Effects of canagliflozin on anaemia in patients with type 2 diabetes and chronic kidney disease: a post-hoc analysis of the randomised, double-blind, multicentre CREDENCE trial

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Research in Context

Evidence before this study

Search criteria: We searched PubMed from January 1, 1990, to July 31, 2020 for all publications with the search terms ‘SGLT2’, ‘SGLT2 inhibitor’, ‘anaemia’, ‘blood transfusion’ ‘chronic kidney disease’, ‘nephropathy’, ‘erythropoietin’, ‘erythropoietin alpha’ and ‘erythropoiesis stimulating agent’.

Anaemia is a frequently occurring complication of chronic kidney disease. Observational studies have shown that anaemia is a strong and independent predictor of progression of chronic kidney disease (CKD), cardiovascular morbidity and mortality. Previous trials have shown that raising haemoglobin levels with erythropoietin stimulating agents did not decrease the risk of cardiovascular or kidney outcomes in patients with CKD with or without type 2 diabetes. Sodium glucose co-transporter 2 (SGLT2) inhibitors have cardiovascular and kidney benefits in people with type 2 diabetes, as demonstrated in large cardiovascular and kidney outcome trials and registries from clinical practice. Small and short-term studies have shown that SGLT2 inhibitors enhance erythropoiesis and increase red cell mass. Additionally, sustained increases in haematocrit and haemoglobin are consistently reported in clinical trials with SGLT2 inhibitors. It is however unknown if long-term treatment with SGLT2 inhibitors decrease the risk of anaemia.

Added value of this study

In this post-hoc analysis of the CREDENCE trial of 4401 participants with type 2 diabetes and CKD, we found that canagliflozin statistically significantly reduced the incidence of anaemia events or initiation of treatment for anaemia compared to placebo over a median follow-up of 2·6 years. These effects were consistent across various subgroups of participants. Furthermore, canagliflozin increased haemoglobin, haematocrit, and erythrocyte count. Proportional effects of canagliflozin on haemoglobin were greater than effects on serum albumin suggesting that direct effects on erythropoiesis contributed to the observed increases in haemoglobin.

Implications of all the available evidence

In addition to the benefits for kidney and cardiovascular outcomes, the available evidence suggests that canagliflozin may reduce the risk of anaemia and minimise the need for erythropoiesis stimulating agents and other treatment for anaemia in patients with type 2 diabetes and CKD.

Abstract

Background

Sodium glucose co-transporter 2 inhibitors may enhance erythropoiesis and red cell mass. We assessed the long-term effects of canagliflozin on anaemia-related outcomes..

Methods

In this post-hoc analysis of the randomised double-blind CREDENCE trial (ClinicalTrials.gov NCT02065791) of 4401 people with type 2 diabetes and chronic kidney disease (CKD), we assessed the effects of canagliflozin (100 mg) versus placebo on haemoglobin and haematocrit using linear mixed effects models. The effect of canagliflozin versus placebo on the composite outcome of investigator reported anaemia or treatment for anaemia was also estimated using Kaplan-Meier analysis and Cox regression models. All analyses were done according to the principle of intention to treat**.**

Findings

Mean haemoglobin was 132·0g/L (SD 17·7), 36% had anaemia (defined as haemoglobin <130g/L in men or <120g/L in women), and 0.7% used erythropoiesis-stimulating agents (ESA) at baseline. During a median follow-up of 2.6 years, mean haemoglobin and haematocrit were 7.1g/L (95%CI 6.4–7.8) and 2.4% (2.2–2.6) higher in participants receiving canagliflozin versus placebo. Overall, 358 participants reported anaemia events, 343 initiated iron preparations, 141 initiated ESA, and 104 received blood transfusion. Compared to placebo, canagliflozin reduced the risk of the composite outcome of anaemia events or initiation of treatment for anaemia by 35% (HR 0·65, 95%CI 0·55–0·77; p<0·0001). Canagliflozin also individually reduced anaemia events (HR 0·58, 95%CI 0·47–0·72; p<0·0001), initiation of iron preparations (HR 0·64, 95%CI 0·52–0·80; p<0·0001), and need for ESA (HR 0·65, 95%CI 0·46–0·91; p=0·012).

Interpretation

These data suggest that canagliflozin reduces the risk of anaemia-related outcomes, including the need for ESA, among patients with type 2 diabetes and CKD.

Funding

Janssen Research & Development, LLC

Introduction

Approximately 40% of patients with type 2 diabetes will develop chronic kidney disease (CKD), and these individuals are at high risk of progression to end-stage kidney disease (ESKD).[1,2] Anaemia occurs frequently in people with type 2 diabetes and CKD and its prevalence increases as estimated glomerular filtration rate (eGFR) declines.[3,4] Anaemia is an independent risk marker for kidney and cardiovascular outcomes and is associated with cognitive impairment and a poor quality of life.[5] The management of anaemia in CKD consists of iron therapy and synthetic derivatives of recombinant erythropoietin (EPO).[6] Although correction of CKD related anaemia with EPO reduces the need for blood transfusion, EPO does not reduce the risks of kidney or cardiovascular outcomes in patients with CKD.[7,8] Novel treatments to manage anaemia in patients with type 2 diabetes and CKD are therefore desired.

Canagliflozin, a sodium glucose co-transporter 2 (SGLT2) inhibitor, increases urinary glucose excretion and improves glycaemic control in patients with type 2 diabetes.[9-11] Because SGLT2 inhibitors simultaneously inhibit reabsorption of sodium and glucose in the proximal tubule, a mild diuretic effect has been observed which has been associated with contraction of plasma volume, improvements in blood pressure and body weight, and increases in haematocrit.[12,13] The elevation in haematocrit is commonly attributed to haemoconcentration, but SGLT2 inhibitors also cause a transient rise in EPO and increase in reticulocyte count, which together also increase haematocrit and haemoglobin in a volume independent manner.[12,14] These effects on erythropoiesis suggest that SGLT2 inhibitors may reduce the incidence of anaemia.

