Association between TNF receptors and KIM-1 with kidney outcomes in early stage CKD

Short running title: TNFR1/2 is associated with kidney outcomes in early CKD

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Abstract

Background and objectives: Clinical trials in nephrology are enriched for patients with micro- or macroalbuminuria to enroll patients at risk of kidney failure. However, patients with normoalbuminuria can also progress to kidney failure. Tumor Necrosis Factor Receptor (TNFR)-1, TNFR-2 and Kidney Injury Marker (KIM)-1 are known to be associated with kidney disease progression in patients with micro- or macroalbuminuria. We assessed the value of TNFR-1, TNFR-2 and KIM-1 as prognostic biomarkers for CKD progression in patients with type 2 diabetes and normoalbuminuria.

Design, setting, participants and measurements: TNFR-1, TNFR-2, and KIM-1 were measured using immunoassays in plasma samples from patients with type 2 diabetes at high cardiovascular risk participating in the CANVAS trial. We used multivariable adjusted Cox proportional hazards analyses to estimate hazard ratios per doubling of each biomarker for the kidney outcome and stratified the population by the 4th quartile of each biomarker distribution and assessed the number of events and event rates.

Results: In patients with normoalbuminuria (N=2,553), 51 kidney outcomes were recorded during a median follow-up of 6.1 years (event rate 3.5 [95%CI 2.6–4.6] per 1,000-patient-years). Each doubling of baseline TNFR-1 (HR 4.16; 95%CI 1.80–9.61) and TNFR-2 (HR 2.35; 95%CI 1.51–3.63) was associated with a higher risk for the kidney outcome. Baseline KIM-1, UACR and eGFR were not associated with kidney outcomes. The event rates in the highest quartile of the TNFR-1 ($\geq 2,992$ ng/ml) or TNFR-2 ($\geq 11,394$ ng/ml) were 5.6 and 7.0 events per 1000-patient-years compared to 2.4 and 2.8 in the lower three quartiles.

Conclusion: TNFR-1 and TNFR-2 are associated with kidney outcomes in patients with type 2 diabetes and normoalbuminuria.

Introduction

Traditional clinical endpoints in clinical trials in patients with chronic kidney disease (CKD) are kidney failure and doubling of serum creatinine. However, these endpoints are late events in the progression of CKD and take a long time to manifest. To ensure sufficient endpoints occur within the duration of a clinical trial, patients at advanced stages of CKD, those with high albuminuria and low estimated glomerular filtration rate (eGFR), are enrolled. Patients at early-stages of CKD are usually excluded from CKD clinical trials because of low event rates. However, although event rates are lower, some patients without traditional risk markers still progress and experience clinical kidney outcomes. Moreover, since the prevalence of early-stage CKD is much higher than later stages of CKD, the total number of kidney outcomes was similar when comparing patients at early and advanced stages of CKD in past cardiovascular outcome trials (1,2). For example, in the DECLARE-TIMI-58 trial, 69.1% of patients had normoalbuminuria and 6.9% had macroalbuminuria, the number of kidney outcomes in both subgroups was 145 versus 106, respectively (1). Identifying patient characteristics or biomarkers that associate with kidney disease progression in early-stages of CKD will enable better tailoring of treatment to those at risk of progressive kidney function loss and may facilitate clinical trial design and conduct.

Biomarkers, including tumor necrosis factor receptor-1 (TNFR)-1, TNFR-2, and kidney injury molecule-1 (KIM-1) are associated with kidney disease progression (3). TNFR-1 and TNFR-2 are circulating receptors of the pro-inflammatory cytokine Tumor Necrosis Factor (TNF)- α . Studies in patients with established CKD demonstrated that higher concentrations of TNFR-1 and TNFR-2 are associated with a higher risk of progressive kidney function loss and kidney failure (4–6). KIM-1 is also associated with the progression of kidney disease. Elevated plasma and urine levels of KIM-1 are associated with progression to kidney failure (5,7–9). However, the association of these biomarkers with kidney disease progression in early-stages

of CKD and their utility to enrich clinical trials for kidney outcomes in patients with normoalbuminuria is unknown.

