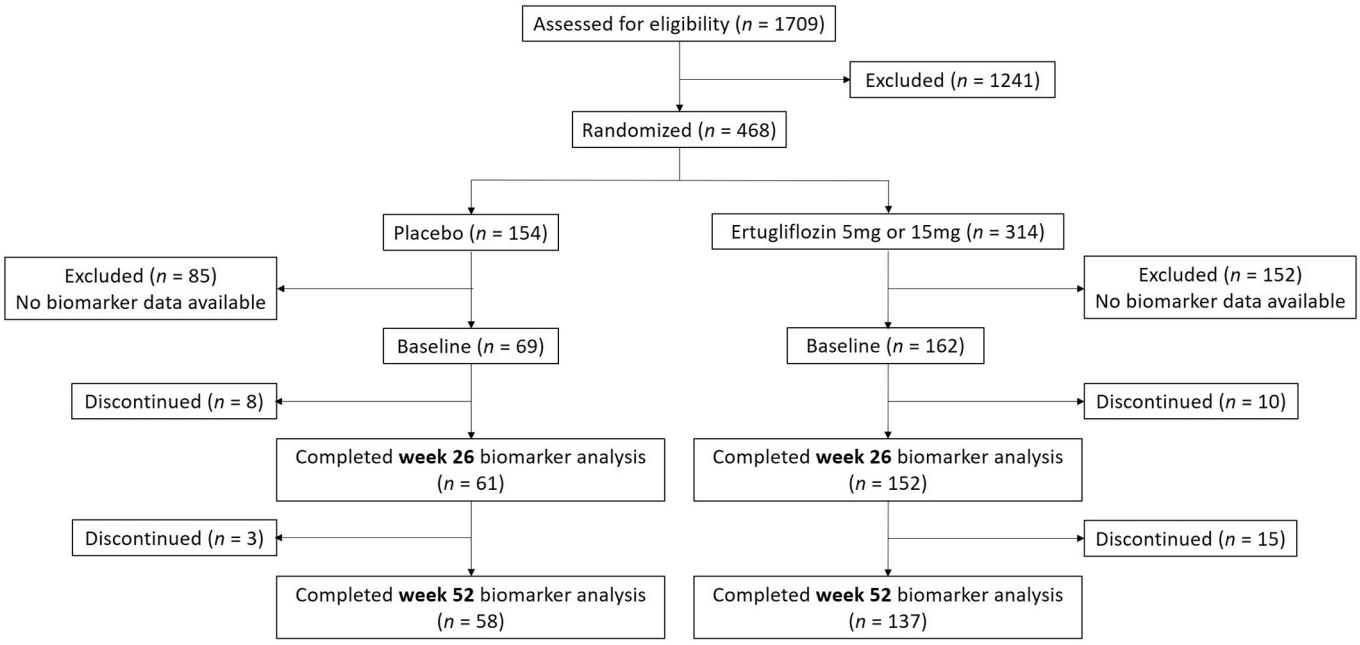

Figure 1. A flow diagram of the study participants.

protocol, with an assay sensitivity ranging from 8 to 12 pg/ml. KIM-1 was quantified using the MILLIPLEX Human Kidney Injury 2-plex kit (Millipore) according to the manufacturer's protocol, with an assay sensitivity of 0.177 ng/ml. Lipocalin-2/neutrophil gelatinase-associated lipocalin was quantified using the MILLIPLEX Human Kidney Injury 1-plex kit (Millipore) according to the manufacturer's protocol, with an assay sensitivity of 0.046 ng/ml. Cystatin C was measured using the MILLIPLEX Human Kidney Injury 1-plex kit according to the manufacturer's protocol, with an assay sensitivity of 0.404 ng/ml.

Enzyme-linked immunosorbent assays were performed using the EnSpire Multilabel Plate Reader system (PerkinElmer, Inc., Waltham, MA) by Eve Technologies Corp. Human 8-hydroxy-2-deoxyguanosine was quantified using the Inc 8-hydroxy 2 deoxyguanosine ELISA Kit (abcam Inc., Toronto, Ontario, Canada) according to the manufacturer's protocol, with an assay sensitivity of 0.59 ng/mL. 8-Isoprostane was quantified using the 8-Isoprostane ELISA Kit (Item No. 516351; Cayman Chemical, Ann Arbor, MI) according to the manufacturer's protocol. The observed imprecision based on quality control data over the 12 runs (over 3 months) was 18% at the 35-pg/ml level. The remainder of the reported markers were measured locally at the participating sites' clinical laboratories.[20]