The aim of this post-hoc analysis from the Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial was to determine the effects of canagliflozin on haematological parameters and anaemia-related outcomes in patients with type 2 diabetes and CKD.

Methods

Study design and participants

This study analysed data from the CREDENCE trial, a multicentre, double-blind, placebo-controlled, randomised trial evaluating the effects of canagliflozin on kidney outcomes in subjects who had type 2 diabetes and CKD and were at least 30 years of age. The CREDENCE trial is registered with

ClinicalTrials.gov, number NCT02065791 and the trial protocol including a detailed description of the trial design and statistical analysis plans has been published previously along with the primary CREDENCE trial article (Supplemental document).[11,15] In brief, 4401 individuals underwent randomisation at 690 sites in 34 countries between March 2014 and May 2017. Patients were eligible if they were ≥30 years of age and had type 2 diabetes with a glycated haemoglobin level of 6.5 to 12.0%. They were also required to have CKD, defined as eGFR of 30 to <90 mL/min/1.73 m^2 and urinary albumin-to-creatinine ratio of >300 to 5000 mg/g (>33.9 to 565.6 mg/mmol). All the participants received a stable dose of renin-angiotensin-aldosterone system inhibitors for at least 4 weeks before randomisation.

Participants were randomised to receive oral canagliflozin 100 mg daily or matching placebo using randomly permuted blocks with stratification by screening eGFR categories (30-<45, 45-<60, and 60-<90 mL/min/1·73 m^2). The use of other background therapy for glycaemic management and control of cardiovascular risk factors were recommended in accordance with local guidelines. The median total follow-up period was 2·6 years until the last trial visits (either in-clinic or telephone) which occurred by October 30, 2018. Local institutional ethics committees approved the trial protocols at each site. All participants provided written informed consent. The trial was conducted according to the principles outlined in the Declaration of Helsinki.

Procedures

MO searched data from the CREDENCE trial database held by The George Institute in Sydney, NSW, Australia. Haemoglobin, haematocrit, erythrocyte count as well as total protein and serum albumin, as surrogates for volume status, were measured during the study by a central laboratory. We used two approaches to identify anaemia outcomes. First, we searched the adverse events database for records including permutations of the word “anaemia” or “cytopenia”. Adverse events of anaemia were spontaneously reported by the investigator. The definition of anaemia includes the preferred terms of “anaemia” in the Medical Dictionary for Regulatory Activities (MedDRA). Second, we searched the concomitant medications database for recorded post-randomisation treatments used for the management of anaemia including iron preparations, erythropoiesis stimulating agents (ESAs), and red blood cell transfusion. The initiation of iron preparations or ESA was defined as use of either post-randomisation with no documented use at baseline.

Outcomes

The primary outcome of this post-hoc analysis was a composite of an investigator reported anaemia event or the initiation of treatment for anaemia; anaemia events alone; initiation of iron preparations; initiation of ESA; or red blood cell transfusion. The secondary outcomes were new-onset anaemia defined as a post-randomisation haemoglobin of <130 g/L in men or <120 g/L in women according to the world health organization [WHO] guideline[16] in those without anaemia or not receiving treatment for anaemia at baseline; severe anaemia defined as a post-randomisation haemoglobin of <100 g/L in those with baseline haemoglobin of ≥100 g/L and not receiving treatment for anaemia; and correction of anaemia defined as post-randomisation haemoglobin of ≥130 g/L in men or ≥120 g/L in women in those with anaemia (haemoglobin of <130 g/L in men or <120 g/L in women) at baseline. These outcomes were chosen prior to this analysis. The primary endpoint was defined in accordance with previous trials for anaemia management.[17,18]

Plasma volume calculation

Baseline plasma volume was estimated using the Kaplan-Hakim formula[19]: plasma volume (1-haematocrit)*(a+[b*weight(kg)]), where haematocrit is a fraction, and a=1530 in males and 864 in females, and b=41 in males and 47·9 in females. This formula was derived from curve-fitting analytics in comparison to direct plasma volume measurement and was validated in a historic cohort of patients with CKD.[20,21]

Statistical analyses

Since this analysis was post hoc, no power calculation was done a priori. However, a study of 4401 individuals with equal randomisation that records 573 events provides more than 90% power (two-sided α=0·05) to detect a 12% or greater reduction in the relative risk ratio of anaemia outcomes. All analyses were done according to the principle of intention to treat.

Baseline characteristics were summarised in the canagliflozin and placebo group. Continuous variables were reported as means with SDs for variables with approximately symmetrical distributions. Results for variables with skewed distributions were presented as median and interquartile range (IQR).

Mean changes from baseline in haemoglobin, haematocrit, erythrocyte count, total protein, and serum albumin over time were analysed using linear mixed effects models with a restricted maximum likelihood (REML)-based repeated measures up to week 182. The models included the fixed, categorical effects of treatment, trial visit, category of eGFR at screening, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline value and baseline value-by-visit interaction. An unstructured covariance structure was used to model the within-patient errors. We also evaluated mean percentage changes from baseline in these values using linear mixed effects models described above. Effects of canagliflozin versus placebo on mean change in haemoglobin were determined for baseline patient subgroups defined by age (<65 or ≥65 years), sex, anaemia (haemoglobin <130 g/L in men or <120 g/L in women), haemoglobin (<100, 100 to <120, 120 to <130, or ≥130 g/L), haematocrit (<median or ≥median; 0·41 in men or 0·38 in women), estimated plasma volume (<median or ≥median; 2952 mL in men or 2791 mL in women), baseline treatment of anaemia (iron preparations or ESA), race, smoking habit, history of hypertension and cardiovascular disease, duration of diabetes (<15 or ≥15 years), body-mass index (<30 or ≥30 kg/m^2), systolic blood pressure (<140 or ≥140 mmHg), glycated haemoglobin (<8 [64] or ≥8% [64 mmol/mol]), total protein (<60 or ≥60 g/L), serum albumin (<35 or ≥35 g/L), eGFR (30-<45, 45-<60, or 60-<90 mL/min/1·73 m^2), urinary albumin-to-creatinine ratio (<1000 or ≥1000 mg/g), and diuretic use. The interaction was assessed by adding treatment by subgroup interaction terms to the relevant models with no correction of multiplicity.