We performed a post hoc analysis of the CANagliflozin cardioVascular Assessment Study (CANVAS) randomized controlled trial that assessed the efficacy and safety of canagliflozin in patients with type 2 diabetes and higher cardiovascular risk. We assessed the performance of TNFR-1, TNFR-2, and KIM-1 as prognostic biomarkers to identify patients with normoalbuminuria at risk of CKD progression.

Materials and Methods

Patients and study design

The CANVAS program was a double-blind, randomized, placebo-controlled trial that assessed the cardiovascular efficacy and safety of the sodium glucose co-transporter 2 (SGLT2) inhibitor canagliflozin in patients with type 2 diabetes at higher cardiovascular risk. The CANVAS program consists of two trials, the CANVAS trial and the CANVAS-Renal (CANVAS-R) trial. Baseline blood samples of the CANVAS trial only were stored for exploratory biomarker analyses. No blood samples from the CANVAS-R trial were stored for biomarker analyses. For our analysis we included all patients with available blood samples for measurement at baseline.

The design and primary results of the CANVAS trial have been published previously (10,11). The CANVAS trial included 4330 patients with type 2 diabetes, who were 30 years or older, had an eGFR >30 ml/min/1.73m², and were at higher cardiovascular risk. Patients needed to have an glycated hemoglobin (HbA1c) level of ≥ 53 mmol/mol (7.0%) and ≤ 91 mmol/mol (10.5%). Higher cardiovascular risk was defined as a history of symptomatic atherosclerotic vascular disease or an age of ≥ 50 years with two or more cardiovascular risk factors, including a diabetes duration of ≥ 10 years, systolic blood pressure (BP) >140 mmHg while using ≥ 1 antihypertensive agent, current smoking, micro- or macroalbuminuria or an HDL cholesterol

level of <1 mmol/L. Eligible patients were randomly assigned to 100 mg/day canagliflozin, 300 mg/day canagliflozin or placebo in a 1:1:1 ratio.

The trial protocol was approved by a local or central ethics committee at all trial sites, and each participant provided written informed consent before entering the study. The trial was conducted according to the Declaration of Helsinki. The CANVAS trial is registered on ClinicalTrials.gov (NCT01032629).

Biomarker measurements

Plasma TNFR-1, TNFR-2, and KIM-1 were measured from baseline blood samples using high-performance electrochemiluminescence immunoassays (MesoScale Quickplex SQ 120 platform [MSD], performed by RenalytixAI, New York, NY, USA). All immunoassays were measured between August 2019 and December 2019. A random sample of 469 blood samples were measured in duplicate, with the following mean (minimum, maximum) coefficient of variation: TNFR-1: 2% (0%, 10%); TNFR-2: 2% (0%, 12%); and KIM-1: 3% (0%, 18%). First morning void urine was collected to determine urinary albumin:creatinine ratio (UACR).

Outcomes

The kidney outcome for this post hoc analysis was defined as a composite of 40% reduction in eGFR sustained for at least two consecutive measurements, kidney failure defined as a sustained eGFR <15 ml/min/1.73m², the need for kidney-replacement therapy and kidney transplantation, or kidney death. The cardiovascular outcome for the current study was defined as hospitalization for heart failure. These endpoints were pre-specified in the study protocol and endpoints were adjudicated by a blinded adjudication committee (10).

Statistical analysis

Baseline characteristics are presented as means and standard deviation. For variables with skewed distribution the median and the interquartile range (IQR) are reported. UACR, TNFR-

1, TNFR-2 and KIM-1 were log-transformed in all analyses to account for their skewed distributions. Categorical variables are presented as percentage of observations. Baseline characteristics are presented for patients with normoalbuminuria (UACR<30 mg/g) and compared with patients with microalbuminuria (UACR $\geq$ 30 and <300 mg/g) and macroalbuminuria (UACR $\geq$ 300 mg/g). BMI was missing in 4 patients, LDL cholesterol was missing in 3 patients and creatinine was missing in 2 patients. We imputed the mean values for these patients. Differences in baseline characteristics across these UACR subgroups were tested with one-way analysis of variance (ANOVA) or chi-squared test where appropriate. eGFR was calculated from the serum creatinine measurements using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (12).