Microparticle analysis was performed at the University of Ottawa Flow Cytometry Core Facility using the CytoFLEX Flow Cytometer (Beckman Coulter, Brea, CA). The data were analyzed using FlowJo (version 7.6.5). Microparticles were defined as particles between ~100 and 1000 nm in size that exhibited significantly more annexin V fluorescence than their negative controls. The results are the number of annexin V+ (total), annexin V+, and CD144+ (endothelial) microparticles/ml plasma.[21]

Statistics

Differences in changes (baseline to 26 and 52 weeks) in biomarker levels between the placebo and pooled (5 and 15mg) treatment groups were analyzed using a mixed-effect model repeated measurement under the missing-at-random framework based on the restricted maximum likelihood method for estimation. The models used the change in biomarker levels at 26 and 52 weeks as the outcome and included baseline biomarkers levels, treatment, visit week, and treatment-by-visit interaction as covariates. Biomarkers were log transformed before analysis to mitigate skew, and different visits were treated as repeated measure units from the same participant. A contrast statement combining treatment and treatment-by-time interaction was used to evaluate the overall differences in the biomarker change between the placebo and treatment groups at different time points. Accordingly, the model least squares means were used to report the differences at particular time points. Additional analyses were performed after stratifying the study cohort by baseline albuminuria (UACR <30 or ≥ 30 mg/g) and kidney function (eGFR <45 or ≥ 45 ml/min per 1.73 m^2) using the same mixed-effect model repeated measurement model. Correlation

Table 1. Baseline characteristics and medications of clinical trial cohort

	Placebo	Ertugliflozin	*P* Value
N	69	162	
Age	66.0 (61.0–73.0)	67.0 (62.0–73.0)	0.7565
Sex–male (%)	30 (43.4)	84 (51.9)	0.2539
BMI	32.8 (29.8–37.1)	31.5 (28.1–35.9)	0.2633
Body weight (kg)	86.7 (75.7–106.9)	88.9 (74.0–101.5)	0.7905
Duration type 2 diabetes (yr)	12.5 (7.6–15.6)	13.1 (7.0–18.8)	0.4528
Race			0.5659
Asian	7 (10.1)	16 (9.9)	
Black	2 (2.9)	7 (4.3)	
Multiple	4 (5.8)	13 (8.0)	
White	55 (79.7)	126 (77.8)	
Others	1 (1.5)	0 (0)	
History of CVD	32 (46.4)	81 (50.0)	0.6141
History of HF	1 (1.4)	3 (1.9)	
Baseline FPG	150.0 (118.0–168.0)	153.0 (121.0–183.0)	0.3224
Baseline eGFR	45.0 (40.0–53.0)	49.0 (44.0–53.0)	0.0885
Baseline HbA1c (%)	8.0 (7.5–8.4)	8.0 (7.5–8.5)	0.9562
Baseline blood albumin (g/dL)	4.3 (4.0–4.5)	4.3 (4.1–4.6)	0.3850
Baseline UACR (mg/g)	32.0 (8.0–103.0)	26.5 (9.0–191.0)	0.4029
Normoalbuminuria (UACR<30 mg/g)	32 (48.5)	81 (50.6)	
Microalbuminuria (UACR >30 mg/g ≤300 mg/g)	26 (39.4)	45 (28.1)	
Macroalbuminuria (UACR >300 mg/g)	8 (12.1)	34 (21.3)	
Baseline medications			
Antihyperglycemic	67 (97.1)	153 (93.2)	0.3538
DPP4i	8 (11.6)	19 (11.7)	
Insulin	43 (62.3)	95 (58.6)	
Sulfonylureas	29 (42.0)	61 (37.7)	
Biguanides	16 (23.2)	35 (21.6)	
Antihypertensive	65 (94.2)	159 (98.2)	0.2011
Alpha/beta blockers	42 (60.9)	102 (63.0)	
Diuretics	46 (66.7)	90 (55.6)	
Calcium channel blockers	22 (31.9)	61 (37.7)	
Other antihypertensive	3 (4.3)	9 (5.6)	
RAS blockade	57 (82.6)	145 (89.5)	0.1919
ACEi	35 (50.7)	82 (50.6)	
ARBs	25 (36.2)	67 (41.4)	
MRAs	3 (4.3)	7 (4.3)	
Lipid lowering	51 (73.9)	136 (84.0)	0.0986
Statins	47 (68.1)	120 (74.1)	
Bile acid sequestrants	1 (1.4)	2 (1.2)	
Fibrates	12 (17.4)	32 (19.8)	
Nicotinic acid and derivatives	1 (1.4)	0 (0.0)	
Other lipid modifying agents	12 (17.4)	10 (6.2)	
Antithrombotic	39 (56.5)	104 (64.2)	0.3015