We estimated the effects of canagliflozin versus placebo on anaemia from a Kaplan-Meier analysis with Cox proportional hazards models to determine hazard ratios (HR) and 95% CIs. Analyses were stratified by eGFR categories at screening as pre-specified for the main analysis.[11] We investigated the consistency of treatment effects across baseline participant subgroups defined above.

To assess the associations between an anaemia event and subsequent risks of kidney, cardiovascular, and safety events, we performed multivariable Cox proportional hazards models to estimate HR and 95% CIs for kidney, cardiovascular, and safety events in participants who experienced the primary anaemia outcome and those who did not.

All analyses were conducted using SAS, version 9.4 and Stata, version 15. A two-sided p value <0.05 was considered statistically significant and no adjustment was made for multiplicity.

Role of Funder

The CREDENCE trial was sponsored by Janssen Research and Development and was overseen by an independent steering committee, which involved non-voting members from the sponsor. The steering committee oversaw the design of the trial, the conduct of the study and the management and analysis of the data. The sponsor was involved in the design of the study, the collection and analysis of the data and the writing of the report. All authors had full access to the data. MO and HJLH had full access to all of the data and the final responsibility for the decision to submit for publication.

Results

The CREDENCE trial population consisted of 4401 participants randomly assigned to canagliflozin (n=2202) or matching placebo (n=2199). At baseline, mean haemoglobin was 132·0 g/L (SD 17·7), haematocrit 40·4% (5·3), and erythrocyte count $4{\cdot}47\times10^{12}$/L (0·58). 1599 (36%) participants had anaemia (haemoglobin <130 g/L in men or <120 g/L in women) and 117 (3%) participants had severe anaemia (haemoglobin <100 g/L) at baseline (table 1). Iron use was recorded at baseline in 233 (5%) participants and ESA in 33 (0·7%) participants. Mean haemoglobin and haematocrit were lower in women (136.4 g/L [SD 17·3] and 38·2% [4·6]) compared with men (136·4 g/L [17·3] and 41·5% [5·3]), and the proportion of participants who had anaemia was higher in women (n=612 [41%]) compared with men (n=987 [34%]).

There were 4395 participants who had a haemoglobin measurement at baseline and 4048 participants who had at least one measurement during follow-up. In both the canagliflozin and placebo group, mean baseline haemoglobin was lower in women, Black or African Americans, Asians, non-smokers, those with lower levels of body-mass index, eGFR, serum albumin, and total protein, higher levels of estimated plasma volume, and albuminuria, no history of cardiovascular disease, and longer duration of diabetes (table 2).

Mean values of haemoglobin, haematocrit, and erythrocyte count increased after randomisation and remained higher in those treated with canagliflozin than those treated with placebo during follow-up. Haemoglobin, haematocrit and erythrocyte count declined in both groups after month 12 (figure 1A-C). Compared with placebo, mean haemoglobin, haematocrit, and erythrocyte count was 7·1 g/L (95% CI 6·4–7·8), 2·4% (2·2–2·6), and $0{\cdot}25\times10^{12}$/L (0·23–0·27) higher respectively in the canagliflozin group. Increase in haemoglobin was greater in men and smokers (p for interaction 0·0004 and 0·016; table 2). The effect of

canagliflozin on haemoglobin was consistent regardless of baseline haemoglobin and haematocrit levels or estimated plasma volume status and independent of the presence or absence of anaemia (all p values for interaction >0·05; table 2). Canagliflozin also increased serum albumin and total protein compared to placebo (figure 1D/1E). The proportional effect of canagliflozin on haemoglobin (+5·40%), haematocrit (+6·00%), and erythrocyte count (+5·58%) were larger compared to the effects on total protein (+1·87%) or serum albumin (+2·60%; figure 1F).

During a median follow-up of 2·62 (IQR 2·11–3·09) years, 573 participants experienced the composite outcome of an investigator reported anaemia event or initiation of treatment for anaemia; 358 experienced an investigator reported anaemia event (of which 38 [10·6%] required or prolonged hospitalization [Supplement table 1]), 343 initiated iron preparations, 141 commenced an ESA, and 104 received red blood cell transfusion. Among participants who had anaemia events, the types of anaemia and drug action were similar between the canagliflozin and placebo groups (Supplemental table 1). There was no documented record of anaemia of malignant disease.

Canagliflozin reduced the risk of anaemia events or initiation of treatment for anaemia compared to placebo (41·2 versus 63·0 patients with an event per 1000 patient-years; HR 0·65, 95% CI 0·55–0·77; p<0·0001; figure 2 and 3). The effect on the anaemia composite outcome was greater in those not receiving a diuretic compared to those receiving a diuretic at baseline and in smokers (p for interaction 0·018 and 0·029 respectively; figure 4). There was no evidence that the effect of canagliflozin varied by other subgroups defined by baseline demographics or clinical characteristics (figure 4; all p values for interaction >0·13). Similar effect estimates were observed when the components of the composite anaemia endpoint were analysed. Compared to placebo, canagliflozin reduced anaemia events alone (23·9 versus 41·1 patients with an event per 1000 patient-years; HR 0·58, 95% CI 0·47–0·72; p<0·0001); the initiation of iron preparations (24·4 versus 37·9 patients with an event per 1000 patient-years, HR 0·64, 95% CI 0·52–0·80; p<0·0001) and the initiation of ESA (9·8 versus 15·1 patients with an event per 1000 patient-years, HR 0·65, 95% CI 0·46–0·91; p=0·012). The numerical effect on the need for blood transfusion was consistent with the other components of the anaemia outcomes although there were very few of these events (9·5 versus 10·6 patients with an event per 1000 patient-years, HR 0·89, 95% CI 0·62–1·29; p=0·55).