We used Cox proportional hazard regression analyses to estimate the hazard ratios (HRs) for each outcome associated with each doubling in baseline TNFR-1, TNFR-2 or KIM-1. We also assessed the association between each doubling in baseline UACR and eGFR with the kidney outcome to compare the performance of the novel biomarkers with established risk markers of CKD progression. Models were adjusted for covariates that were added in a stepwise manner in three consecutive models. In the first model we adjusted for treatment allocation (canagliflozin or placebo). In the second model we additionally adjusted for age, sex, race, BMI, history of cardiovascular disease (yes or no), systolic and diastolic blood pressure, LDL-cholesterol, and HbA1c. The third model was additionally adjusted for UACR and eGFR. Estimations of the association of doubling in UACR and eGFR with the kidney outcome were performed in the final model without adjustments for UACR and eGFR, respectively. In addition, we divided the population into quartiles by TNFR-1 and TNFR-2 level and determined the HR for each quartile using the first quartile as a common reference.

To assess if the biomarkers of interest can be used as risk selection markers in patients with normoalbuminuria, we divided the population above or below the 4th quartile of baseline TNFR-

1 and TNFR-2, respectively. We assessed in both subgroups the number of events and Poisson event rates, calculated as the number of events per 1,000 patient years.

The effect of using the biomarkers for prognostic enrichment on sample size, total number of screenings and costs of a future clinical trial was evaluated using the Biomarker Prognostic Enrichment Tool (BioPET, <http://prognosticenrichment.com/orig/>) (13). As inputs we used the event rate of the placebo group and the performance of the association of TNFR-1, TNFR-2 and UACR with the kidney outcomes among patients with normoalbuminuria. We assumed a 90% power to detect a relative risk reduction for the kidney outcome of 40%, consistent with the observed effect of canagliflozin in the CANVAS program, and a type 1 error rate of 0.05. To estimate the implications of prognostic enrichment on trial costs, we set the screening costs per patient at \$1,000 and the costs per patient during the trial at \$50,000.

We performed Cox proportional hazard regression analysis to assess if the treatment effect of canagliflozin relative to placebo in patients with normoalbuminuria and TNF receptors above or below the 4th quartile were consistent with the main CANVAS trial findings. Heterogeneity of treatment effects across TNF receptor subgroups was tested by adding an interaction term between the TNF receptor subgroup and treatment assignment to the model.

All statistical analyses were performed in Stata 14.2 SE (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Of the 4,330 participants of the CANVAS trial, 3,532 (82%) had available blood samples for measurements at baseline. Of these, 2,553 (72%) had normoalbuminuria, 778 (22%) had microalbuminuria and 201 (6%) had macroalbuminuria. Median follow-up time was 6.1 (IQR 5.8 to 6.4) years. Baseline characteristics stratified by baseline albuminuria are shown in Table 1. Patients with macroalbuminuria had a higher blood pressure, higher HbA1c and lower eGFR

compared to patients with micro- or normoalbuminuria. TNFR-1, TNFR-2, and KIM-1 levels were also higher in those with macroalbuminuria (Table 1).

The number of kidney outcomes was similar across albuminuria subgroups: 51, 45, and 41 outcomes were recorded in patients with normo-, micro-, and macroalbuminuria, respectively (Table 1). The event rate was however markedly lower in the normo- compared to micro- or macroalbuminuria subgroups with corresponding rates of 3.5 (95% CI 2.6 to 4.6), 10.5 (7.8 to 14.1), and 43.1 (31.7 to 58.5) per 1,000 patient years, respectively.

Association of biomarkers with the kidney outcomes in patients with normoalbuminuria

We observed a wide variation in TNFR-1, TNFR-2, and KIM-1 levels in patients with normoalbuminuria; the mean and 2.5th to 97.5th percentile for each biomarker was 2600 [1,439 to 4,500], 9,922 [5,101 to 17,729], and 121 [31 to 360] pg/ml (Supplemental Figure S1). In unadjusted analyses TNFR-1, TNFR-2 and KIM-1 were statistically significantly associated with the kidney outcome (Table 2). When models were adjusted for baseline characteristics and cardiovascular and kidney risk markers, each doubling of TNFR-1 and TNFR-2 was significantly associated with a higher risk of kidney outcomes, with corresponding HRs of 4.2 (95%CI 1.8 to 9.6; P=0.001) and 2.3 (95%CI 1.5 to 3.6; p<0.001), respectively. In the fully adjusted model, we did not observe a statistically significant association between doubling of baseline KIM-1 and the kidney outcome (HR 1.4; 95%CI 1.0 to 1.9; P=0.066). Baseline eGFR and UACR were also not associated with the kidney outcome in the fully adjusted model (Table 2).