ARBs, angiotensin II receptor blockers; ACEi, angiotensin-converting enzyme inhibitor; BMI, body mass index; CVD, cardiovascular disease; DPP4i, dipeptidyl peptidase 4 inhibitor; eGFR, glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HF, heart failure; MRAs, mineralocorticoid receptor antagonists; UACR, urine albumin to creatine ratio.
Continuous variables are presented as medians (interquartile range), and categoric variables are presented as *n* (%). Continuous variables: Kruskal-Wallis test (multilevel Wilcoxon rank sum test) across 2 categories; categoric variables: chi-square test. Antithrombotic medications include direct factor Xa inhibitors, direct thrombin inhibitors, salicylic acid and derivatives, and platelet aggregation inhibitors, excluding heparin. Diuretics include loop diuretics (42%) and thiazide diuretics (58%).

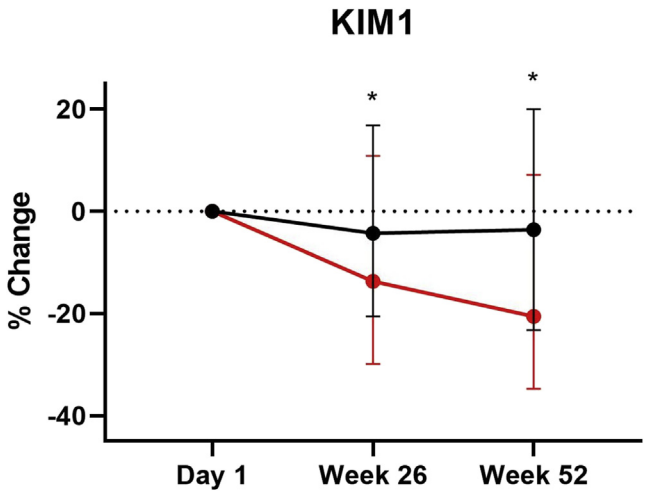

Figure 2. The change in kidney injury molecule-1 (KIM1) after ertugliflozin treatment and placebo. Black lines/circles, placebo; red lines/circles, ertugliflozin (pooled 5 and 15 mg/d). Data are presented as median % change ± interquartile range for presentation purposes. Data were analyzed using the mixed-effect regression model and post-hoc least squares mean results for individual time points, $^*P \leq 0.05$.

analyses between changes in KIM-1 and changes in albuminuria and HbA1c as well as baseline KIM-1 and changes in eGFR were conducted using the Pearson correlation coefficient. All statistical analyses evaluating treatment effects used a 5% significance level and were 2 sided. We did not correct for multiple hypotheses testing because this was an exploratory analysis. All analyses were performed using SAS 9.4 for Windows (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics of the 467 participants enrolled in the VERTIS RENAL randomized clinical trial have been previously published.[20] This exploratory post-hoc analysis included 231 participants from the VERTIS RENAL trial who consented to have samples drawn for exploratory analysis. Briefly, participants with T2D and stage 3 CKD (eGFR 30–60 ml/min per 1.73 m^2) were randomized to ertugliflozin (5 or 15 mg once daily, data pooled for this analysis) or placebo. Baseline clinical characteristics of this cohort are shown in Table 1 and were generally similar to the baseline characteristics of the original VERTIS RENAL cohort (Supplementary Table S1), aside from a small but significant ($P = 0.004$) difference in HbA1c between the original (8.2% ± 0.9%) and post-hoc analysis cohorts (8.0% ± 0.8%). The median UACR and eGFR were 29.5 mg/g and 48 ml/min per 1.73 m^2, respectively. As reported in the original trial, there were no sustained differences in HbA1C, eGFR, albuminuria, or blood pressure over the