In participants without anaemia and not receiving treatment for anaemia at baseline, canagliflozin reduced the risks of new-onset anaemia (84·2 versus 165·6 patients with an event per 1000 patient-years, HR 0·48, 95% CI 0·41–0·55; p<0·0001; supplemental figure 1). Canagliflozin also reduced the risk of severe anaemia (19·2 versus 37·8 patients with an event per 1000 patient-years, HR 0·50, 95% CI 0·39–0·64; p<0·0001) compared to placebo. In contrast, among participants with anaemia at baseline, correction of anaemia occurred more frequently in participants assigned to canagliflozin (275·6 versus 109·8 patients with an event per 1000 patient-years; HR 2·59, 95% CI 2·18–3.08; p<0·0001).

In the additional analysis, kidney, cardiovascular, and safety events occurred more frequently in participants who experienced the primary anaemia outcome compared with those who did not (Supplemental table 2).

Discussion

Previous generally short-term and small studies demonstrated that SGLT2 inhibitors increase haemoglobin and haematocrit in patients with type 2 diabetes and relatively normal kidney function.[12-14,22] Whether these effects persist during long-term treatment and reduce the risk of anaemia in patients with type 2 diabetes and CKD has been unknown. In this post-hoc analysis of the CREDENCE trial, we observed that the effects of canagliflozin on haemoglobin and haematocrit persist over a median of 2.6 years and that canagliflozin reduces the risk of anaemia related outcomes in patients with type 2 diabetes and CKD, including initiation of erythrocyte stimulating agents.

The main finding in this study was that canagliflozin statistically significantly reduced the risk of anaemia or initiation of treatment for anaemia. No study has yet assessed the impact of SGLT2 inhibitors on anaemia related clinical events. The CREDENCE trial, the largest and only kidney outcome study of an SGLT2 inhibitor to date, enrolled participants with established CKD who were therefore at high risk of developing anaemia. This allowed us to robustly assess the effect of canagliflozin on a range of anaemia related outcomes. Although this study is a post-hoc analysis, the strength and consistency of the observed effects on a range of anaemia related outcomes and the comparability of findings across participants with different baseline characteristics suggest a real and long-term protective effect of canagliflozin on anaemia in people with type 2 diabetes, including minimising the need for erythrocyte stimulating agents. These findings also raise the possibility that a drug originally developed as a glucose lowering agent may have an

additional value as an adjunct therapy for anaemia in patients with CKD. To provide the evidence required to support clinical practice, these data require confirmation in a prospective clinical trial.

SGLT2 inhibition leads to a contraction in plasma volume as a result of mild diuretic effects.[12,23] The increase in haemoglobin and haematocrit can thus be attributed at least in part to plasma volume contraction. Notably, there was only a small increase in serum albumin and total protein in the canagliflozin group. The proportional larger effects of canagliflozin on haemoglobin and haematocrit compared to serum albumin and total protein were observed in a pooled analysis of clinical trials with the SGLT2 inhibitor dapagliflozin.[24] These data suggest that the increases in haemoglobin and haematocrit are not attributable to plasma volume contraction alone and support a possible direct effect of canagliflozin on erythropoiesis. This is further supported by the consistent effects of canagliflozin on haemoglobin regardless of baseline haemoglobin levels or estimated plasma volume.

SGLT2 inhibitors have been reported to cause a transient increase in EPO 2 to 4 weeks after treatment initiation in patients with type 2 diabetes which is accompanied by an increase in red cell mass and an increase in reticulocyte count,[12,14,25] although EPO and reticulocyte count were not measured in the CREDENCE trial. Increased EPO release has been hypothesized to result from improvements in the hypoxic microenvironment in the proximal tubule.[26] SGLT2 inhibitors decrease adenosine triphosphate (ATP) dependent workload of the proximal tubule cells thereby alleviating metabolic stress and tubulointerstitial hypoxia.[26] This reduction in ATP dependent workload has been thought to reverse EPO non-producing myofibroblasts into EPO producing fibroblasts thereby enhancing erythropoiesis and haematocrit. SGLT2 inhibitors also suppress hepcidin which may lead to an increase in bioavailability and utilization of iron and increased erythropoiesis.[27] Additionally, SGLT2 inhibition causes a shift in sodium reabsorption to the medullary thick ascending limb which increases oxygen demands in distal parts of the tubule. A decrease in oxygen tension in the medulla stimulates hypoxia inducible factor (HIF)-1α which can increase EPO production in a volume independent manner.[28] In this respect it is interesting that HIF-1α stabilisers have been proven effective in treating anaemia in patients with CKD.[29] Finally, erythropoiesis may be stimulated in a vasopressin dependent manner. As a result of mild diuretic effects, SGLT2 inhibitors increase copeptin, a surrogate marker of vasopressin.[30] In experimental settings of anaemia, vasopressin stimulates EPO release and red cell mass production providing another explanation for the observed increase in haemoglobin and haematocrit in our study.[31] Furthermore, SGLT2 inhibitors restore impaired

tubuloglomerular feedback and reduce hyperfiltration in diabetes, which could preserve long-term kidney function and reduce hypoxia.[32,33]

Prior long-term and large clinical trials of SGLT2 inhibitors have reported similar increase in haemoglobin and haematocrit over follow-up in patients with type 2 diabetes and relatively normal kidney function[13,34,35,36] These studies also demonstrated that changes in haemoglobin and haematocrit from baseline explained nearly half of the effects of SGLT2 inhibitors versus placebo on the risk of cardiovascular death, heart failure, and kidney failure.[34-36] These data suggest a strong association between erythropoiesis parameters with cardiovascular and kidney outcomes which is supported by the findings from the current analysis demonstrating that development of anaemia was statistically significantly associated with subsequent risk of kidney and cardiovascular outcomes.