Since KIM-1 was not significantly associated with the kidney outcome after multivariable adjustment, we restricted further analyses to TNFR-1 and TNFR-2. Analyses of quartiles of baseline TNFR-1 and TNFR-2 showed that in the upper quartile of the TNFR-1 and TNFR-2 distribution the largest number of events occurred. Accordingly, the HR was larger at higher quartiles of TNFR-1 and TNFR-2, with a HR of 5.2 (95%CI 1.7 to 15.5) for TNFR-1 and 3.6

(95%CI 1.5 to 8.8) for TNFR-2 in the highest compared to the first quartile in the fully adjusted model (Supplemental Figure S2).

Performance of TNFR-1 and TNFR-2 as risk selection markers for future clinical trials

To identify patients with normoalbuminuria at highest risk for kidney outcomes for a future clinical trial and assess the performance of the TNF receptors to enrich clinical trial populations with participants likely to experience events, we selected patients with a TNFR-1 or TNFR-2 level in the highest (4th) quartile (Supplemental Figure S3). Because of the strong correlation between TNFR-1 and TNFR-2, the combination of these biomarkers was not tested (Pearson correlation 0.7, Supplemental Figure S4).

Patients with normoalbuminuria and TNFR-1 or TNFR-2 in the 4th quartile had a median TNFR-1 level of 3448 (IQR 3,181 to 3,855) pg/ml and a median TNFR-2 of 13,144 (IQR 12,080 to 14,923) pg/ml. The kidney endpoint occurred in 20 out of 638 patients (3%) in the 4th quartile of TNFR-1, corresponding to an event rate of 5.6 (95%CI 3.6 to 8.6) per 1,000 patient-years. In the 4th quartile of TNFR-2, 25 of 638 patients (4%) experienced the kidney outcome, the corresponding event rate was 7.0 (95%CI 4.7 to 10.3) per 1,000 patient-years. In comparison, in the lower three quartiles of TNFR-1 and TNFR-2 the event rates were 2.8 (95%CI 2.0 to 4.0) and 2.3 (95%CI 1.6 to 3.4), respectively (Table 3).

We evaluated the impact of using TNF receptor concentrations as prognostic enrichment marker on sample size requirements and costs for a hypothetical future clinical trial. Enrichment with TNFR-1 or TNFR-2 resulted in a higher event rate, and a decrease in clinical trial sample size. Although enrichment with either TNF receptor resulted in a larger number of patients required to be screened, the overall clinical trial costs decreased (Figure 1). Specifically, using a threshold to include patients with the 25% highest TNFR-2 concentrations (i.e., 4th quartile) would reduce the sample size of the hypothetical trial from 3692 to 2046 (45% reduction), increase the number of patients needed to screen for the trial from 3,692 to 8,183, but reduce

costs by 40% (Supplemental Table S1). Similar results are observed when using TNFR-1 for prognostic enrichment (Figure 1, Supplemental Table S1). Prognostic enrichment with KIM-1 did not lead to a reduction in sample size or a reduction in costs in comparison with UACR (Supplemental Figure S5).

Subgroup analyses by the baseline biomarker levels were performed to ascertain that the benefit of canagliflozin in reducing the risk of clinical outcomes was preserved in patients in the 4th quartile of baseline TNFR-1 and TNFR-2. We observed that the treatment effect of canagliflozin on the kidney outcome and hospitalization for heart failure is consistent in patients with a baseline TNFR-1 or TNFR-2 level above or below the 4th quartile as well as in patients with micro- or macroalbuminuria (both *p* for interaction > 0.391, Supplemental Table S2).