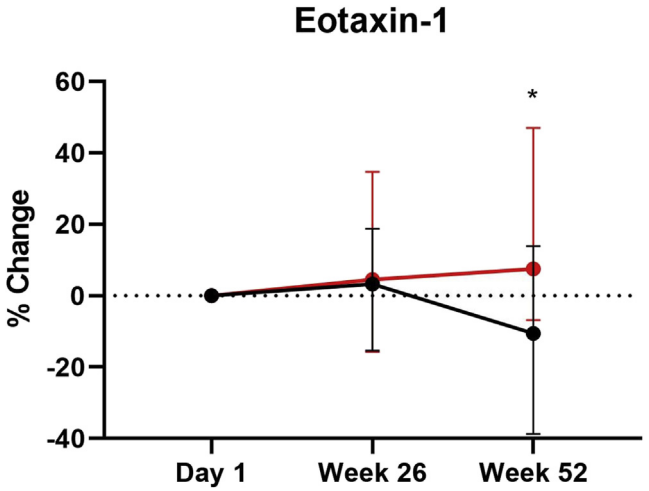

Figure 3. The change in eotaxin-1 after ertugliflozin treatment and placebo. Black lines/circles, placebo; red lines/circles, ertugliflozin (pooled 5 and 15 mg/d). Data are presented as median % change ± interquartile range for presentation purposes. Data were analyzed using the mixed-effect regression model and post-hoc least squares means results for individual time points, $*P \leq 0.05$.

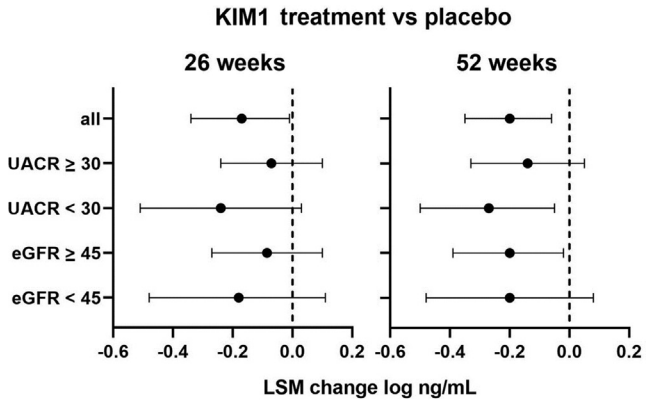

Figure 4. The change in kidney injury molecule-1 (KIM1) after ertugliflozin treatment stratified by urine albumin to creatine ratio (UACR) (≥30 mg/g, $n = 112$ or <30 mg/g, $n = 111$; P interaction = 0.089) and estimated glomerular filtration rate (eGFR) (≥45 ml/min per 1.73 m^2, $n = 158$ or <45 ml/min per 1.73 m^2, $n = 68$; P interaction = 0.973) at 26 and 52 weeks. Data are presented as least square mean (LSM) change ± 95% confidence interval. Data were analyzed using the mixed-effect regression model and post-hoc least squares means results for individual time points.

course of the 52-week treatment with ertugliflozin in this cohort.[20]

Effect of Ertugliflozin on Plasma Biomarkers of Tubular Injury, Inflammation, Fibrosis, Oxidative Stress, and Endothelial Dysfunction

Changes in all measured biomarkers (fibroblast growth factor 2; eotaxin-1; granulocyte-macrophage colony-stimulating factor; interferon alpha-2; macrophage-derived chemokine; soluble cluster of differentiation 40-ligand; regulated on activation, T cell expressed, and secreted; tumor necrosis factor-α, soluble tumor necrosis factor receptor 1, soluble tumor necrosis factor receptor 2, monocyte chemoattractant protein-1, monocyte chemoattractant protein-3, macrophage inflammatory protein 1α, 8-hydroxy-2-deoxyguanosine, platelet-derived growth factor AA, platelet-derived growth factor AB/BB, isoprostane, interleukin-12, interleukin-12p40, interleukin-2, interleukin-6, interleukin-8, lipocalin-2/neutrophil gelatinase-associated lipocalin, KIM-1, cystatin, endothelial microparticles, and total microparticles) are presented in Supplementary Table S2. Plasma KIM-1 levels were reduced in the ertugliflozin group at both 26 and 52 weeks ($P = 0.044$ and $P = 0.007$, respectively; Figure 2) compared with placebo. The rise in eotaxin-1 was greater in the ertugliflozin group compared with the placebo group at 52 weeks ($P = 0.007$; Figure 3). There were no other significant differences in changes to the markers of tubular injury (including plasma neutrophil gelatinase-associated lipocalin), inflammation, fibrosis, oxidative stress, and endothelial dysfunction. Although MCP-3 and isoprostane were found to be significantly different between the ertugliflozin and placebo groups at 52 weeks based on the post-hoc analysis using least squares means ($P = 0.035$ and $P = 0.047$, respectively), the overall mixed-effect model repeated measurement analysis for these biomarkers did not show any significant changes ($P = 0.089$ and $P = 0.1264$, respectively). Interleukin-12p40 was found to be significantly different between treatment groups by the overall mixed-effect model repeated measurement analysis, but the trend P value was found to be largely driven by comparisons other than treatment versus placebo at 26 ($P = 0.075$) and 52 weeks ($P = 0.183$).