The strengths of our study include the large number of participants, long duration of follow-up, sequential measurements of haemoglobin, and substantial number of anaemia related events. However, our study has several limitations. First, this study was post-hoc and may increase the risk of chance findings resulting from the multiple analyses done. All conclusions can thus only be considered hypothesis generating; as such reported p values were nominal in nature and no adjustment for multiplicity was applied. Another limitation is that the primary outcome consists of a composite of anaemia thresholds as surrogate events as well as initiation of treatment for anaemia which is clinically meaningful to patients and clinicians. Third, we did not measure EPO, reticulocyte count, and iron status, and could not therefore more directly evaluate erythropoiesis. However, several studies have already reported on the erythropoiesis stimulating effects of SGLT2 inhibitors. In addition, the effects of canagliflozin on anaemia outcomes could be in part explained by slowing progressive loss of kidney function rather than direct effects on erythropoiesis. However, the effects of canagliflozin on haematopoietic parameters persisted until the end of the trial despite an approximately 3 mL/min/1·73 m^2/year decline in eGFR during canagliflozin treatment suggesting that improvements in anaemia outcomes were not solely achieved through preservation of kidney function. Finally, anaemia was not a prespecified or adjudicated endpoint and was captured as part of routine adverse event reporting during the trial. Some anaemia events may have been missed, but such errors would be expected to have occurred non-differentially across active and placebo groups and relative effect estimates should therefore be unbiased.

In conclusion, canagliflozin reduced the risk of anaemia related outcomes, including the need for ESA, and increased haemoglobin in patients with type 2 diabetes and CKD. These findings suggest that canagliflozin could be a novel adjunct therapy for anaemia management in this population. Further prospective studies are required to confirm these observations.

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Author contributions

MO, MJ and HJLH designed the study. MO analysed the data. MO and HJLH wrote the first draft of the manuscript. All authors contributed to the design and conduct of the study and the interpretation of the data. All authors provided input into subsequent drafts and approved the final version for submission. All authors reviewed and approved the manuscript. MO and HJLH had the final responsibility for the decision to submit for publication. All authors reviewed and approved the manuscript.

Data sharing

Data from this study will be made available in the public domain via the Yale University Open Data Access Project (http://yoda.yale.edu/) once the product and relevant indication studied have been approved by regulators in the United States and European Union and the study has been completed for 18 months.

Declaration of interest

M. Oshima is supported by the Japan Society for the Promotion of Science Program for Fostering Globally Talented Researchers.

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M.J. Jardine is supported by a Medical Research Future Fund Next Generation Clinical Researchers Program Career Development Fellowship; is responsible for research projects that have received unrestricted funding from Baxter, Amgen, Eli Lilly, and Merck Sharp & Dohme; serves on a steering committee sponsored by CSL; has served on advisory boards sponsored by Akebia, Baxter, Boehringer Ingelheim, MSD, and Vifor; and has spoken at scientific meetings sponsored by Janssen and Amgen, with any consultancy, honoraria, or travel support paid to her institution.

G. Bakris has received research funding paid to the University of Chicago for serving as principal investigator on national clinical trials for Bayer, Janssen, and Novo Nordisk; has served as a consultant for Merck, Relypsa, Novo Nordisk, and AstraZeneca; has served on a steering committee for Vascular Dynamics; has served as Editor of the American Journal of Nephrology and Nephrology, Editor-in-Chief of UpToDate, and Nephrology and Hypertension Section Editor of UpToDate; and has served as Associate Editor of Diabetes Care, Hypertension Research, and Nephrology, Dialysis, and Transplantation.

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N. Rosenthal is a full-time employee of Janssen Research & Development, LLC.

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References

1. United States Renal Data System. 2017 USRDS annual data report: Epidemiology of kidney disease in the United States. . Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2017.
2. International Diabetes Federation. IDF Diabetes Atlas. 8th edition ed. Brussels, Belgium: International Diabetes Federation; 2017.
3. El-Achkar TM, Ohmit SE, McCullough PA, et al. Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. *Kidney Int* 2005; **67**(4): 1483-8.
4. Idris I, Tohid H, Muhammad NA, et al. Anaemia among primary care patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD): a multicentred cross-sectional study. *BMJ Open* 2018; **8**(12): e025125.
5. Fishbane S, Spinowitz B. Update on Anemia in ESRD and Earlier Stages of CKD: Core Curriculum 2018. *Am J Kidney Dis* 2018; **71**(3): 423-35.
6. Mehdi U, Toto RD. Anemia, diabetes, and chronic kidney disease. *Diabetes Care* 2009; **32**(7): 1320-6.
7. Pfeffer MA, Burdmann EA, Chen CY, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med* 2009; **361**(21): 2019-32.
8. Palmer SC, Saglimbene V, Mavridis D, et al. Erythropoiesis-stimulating agents for anaemia in adults with chronic kidney disease: a network meta-analysis. *Cochrane Database Syst Rev* 2014; (12): CD010590.
9. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**(7): 644-57.
10. Perkovic V, de Zeeuw D, Mahaffey KW, et al. Canagliflozin and renal outcomes in type 2 diabetes: results from the CANVAS Program randomised clinical trials. *Lancet Diabetes Endocrinol* 2018; **6**(9): 691-704.
11. Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* 2019; **380**(24): 2295-306.
12. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes Obes Metab* 2013; **15**(9): 853-62.
13. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**(22): 2117-28.
14. Maruyama T, Takashima H, Oguma H, et al. Canagliflozin Improves Erythropoiesis in Diabetes Patients with Anemia of Chronic Kidney Disease. *Diabetes Technol Ther* 2019; **21**(12): 713-20.
15. Jardine MJ, Mahaffey KW, Neal B, et al. The Canagliflozin and Renal Endpoints in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) Study Rationale, Design, and Baseline Characteristics. *Am J Nephrol* 2017; **46**(6): 462-72.
16. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. https://apps.who.int/iris/handle/10665/85839 (Last accessed July 1, 2020).
17. Macdougall IC, Bock A, Carrera F, et al. The FIND-CKD study--a randomized controlled trial of intravenous iron versus oral iron in non-dialysis chronic kidney disease patients: background and rationale. *Nephrol Dial Transplant* 2014; **29**(4): 843-50.
18. O'Lone EL, Hodson EM, Nistor I, Bolignano D, Webster AC, Craig JC. Parenteral versus oral iron therapy for adults and children with chronic kidney disease. *Cochrane Database Syst Rev* 2019; **2**: CD007857.
19. Daugirdas JT, Blake PG, Ing TS. Handbook of dialysis. 4th edition. Philadelphia: Lippincott, Williams and Wilkins; 2001.
20. Ling HZ, Flint J, Damgaard M, et al. Calculated plasma volume status and prognosis in chronic heart failure. *Eur J Heart Fail* 2015; **17**(1): 35-43.
21. Fudim M, Miller WL. Calculated Estimates of Plasma Volume in Patients With Chronic Heart Failure-Comparison With Measured Volumes. *J Card Fail* 2018; **24**(9): 553-60.
22. Mazer CD, Hare GMT, Connelly PW, et al. Effect of Empagliflozin on Erythropoietin Levels, Iron Stores, and Red Blood Cell Morphology in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease. *Circulation* 2020; **141**(8): 704-7.
23. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* 2016; **134**(10): 752-72.