Discussion

In this post hoc analysis of the CANVAS trial, we demonstrated that baseline TNFR-1 and TNFR-2 levels can be used to identify patients with normoalbuminuria at elevated risk of CKD progression. In these individuals, there was a strong association between TNFR-1 and TNFR-2 with the kidney outcome. The association between KIM-1 and the kidney outcome did not reach significance after multivariable adjustment. These data highlight the potential use of TNFR-1 or TNFR-2 as biomarkers to identify individuals with normoalbuminuria at risk of CKD progression.

TNFR-1 and TNFR-2 are receptors of the pro-inflammatory cytokine TNF α , and have been shown to be associated with kidney outcomes in patients with and without diabetes at various stages of CKD (4–6,14–16). Here we confirm and extend these findings in a large, heterogeneous population with type 2 diabetes, normoalbuminuria and high cardiovascular risk, treated according to contemporary treatment guidelines. In our study, participants in the highest quartile of TNFR-1 and TNFR-2 had a 5-fold higher risk compared to the lowest quartile to develop the kidney outcome. This finding is in accordance with an earlier study demonstrating

that the vast majority of kidney events occurred in the highest quartile of the TNFR-1 distribution (4). Taken together, TNF receptors are associated with kidney disease progression across a range of patients and in patients without obvious signs of kidney damage, such as those with normal eGFR and normoalbuminuria.

Prior studies have also evaluated the applicability of TNFR-1 to improve clinical trial design (17). A study by Yamanouchi *et al.* evaluated the use of TNFR-1 to enrich the clinical trial cohort for patients with diabetes and CKD stage 3 or 4 more likely to reach kidney failure. This study showed that TNFR-1 levels $>4,300$ pg/ml or between 2,900-4,300 pg/ml in combination with a UACR $>1,900$ mg/g are the optimal thresholds for high risk selection (17). Here we extend these criteria and show that in people with type 2 diabetes and normoalbuminuria, TNFR-1 ($\geq 2,992$ pg/ml) and TNFR-2 ($\geq 11,394$ pg/ml) could be used for enrichment in a clinical trial. In addition, because the prevalence of early-stage CKD is much higher than later stages of CKD, the use of biomarkers, such as TNFR-1 and TNFR-2, would significantly increase the number of potential eligible clinical trial participants at risk of kidney function decline beyond the commonly used selection criteria such as the presence of macroalbuminuria.

KIM-1 is a marker for damage to the proximal tubule and has been proposed as a risk marker for progression of kidney disease (5,7–9). In our study, we did not observe a clear association between plasma KIM-1 and kidney outcomes in patients with normoalbuminuria. Previous studies in patients with type 1 and type 2 diabetes showed strong associations between plasma KIM-1 levels and kidney outcomes, including eGFR decline and progression of CKD (5,8,9). A recent trial confirmed this association in a general population cohort with preserved kidney function. Compared to our findings, in this study baseline plasma KIM-1 was associated with kidney outcomes, whereas we found no significant association (18). These differences may be explained by differences in design and analytical methods of the studies. Specifically, the longer follow-up and difference in outcome definition (incident CKD defined as $\text{eGFR} < 60$

mL/min/1.73m²) in the study by Schulz et.al. may have increased statistical power to detect a significant association in their study. More importantly perhaps, no data on albuminuria was collected in the study by Schulz et al and therefore, associations were not adjusted for albuminuria (18).

Albuminuria is a well-known risk marker for kidney failure. Multiple studies in varying patient populations have shown a strong and continuous association between higher degrees of albuminuria and risk for kidney outcomes without a threshold below which this association disappears (19,20). This indicates that even subtle increases in albuminuria, even within the currently considered normoalbuminuric range, are already associated with a higher risk of CKD progression and clinical events. In the current study we did not observe a statistically significant association between albuminuria and the kidney outcome in subjects with normoalbuminuria. The relatively low number of kidney outcomes may have resulted in insufficient statistical power to detect and confirm this association. An increase in albuminuria usually precedes the development of severe kidney damage (19,20). However, recent studies show that the prevalence of non-albuminuric CKD is increasing (21–23). Patients with high GFR and low albuminuria have been excluded from nearly all recent clinical trials because of concerns of low event rates and large sample size requirements. TNFR-1 and TNFR-2 could be considered as risk markers for these patients for whom evidence-based treatments are available (24).