Effect of Ertugliflozin on Markers of Tubular Injury Stratified by Baseline Albuminuria and Kidney Function

Changes in plasma KIM-1 levels were similar in subgroups analyzed by baseline UACR status (<30 mg/g, $n = 111$ or ≥30 mg/g, $n = 112$; P interaction = 0.089) and baseline eGFR stage (<45 ml/min per 1.73 m^2, $n = 68$ or ≥45 ml/min per 1.73 m^2, $n = 158$, P interaction = 0.973) in participants treated with ertugliflozin. In all stratification groups, there was a trend toward a reduction in KIM-1 at 26 and 52 weeks (Figure 4). When stratified by UACR, the reduction in KIM-1 from baseline was significant among participants with UACR <30 mg/g at 52 weeks ($P = 0.0167$). When stratified by baseline eGFR, the reduction in KIM-1 from baseline was significant among participants with eGFR ≥45 ml/min per 1.73 m^2 at 52 weeks ($P = .0303$).

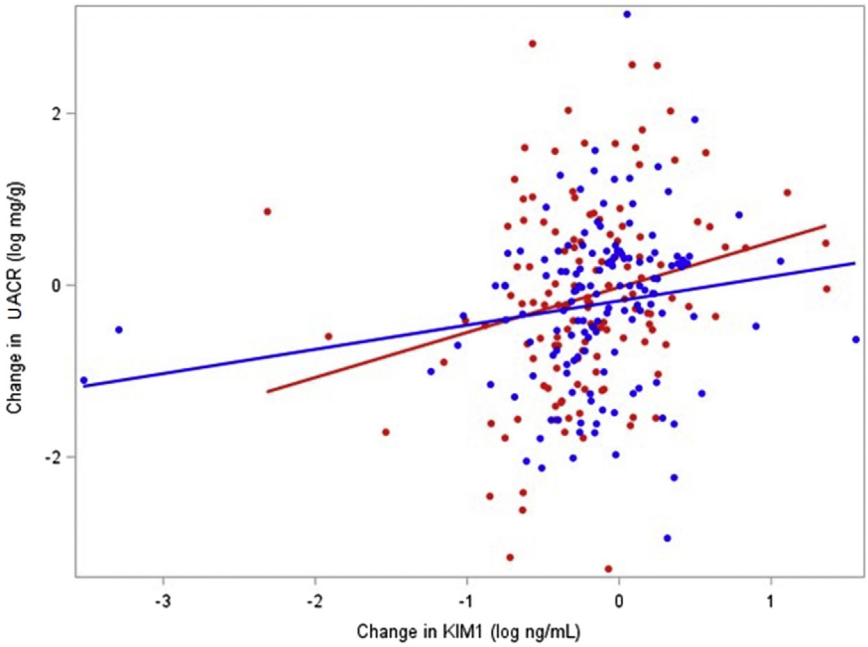

Figure 5. The correlation of the change in kidney injury molecule-1 (KIM1) and the change in urine albumin to creatinine ratio (UACR) from baseline to 26 weeks (blue lines/dots, Pearson's $r = 0.1716$, $P = 0.0442$) and baseline to 52 weeks (red lines/dots, Pearson's $r = 0.2334$, $P = 0.0071$) in the ertugliflozin group.

Correlating KIM-1 With Albuminuria, eGFR, and HbA1c

The change in KIM-1 positively correlated with the change in UACR among participants treated with ertugliflozin from baseline to 26 weeks and baseline to 52 weeks (Pearson's $r = 0.17$, $P = 0.0442$ and Pearson's $r = 0.23$, $P = 0.0071$, respectively; Figure 5). No significant correlation was observed within the placebo group. Baseline KIM-1 negatively correlated with the change in eGFR from baseline to 52 weeks (Pearson's $r = -0.22$, $P = 0.0031$) in placebo- and ertugliflozin-treated participants (Figure 6). A similar trend was noted comparing baseline KIM-1 with the change in eGFR from 26 to 52 weeks, although this did not reach statistical significance (Pearson's $r = -0.14$, $P = 0.06$). Changes in KIM-1 were correlated with changes in HbA1c from baseline to 52 weeks (Pearson's $r = 0.2386$, $P = 0.0055$) in the ertugliflozin group (Supplemental Figure S1).