24. Steffanson BV, Heerspink HJ, Wheeler DC, et al. Correction of anemia by dapagliflozin in patients with type 2 diabetes. Washington DC, Kidney Week 2019. https://www.asn-online.org/education/kidneyweek/2019/program-abstract.aspx?controlId=3236721 (Last accessed July 1, 2020).
25. Ferrannini E, Baldi S, Frascerra S, et al. Renal Handling of Ketones in Response to Sodium-Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes. *Diabetes Care* 2017; **40**(6): 771-6.
26. Sano M, Goto S. Possible Mechanism of Hematocrit Elevation by Sodium Glucose Cotransporter 2 Inhibitors and Associated Beneficial Renal and Cardiovascular Effects. *Circulation* 2019; **139**(17): 1985-7.
27. Ghanim H, Abuaysheh S, Hejna J, et al. Dapagliflozin Suppresses Hepcidin And Increases Erythropoiesis. *J Clin Endocrinol Metab* 2020; **105**(4).
28. Gupta N, Wish JB. Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors: A Potential New Treatment for Anemia in Patients With CKD. *Am J Kidney Dis* 2017; **69**(6): 815-26.
29. Chen N, Hao C, Peng X, et al. Roxadustat for Anemia in Patients with Kidney Disease Not Receiving Dialysis. *N Engl J Med* 2019; **381**(11): 1001-10.
30. Eickhoff MK, Dekkers CCJ, Kramers BJ, et al. Effects of Dapagliflozin on Volume Status When Added to Renin-Angiotensin System Inhibitors. *J Clin Med* 2019; **8**(6).
31. Mayer B, Nemeth K, Krepuska M, et al. Vasopressin stimulates the proliferation and differentiation of red blood cell precursors and improves recovery from anemia. *Sci Transl Med* 2017; **9**(418).
32. Cherney DZ, Perkins BA, Soleymanlou N, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* 2014; **129**(5): 587-97.
33. van Bommel EJM, Muskiet MHA, van Baar MJB, et al. The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial. *Kidney Int* 2020; **97**(1): 202-12.
34. Inzucchi SE, Zinman B, Fitchett D, et al. How Does Empagliflozin Reduce Cardiovascular Mortality? Insights From a Mediation Analysis of the EMPA-REG OUTCOME Trial. *Diabetes Care* 2018; **41**(2): 356-63.
35. Li J, Neal B, Perkovic V, et al. Mediators of the effects of canagliflozin on kidney protection in patients with type 2 diabetes. *Kidney Int* 2020.
36. Li J, Woodward M, Perkovic V, et al. Mediators of the Effects of Canagliflozin on Heart Failure in Patients With Type 2 Diabetes. *JACC Heart Fail* 2020; **8**(1): 57-66.

Table 1: Baseline characteristics

Characteristic	Canagliflozin (n=2202)	Placebo (n=2199)
Age, years	62·9 (9·2)	63·2 (9·2)
Sex		
Male	1440 (65%)	1467 (67%)
Female	762 (35%)	732 (33%)
Race or ethnic group *[1]		
White	1487 (68%)	1444 (66%)
Black	112 (5%)	112 (5%)
Asian	425 (19%)	452 (21%)
Other	178 (8%)	191 (9%)
Current smoker	341 (16%)	298 (14%)
History of hypertension	2131 (97%)	2129 (97%)
Duration of diabetes, years	15·5 (8·7)	16·0 (8·6)
Nephropathy	2202 (100%)	2199 (100%)