Canagliflozin reduces the relative risks of kidney failure and heart failure hospitalization in patients with type 2 diabetes at high cardiovascular risk and with established kidney disease (11,25). In this study we demonstrated that canagliflozin reduced the kidney outcome and hospitalization for heart failure consistently across patients with normoalbuminuria irrespective of baseline TNFR-1 or TNFR-2 levels. Thus, no effect modification occurred when selecting patients on the basis of TNFR-1 or TNFR-2 levels supporting the utility of these markers for future trials. Using TNFR-1 or TNFR-2 as biomarkers for prognostic enrichment for a clinical

trial decreases the sample size and costs leading to an increase in feasibility of a clinical trial. However, the disadvantage of using enrichment criteria is that it reduces generalizability.

The strength of this study includes that CANVAS is a large trial with a heterogeneous population of patients with type 2 diabetes at higher cardiovascular risk with a long follow-up. Also, the CANVAS trial had clear, pre-specified endpoints that were assessed by a blinded endpoint adjudication committee. This study also has some limitations. First, it is a post hoc analysis prone to chance findings. Second, there is only a small number of events in the normoalbuminuria group limiting the statistical power. However, the coherence with prior data suggests that our findings are unlikely the result of chance. Because of day-to-day variability in albuminuria, guidelines recommend assessment of albuminuria in three consecutively collected first morning void urine samples. In the CANVAS trial urinary albumin and creatinine were measured in single first morning void urine samples, which may have led to misclassification of the albuminuria status. Finally, the CANVAS trial enrolled patients with type 2 diabetes with established cardiovascular disease or high cardiovascular risk. The results cannot be generalized to patients without type 2 diabetes.

In conclusion, higher plasma concentrations of TNFR-1 and TNFR-2 are associated with a higher risk of kidney disease progression in patients with type 2 diabetes and normoalbuminuria.

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Data sharing

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

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Tables and Figures

Table 1: Baseline characteristics stratified by baseline UACR.*

	Normoalbuminuria (n=2,553)	Microalbuminuria (n=778)	Macroalbuminuria (n=201)
Endpoint			
Kidney outcome	51	45	41
Baseline Characteristics			
Age (years)	63 (8)	63 (8)	63 (7)
Sex			
Male	1660 (65%)	564 (72%)	143 (71%)
Female	893 (35%)	214 (28%)	58 (29%)
Race			
Asian	243 (10%)	92 (12%)	18 (9%)
Black	54 (2%)	18 (2%)	9 (4%)
White	2085 (82%)	624 (80%)	163 (81%)
Other	171 (7%)	44 (6%)	11 (5%)
BMI (kg/m ²) [†]	32.6 (6.2)	32.9 (6.1)	32.4 (5.8)
Cardiovascular disease history			
Systolic blood pressure (mmHg)	135 (15)	139 (16)	146 (18)
Diastolic blood pressure (mmHg)	77 (10)	78 (10)	80 (10)
LDL cholesterol (mg/dl) [†]	2.3 (0.9)	2.2 (0.9)	2.5 (1.0)
HbA1c (%)	8.1 (0.9)	8.3 (0.9)	8.4 (0.9)
eGFR (ml/min/1.73m ²) [†]	80 (17)	78 (18)	70 (21)
UACR (mg/g)	8.2 (5.5 to 13.2)	66.8 (43.6 to 121.4)	675.4 (436.8 to 1167.1)
TNFR-1 (pg/ml)	2,477 (2,068 to 2,990)	2,781.5 (2,290 to 3,484)	3,495 (2,792 to 4,414)
TNFR-2 (pg/ml)	9,323 (7,642 to 11,392)	10,475 (8,403 to 13,185)	12,834 (10,338 to 17,459)
KIM-1 (pg/ml)	97 (64 to 145)	143.5 (98 to 225)	281 (186 to 474)
Treatment allocation			
Placebo	847 (33%)	257 (33%)	81 (40%)
Canagliflozin	1,706 (67%)	521 (67%)	120 (60%)

*Data are n (%), mean (SD), or median [25th to 75th Percentile].

[†]Mean BMI (4 patients), LDL cholesterol (3 patients) and creatinine (2 patients) values were imputed for patients with missing baseline values.

eGFR, estimated glomerular filtration rate; UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2; KIM-1, kidney injury molecule-1.