DISCUSSION

SGLT2 inhibition reduces the risk of hard kidney outcomes in cardiovascular outcome trials and in dedicated kidney outcome trials.[3,5,22–24] However, the mechanisms responsible for these protective effects remain incompletely understood.[25] In this exploratory post-hoc analysis of the VERTIS RENAL trial, we assessed the association between ertugliflozin and circulating biomarkers of kidney tubular injury, inflammation, fibrosis, oxidative stress, and endothelial dysfunction in participants with T2D and stage 3 CKD. Our main observation was that ertugliflozin treatment was associated with a reduction in circulating levels of the tubular injury marker KIM-1 compared with placebo at 26 weeks and 52 weeks regardless of baseline kidney function, suggesting tubular protective effects. Additionally, baseline KIM-1 levels were negatively correlated with the overall change in eGFR over the study duration, with subsequent changes positively correlating with changes in albuminuria among ertugliflozin-treated participants, highlighting the role of KIM-1 as a clinical biomarker in people with T2D and CKD.

KIM-1 is a sensitive and specific biomarker of kidney injury. In health, KIM-1 is minimally expressed in human kidney tissue.[26] However, under conditions of hypoxic injury within tubular cells of the kidney, such as in the setting of diabetes, KIM-1 expression is increased within proximal tubular epithelial cells, and its extracellular section is shed into urine and the systemic circulation.[27] KIM-1 is also a marker of chronic tubular injury, correlating with the onset and progression of CKD.[27] In individuals with type 1 diabetes with and without evidence of DKD, baseline plasma levels of KIM-1 were predictive of future eGFR decline and end-stage kidney disease,[19,26] independent of baseline variables including albuminuria or serum tumor necrosis factor receptor.[19] In a separate study from the Joslin Diabetes Center, lower baseline urinary KIM-1 levels were associated with a regression of microalbuminuria in participants with type 1

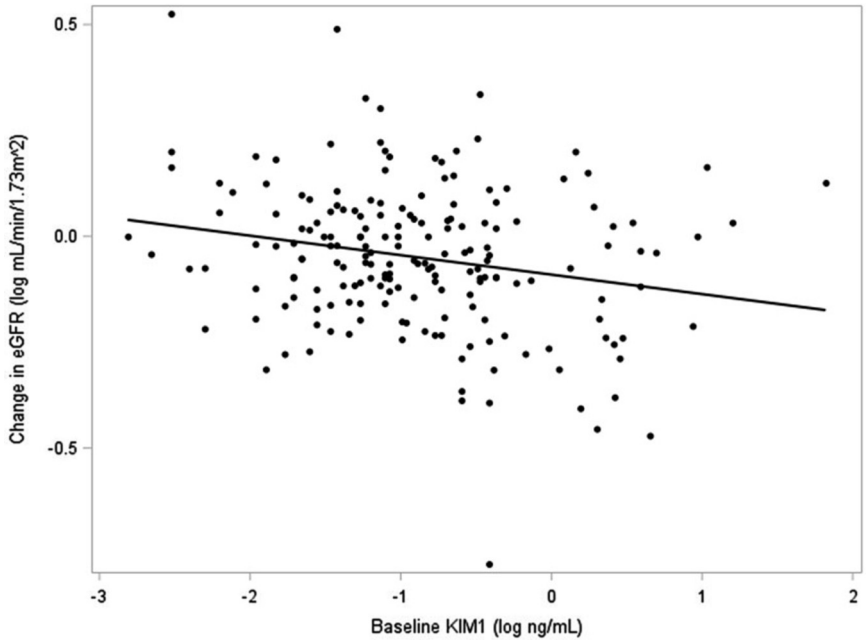

Figure 6. The correlation of the baseline kidney injury molecule-1 (KIM1) with the change in estimated glomerular filtration rate (eGFR) from baseline to 52 weeks in placebo- and ertugliflozin-treated participants (Pearson's $r = -0.21508$, $P = 0.0031$).

diabetes.[28] Although the prognostic implications of baseline KIM-1 levels have been demonstrated in several studies, the clinical significance of KIM-1 reductions in the setting of therapeutic interventions is less clear.