History of cardiovascular disease	1113 (51%)	1107 (50%)
Haemoglobin, g/L *[2]	132·6 (17·7)	131·1 (17·7)
<100 g/L	59 (2·7%)	58 (2·6%)
100 to <120 g/L	462 (21·0%)	513 (23·4%)
120 to <130 g/L	438 (19·9%)	449 (20·5%)
≥130 g/L	1240 (56·4%)	1176 (53·6%)
Anaemia *[3]	769 (35%)	830 (38%)
Haematocrit, % *[4]	40·6 (5·3)	40·2 (5·3)
Erythrocyte count, $\times 10^{12}$/L *[3]	4·49 (0·58)	4·45 (0·59)
Estimated plasma volume, mL	2992·1 (623·7)	2996·7 (619·8)
Body-mass index, kg/m^2	31·4 (6·2)	31·3 (6·2)
Systolic blood pressure, mmHg	139·8 (15·6)	140·2 (15·6)
Diastolic blood pressure, mmHg	78·2 (9·4)	78·4 (9·4)
Glycated haemoglobin, mmol/mol	67 (14·2)	67 (15·3)
Glycated haemoglobin, %	8·3 (1·3)	8·3 (1·4)
Total protein, g/L *[5]	70·7 (5·4)	70·8 (5·5)
Serum albumin, g/L *[5]	39·5 (3·8)	39·5 3·8)
Estimated glomerular filtration rate, mL/min/1·73 m^2	56·3 (18·2)	56·0 (18·3)
Urinary albumin:creatinine ratio (IQR), mg/g	923·0 (459–1794)	931·0 (473–1868)
Iron preparation	123 (6%)	110 (5%)
Erythropoiesis-stimulating agent	18 (0·8%)	15 (0·7%)
Vitamin B12 or folic acid	93 (4·2%)	96 (4·4%)
Diuretic	1026 (47%)	1031 (47%)

Data are mean (SD), n (%), or n/N (%). Percentages might not sum to 100% because of rounding. IQR denotes interquartile range.

*[1] Race or ethnic group was reported by the patients. The designation “other” includes American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, multiple, other, unknown, and not reported.

*[2] Six patients (3 in each group) did not have baseline haemoglobin and erythrocyte measurements.

*[3] Anaemia was defined as <130g/L in men and <120g/L in women according to the WHO guideline.

*[4] Twenty-five patients (17 in the canagliflozin and 8 in the placebo group) did not have baseline haematocrit measurements.

*[5] One and two patients (all in the canagliflozin group) did not have baseline total protein and albumin measurements.

Table 2: Effects of canagliflozin on haemoglobin in participant subgroups defined by baseline characteristics

		N	Mean (SD) haemoglobin at baseline, g/L		Mean difference (95% CI) in haemoglobin during follow-up, g/L	P for interaction
			Canagliflozin	Placebo		
All participants			132·9 (17·6)	131·5 (17·4)	7·1 (6·4–7·8)	
Age (years)	<65		130·8 (18·5)	132·4 (17·8)	7·3 (6·3–8·2)	0·61
	≥65		132·3 (16·3)	133·6 (17·4)	6·9 (6·0–7·8)	
Sex	Male		137·7 (16·9)	135·6 (17·3)	8·0 (7·1–8·8)	<0·001
	Female		124·0 (15·2)	123·4 (14·8)	5·5 (4·5–6·6)	
Anaemia	No		142·3 (12·8)	141·6 (12·7)	7·3 (6·5–8·1)	0·54
	Yes		114·8 (9·8)	114·7 (9·7)	6·8 (5·7–8·0)	
Haemoglobin (g/L)	<100		92·8 (4·8)	92·5 (6·5)	7·4 (0·8–14·1)	0·26
	100 to 120		112·1 (5·3)	111·7 (5·5)	5·9 (4·4–7·3)	
	120 to 130		124·7 (2·9)	124·7 (2·9)	7·0 (5·6–8·5)	
	≥130		145·1 (11·4)	144·4 (11·5)	7·6 (6·7–8·4)	
Haematocrit	<median		118·6 (11·9)	118·4 (11·6)	7·5 (6·4–8·5)	0·45
	≥median		143·0 (13·4)	142·7 (13·4)	7·0 (6·1–7·8)	
Estimated plasma volume	<median		136·8 (18·0)	135·7 (18·5)	6·7 (5·7–7·6)	0·16
	≥median		129·2 (16·3)	127·9 (15·6)	7·7 (6·7–8·6)	
Iron preparation	No		133·8 (17·3)	132·3 (17·3)	7·2 (6·5–7·9)	0·41
	Yes		118·8 (16·2)	116·4 (14·6)	6·2 (3·3–9·1)	
Erythropoiesis stimulating agent	No		133·1 (17·6)	131·7 (17·4)	7·1 (6·5–7·8)	0·70
	Yes		115·9 (12·3)	110·3 (15·6)	7·7 (-1·5–17·0)	
Race or ethnic group	White		134·6 (17·1)	133·8 (16·6)	6·9 (6·1–7·7)	0·54
	Black		124·9 (17·0)	126·9 (17·9)	5·6 (2·9–8·3)	
	Asian		129·1 (17·7)	123·9 (17·4)	7·9 (6·5–9·3)	
	Other		132·8 (19·3)	130·2 (19·7)	7·4 (4·9–9·9)	
Smoker	No		131·9 (17·5)	130·9 (17·2)	6·8 (6·0–7·5)	0·02
	Yes		138·4 (17·1)	135·8 (8·7)	8·9 (7·2–10·5)	
History of hypertension	No		126·6 (17·9)	129·2 (19·7)	8·9 (5·6–12·1)	0·35
	Yes		133·2 (17·6)	131·6 (17·4)	7·0 (6·4–7·7)	
History of cardiovascular disease	No		132·1 (18·0)	130·5 (17·3)	6·9 (6·0–7·8)	0·57
	Yes		133·8 (17·2)	132·5 (17·7)	7·3 (6·5–8·2)	
Duration of diabetes (years)	<15		135·2 (18·1)	133·4 (18·2)	7·7 (6·7–8·7)	0·08
	≥15		130·8 (16·9)	129·9 (16·7)	6·5 (5·6–7·4)	
Body-mass index (kg/m^2)	<30		131·7 (17·8)	129·7 (17·9)	6·5 (5·5–7·5)	0·12
	≥30		134·0 (17·4)	133·2 (16·9)	7·6 (6·7–8·5)	
Systolic blood pressure (mmHg)	≤140		133·6 (17·4)	132·5 (17·9)	6·9 (6·0–7·8)	0·47
	>140		132·1 (17·9)	130·5 (17·0)	7·3 (6·4–8·3)	
Glycated haemoglobin (%) [mmol/mol])	<8 (64)		132·1 (17·5)	130·8 (17·7)	6·8 (5·9–7·8)	0·39
	≥8 (64)		133·7 (17·7)	132·2 (17·3)	7·3 (6·4–8·2)	
Total protein (g/L)	<60		124·2 (22·1)	116·5 (16·3)	7·5 (1·6–13·3)	0·80
	≥60		133·1 (17·4)	131·8 (17·4)	7·1 (6·4–7·8)	
Serum albumin (g/L)	<35		125·0 (21·3)	120·7 (17·2)	6·9 (6·0–7·8)	0·47
	≥35		133·6 (17·1)	132·7 (17·1)	7·1 (6·4–7·8)	
Estimated glomerular filtration rate ($mL/min/1·73\ m^2$)	30-<45		128·7 (18·1)	127·4 (17·6)	7·6 (6·3–8·9)	0·61
	45-<60		131·7 (17·9)	130·5 (18·0)	7·2 (6·0–8·4)	
	60-<90		136·8 (16·2)	135·2 (16·3)	6·7 (5·7–7·8)	
Urinary albumin:creatinine ratio (mg/g)	<1000		134·4 (16·8)	132·9 (17·2)	6·8 (6·0–7·6)	0·34
	≥1000		131·2 (18·4)	129·9 (17·7)	7·5 (6·4–8·5)	
Diuretic	No		133·9 (17·6)	131·8 (17·8)	6·8 (5·9–7·6)	0·26