Table 2: Associations of doubling in baseline TNFR-1, TNFR-2, KIM-1, UACR and eGFR with the kidney outcome in patients with normoalbuminuria.

Biomarker	Model 1	Model 2	Model 3
	Hazard ratio (95% CI)	Hazard ratio (95% CI)	Hazard ratio (95% CI)
TNFR-1	2.89 (1.52 to 5.50)	2.07 (1.04 to 4.15)	4.18 (1.81 to 9.64)
TNFR-2	2.05 (1.40 to 3.01)	1.92 (1.13 to 3.26)	2.35 (1.51 to 3.65)
KIM-1	1.42 (1.06 to 1.91)	1.32 (0.96 to 1.81)	1.36 (0.98 to 1.88)
UACR	1.39 (1.01 to 1.91)	1.26 (0.90 to 1.75)	1.25 (0.89 to 1.74)
eGFR	0.93 (0.40 to 2.17)	2.39 (0.84 to 6.78)	2.33 (0.82 to 6.59)

Models are adjusted for the following covariates.

Model 1: treatment allocation.

Model 2: covariates model 1 and age, sex, race, BMI, history of cardiovascular disease systolic blood pressure, diastolic blood pressure, LDL cholesterol, and HbA1c.

Model 3: covariates model 2 and UACR and eGFR. When analyzing the association of UACR or eGFR with the outcome model 3 is not adjusted for UACR and eGFR respectively. TNFR-1, TNFR-2, KIM-1 and UACR are log transformed for the analysis.

eGFR, estimated glomerular filtration rate; UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2; KIM-1, kidney injury molecule-1.

Table 3: Number of patients, events, and event rate for the kidney outcome in patients with normoalbuminuria stratified by the 4th quartile of TNFR-1 or TNFR-2 and in patients with normoalbuminuria, microalbuminuria and macroalbuminuria.

	Cut-off value	Number of patients	Number of events	Event rate (per 1,000 patient-years)
TNFR-1 < 4 th quartile + normoalbuminuria	< 2992 pg/ml	1915	31	2.80 (1.97 to 3.98)
TNFR-1 ≥ 4 th quartile + normoalbuminuria	≥ 2992 pg/ml	638	20	5.57 (3.59 to 8.63)
TNFR-2 < 4 th quartile + normoalbuminuria	< 11394 pg/ml	1915	26	2.35 (1.60 to 3.45)
TNFR-2 ≥ 4 th quartile + normoalbuminuria	≥ 11394 pg/ml	638	25	6.98 (4.72 to 10.33)
Normoalbuminuria*	< 30 mg/g	2553	51	3.48 (2.64 to 4.58)
Microalbuminuria	< 300 mg/g	778	45	10.49 (7.83 to 14.05)
Macroalbuminuria	≥ 300 mg/g	201	41	43.06 (31.71 to 58.48)

*Event rate was 5.44 (95%CI 3.70 to 7.99) per 1,000 patient years in the placebo group and 2.53 (95%CI 1.71 to 3.75) per 1,000 patient years in the canagliflozin group.
TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2.

Figure Legends

Figure 1: Effect of prognostic enrichment with TNFR-1 or TNFR-2 on sample size, number of screenings, and costs for a future trial in the normoalbuminuria population.

UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2.

Figure 1: Effect of prognostic enrichment with TNFR-1 or TNFR-2 on sample size, number of screenings, and costs for a future trial in the normoalbuminuria population.

UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2.

Supplemental Materials

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Supplemental Table S1: Effect of prognostic enrichment with TNFR-1 or TNFR-2 on sample size, number of screenings and costs for a future trial in the normoalbuminuria population.

Percent of Patients Screened from Trial	Event Rate Among Biomarker- Positive Patients	Sample Size	NNS	Total Screened	Total Costs for Screening and Patients in Trial (million dollars)	Percent Reduction in Total Cost
TNFR-1						
0%	0.05	3,692	1	3,692	184.6	0%
25%	0.06	3,200	1.3	4,267	164.3	11%
50%	0.07	2,769	2	5,538	144.0	22%
75%	0.09	2,280	4	9,121	123.1	33.30%
95%	0.12	1,618	20	32,367	113.3	38.60%
TNFR-2						
0%	0.05	3,692	1	3,692	184.6	0%
25%	0.06	3,286	1.3	4,381	168.7	8.60%
50%	0.07	2,758	2	5,516	143.4	22.30%
75%	0.09	2,046	4	8,183	110.5	40.20%
95%	0.18	1,020	20	20,402	71.4	61.30%

TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2; NNS, number needed to screen.