In addition to acting as a predictor of future risk, KIM-1 levels may reflect kidney protective effects of new therapies such as SGLT2 inhibition. In people with T2D and normal kidney function, Dekkers *et al.*[12] demonstrated that dapagliflozin decreased urinary KIM-1 excretion by 22.6% (95% confidence interval, 0.3%–39.8%; $P = 0.05$) after a 6-week period in participants with T2D and normal kidney function.[12] In this study, we demonstrated an association between ertugliflozin and lower plasma levels of KIM-1 in participants with T2D and stage 3 CKD. However, it is important to note that we did not observe decreases in other markers of tubular injury, such as plasma neutrophil gelatinase-associated lipocalin. SGLT2 inhibitors may reduce proximal tubular injury in DKD by alleviating tubular hypoxia via reducing the reabsorptive requirements at this level of the nephron. By inhibiting SGLT2 and normalizing the tubular workload, SGLT2 inhibitors may reverse tubular hypoxic injury and the associated release of KIM-1. In support of this hypothesis, animal studies have demonstrated a reduction in oxygen demand of renal tubular cells in diabetic rats with the combined SGLT inhibitor phlorizin.[29] Additionally, a decrease in tubular cell hypoxia-inducible factor-α expression was found in patients with diabetes, suggesting an amelioration in tubular hypoxia with SGLT2 inhibition, which may reduce levels of tubular injury biomarkers.[30,31] Lastly, hematocrit has been found to be increased after ertugliflozin treatment in the same cohort of patients, further suggesting that SGLT2 inhibitors may lead to improved oxygenation.[32]

When stratified by baseline albuminuria and eGFR, the reduction in KIM-1 levels in the ertugliflozin group versus placebo remained significant in participants with UACR <30 mg/g and in those with eGFR ≥ 45 ml/min per 1.73 m^2. Although the smaller group sizes after stratification limited the power of the analysis, the significant effect in lower-risk participants with preserved eGFR and less albuminuria raises the intriguing possibility that benefits were more pronounced in lower-risk participants. Therefore, the reduction in KIM-1 may reflect an early kidney protection pathway with ertugliflozin before the onset of significant albuminuria or kidney function loss. Nevertheless, analyses from larger clinical trials are required to assess if reductions in KIM-1 induced by SGLT2 inhibitors contribute to observed reductions in clinical kidney outcomes because we were unable to in this data set. Beyond associations with long-term kidney outcomes, KIM-1 is also a marker of acute kidney injury. Importantly, SGLT2 inhibitors are associated with a reduced risk of acute kidney injury, which may also be on the basis of protection against renal hypoxia-related pathways.[33]

Changes in KIM-1 positively correlated with changes in albuminuria among participants treated with ertugliflozin at 26 weeks and 52 weeks. Favorable energy dynamics within the proximal tubule with SGLT2

inhibition may improve tubular integrity and, in turn, improve tubular reabsorption of albumin. In post-hoc analyses of the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) and Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes (CANVAS Program) trials, SGLT2 inhibition reduced albuminuria in participants with normoalbuminuria, microalbuminuria, or macroalbuminuria.[4,22,23] These albuminuria reductions persisted after a brief washout period, suggesting more permanent structural changes within the kidney.[34] Despite the relatively short period of follow-up, baseline KIM-1 was negatively correlated with the overall change in eGFR from baseline to 52 weeks, which was consistent with the ability of KIM-1 to prognosticate kidney function. When adjusted for the acute ertugliflozin-associated decline in eGFR by limiting the data to eGFR changes from 26 to 52 weeks, the interaction was no longer statistically significant but followed the same trend. In light of these observations, as well as data from Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants With Diabetic Nephropathy (CREDENCE) and A Study to Evaluate the Effect of Dapagliflozin on Renal Outcomes and Cardiovascular Mortality in Patients With Chronic Kidney Disease (DAPA-CKD) demonstrating reductions in the risk of composite clinical kidney outcomes, SGLT2 inhibitor–induced changes in KIM-1 may offer some mechanistic insight into the physiological basis for kidney protection and suggest suppression of tubular injury pathways.

Changes in KIM-1 levels were weakly but significantly correlated with changes in HbA1c from baseline to 52 weeks in the ertugliflozin group. A similar trend was noted in the placebo group, although it was not significant. Although one can speculate that worse glycemic control may be associated higher KIM-1 levels, there are caveats to consider. The change in HbA1c was not significantly different between the ertugliflozin and placebo groups after the treatment period, with similar reductions in both groups.[20] However, as we have described previously, KIM-1 levels were only reduced in the ertugliflozin groups, which is suggestive of glucose-independent mechanisms, as has been previously described elsewhere.[12]