Supplemental Table S2: Treatment effect of canagliflozin in patients with normoalbuminuria and TNFR-1 or TNFR-2 biomarker level above or below the 4th quartile compared to micro- or macroalbuminuria. P for interaction was calculated across the three subgroups: micro- or macroalbuminuria, TNFR-1/2 <4th quartile and normoalbuminuria and TNFR-1/2 ≥4th quartile and normoalbuminuria.

	Number of patients	Number of events	Hazard ratio (95% CI)	P value	P for interaction
40% decrease in eGFR, kidney failure, or kidney death					
Micro- or macroalbuminuria	979	86	0.64 (0.42 to 0.98)	0.040	
TNFR-1 < 4 th quartile and normoalbuminuria	1,915	31	0.41 (0.20 to 0.82)	0.012	0.546
TNFR-1 ≥ 4 th quartile and normoalbuminuria	638	20	0.52 (0.22 to 1.27)	0.152	
TNFR-2 < 4 th quartile and normoalbuminuria	1,915	26	0.35 (0.16 to 0.76)	0.008	0.391
TNFR-2 ≥ 4 th quartile and normoalbuminuria	638	25	0.61 (0.28 to 1.35)	0.222	
Hospitalization for heart failure					
Micro- or macroalbuminuria	979	61	0.95 (0.56 to 1.62)	0.858	
TNFR-1 < 4 th quartile and normoalbuminuria	1,915	49	0.72 (0.41 to 1.27)	0.259	0.764
TNFR-1 ≥ 4 th quartile and normoalbuminuria	638	19	0.75 (0.29 to 1.90)	0.541	
TNFR-2 < 4 th quartile and normoalbuminuria	1,915	42	0.70 (0.38 to 1.30)	0.259	0.758
TNFR-2 ≥ 4 th quartile and normoalbuminuria	638	26	0.79 (0.36 to 1.75)	0.563	

eGFR, estimated glomerular filtration rate; TNFR-1, tumor necrosis factor receptor-1; TNFR-2: tumor necrosis factor receptor-2.

Supplemental Figure S1: Histograms of TNFR-1, TNFR-2, KIM-1, UACR and eGFR in patients with normoalbuminuria. TNFR-1, UACR and eGFR are not truncated, TNFR-2 is truncated at 30,000 pg/ml and KIM-1 is truncated at 800 pg/ml.

eGFR, estimated glomerular filtration rate; UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2; KIM-1, kidney injury molecule-1.

Supplemental Figure S2: Hazard ratios per quartile of TNFR-1 (panel a) and TNFR-2 (panel b) for the kidney outcome in patients with normoalbuminuria. The number above each circle represents the number of outcomes and the number of patients in each quartile. On the x-axis is the median biomarker value of each quartile.

Models are adjusted for treatment allocation, age, sex, race, BMI, history of cardiovascular disease, systolic blood pressure, diastolic blood pressure, LDL cholesterol, HbA1c, UACR, and eGFR.

eGFR, estimated glomerular filtration rate; UACR, urinary albumin:creatinine ratio; TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2; UACR urinary albumin:creatinine ratio

Supplemental Figure S3: Kaplan-Meier curve for the kidney outcome in patients with normoalbuminuria below or above the 4th quartile for TNFR-1 and TNFR-2.

TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2.

Supplemental Figure S4: Associations between TNFR-1 and TNFR-2 in patients with normoalbuminuria. TNFR-1 is not truncated and TNFR-2 is truncated at 30.000

TNFR-1, tumor necrosis factor receptor-1; TNFR-2, tumor necrosis factor receptor-2.

Supplemental Figure S5: Effect of prognostic enrichment with UACR or KIM-1 on sample size, number of screenings, and costs for a future trial in the normoalbuminuria population.

UACR, urinary albumin: creatinine ratio; KIM-1, kidney injury molecule-1.