In contrast with our initial hypothesis of lower levels of cytokines/chemokines after SGLT2 inhibition, ertugliflozin was associated with an increase in plasma levels of eotaxin-1, a chemokine that has been found to be elevated in patients with DKD.[35] Ertugliflozin was not associated with significant changes in other markers of inflammation, fibrosis, oxidative stress, or endothelial dysfunction. Insufficient power and/or observation time may have limited our ability to detect a significant reduction in these other markers of inflammation. Several animal models of diabetes demonstrate reductions in markers of inflammation, fibrosis, and oxidative stress with other SGLT2 inhibition. In humans, Heerspink *et al.*[11] reported that canagliflozin lowered levels of tumor necrosis factor receptor 1, interleukin-6, and fibronectin 1 in participants with T2D, suggesting an amelioration in inflammation and fibrosis with this agent. Dekkers *et al.*[12] also demonstrated reductions in the urinary excretion of interleukin-6 with dapagliflozin in participants with T2D. Both study cohorts were composed of otherwise healthy individuals and were not enriched with participants with CKD or cardiovascular disease. These differences may explain why we did not see a reduction in inflammatory markers in the current analysis. Additionally, it is possible that ertugliflozin is not as effective as other SGLT2 inhibitors for reducing levels of proinflammatory and profibrotic factors, although this seems less likely based on similarities across the class for reducing the risk of hospitalization for heart failure and the risk of a sustained $\geq$40% or more decline in eGFR.[3,4,36] Accordingly, the lack of association of ertugliflozin and markers of inflammation and fibrosis in this post-hoc analysis emphasizes the need for further investigation with longer-term follow-up in larger cohorts with different risk profiles to determine if ertugliflozin reduces inflammatory biomarkers and to establish where changes in biomarkers can act as surrogates for clinical outcomes.

Our analysis does have limitations. This is a post-hoc analysis; thus, the findings are exploratory and hypothesis generating. Accordingly, we did not control for multiple hypothesis testing and acknowledge the possibility of type 1 error. Study participants in the VERTIS RENAL trial were only followed for 1 year. This, along with a relatively small sample size, may have limited power to detect significant differences between groups. Notwithstanding these issues, the size of the cohort used in this study is comparable with similar published analyses. Next, because of the limited number of eGFR time points available, a correlation analysis between the change in KIM-1 and the change in eGFR was not performed due to an inability to account for the the acute ertugliflozin-associated "dip" in eGFR. Moreover, although this study provides unique insight into the effects of SGLT2 inhibitors in patients with moderate CKD, these results may be limited in their ability to be generalized to other populations, such as those with normal kidney function or with more advanced CKD.

In conclusion, in people with T2D and stage 3 CKD, ertugliflozin was associated with a sustained lowering of the tubular injury marker KIM-1, an effect that may

contribute to kidney protection found in clinical trials. Further investigation is warranted to examine the role of hypoxia on tubular injury and whether or not SGLT2 inhibitors mitigate the risk of DKD through these pathways.

DISCLOSURE

DZIC has received honoraria from Boehringer Ingelheim-Lilly, Merck, AstraZeneca, Sanofi, Mitsubishi-Tanabe, Abbvie, Janssen, Bayer, Prometic, BMS, Maze and Novo-Nordisk and has received operational funding for clinical trials from Boehringer Ingelheim-Lilly, Merck, Janssen, Sanofi, AstraZeneca and Novo-Nordisk. DZIC is supported by a Department of Medicine, University of Toronto Merit Award and receives support from the CIHR, Diabetes Canada, and the Heart and Stroke Richard Lewar Centre of Excellence. PRL is supported by the Department of Medicine, University of Toronto and receives unrelated funding from the Canadian Institutes for Health Research, the Peter Munk Cardiac Centre, the LifeArc Foundation, the Province of Ontario, and the Ted Rogers Centre for Heart Research. PRL also receives unrelated consulting fees from Brigham and Women's Hospital, Novartis, and Corrona LLC; unrelated royalties from McGraw-Hill; and unrelated research funding from the Thistledown Foundation. VSS is supported by the Department of Medicine Eliot Phillipson Clinician Scientist Training Program and a Banting and Best Diabetes Centre Postdoctoral fellowship at the University of Toronto. LEL receives support from a CIHR Canada Graduate Scholarship Doctoral Award. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

HL, VSS, LEL, and DZIC contributed to the study concept, design, analysis, interpretation of data, and drafting the manuscript. HL, VSS, LEL, YL, DBu, KB, DBr, PRL, and DZIC contributed to data analysis, provided critical edits, reviewed, and approved the final manuscript. No writing assistance was received by the authors. DZIC is the guarantor of this work, had full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the analysis.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline characteristics and medications of post-hoc analysis cohort compared to the original VERTIS RENAL cohort.

Table S2. Least squares mean differences in biomarkers between ertugliflozin pooled treatments versus placebo.

Figure S1. Correlation between change in KIM-1 and change in HbA1c from baseline to week 52 in placebo (blue line/dots, $r = 0.1957$, $P = 0.1446$) and ertugliflozin treatment groups (red line/dots, $r = 0.2386$, $P = 0.0055$).

STOBE Checklist.

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