Yes	131·9 (7·6)	131·2 (17·1)	7·5 (6·5–8·5)

Figure 1: Effects of canagliflozin on mean change in (A) haemoglobin, (B) haematocrit, (C) erythrocyte count, (D) total protein, (E) serum albumin, and (F) percentage change in these values

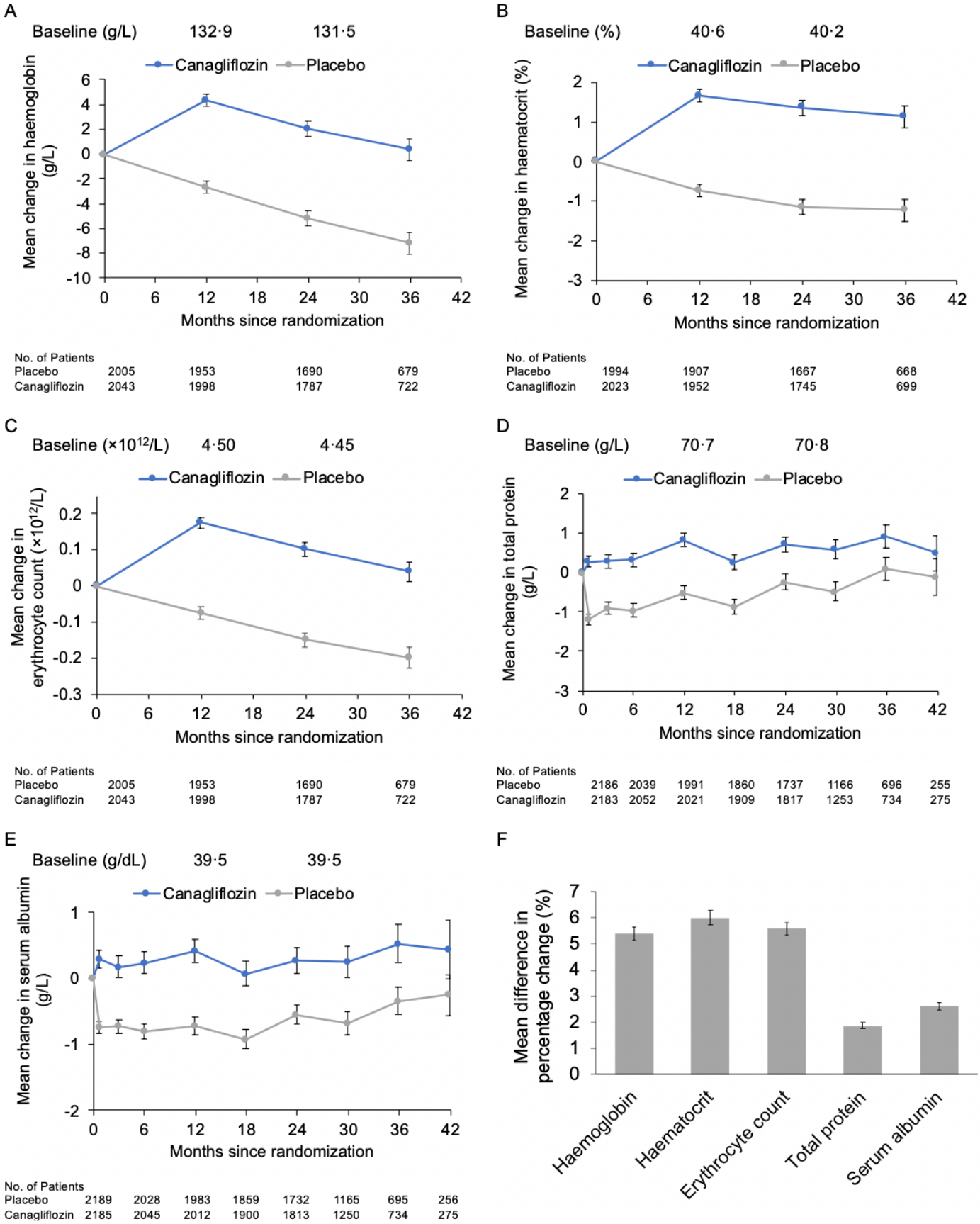


Figure 2: Kaplan-Meier curves of effects of canagliflozin versus placebo on (A) anaemia or initiation of treatment for anaemia, (B) anaemia, (C) initiation of iron preparation, (D) initiation of erythropoiesis stimulating agent, and (E) blood transfusion.

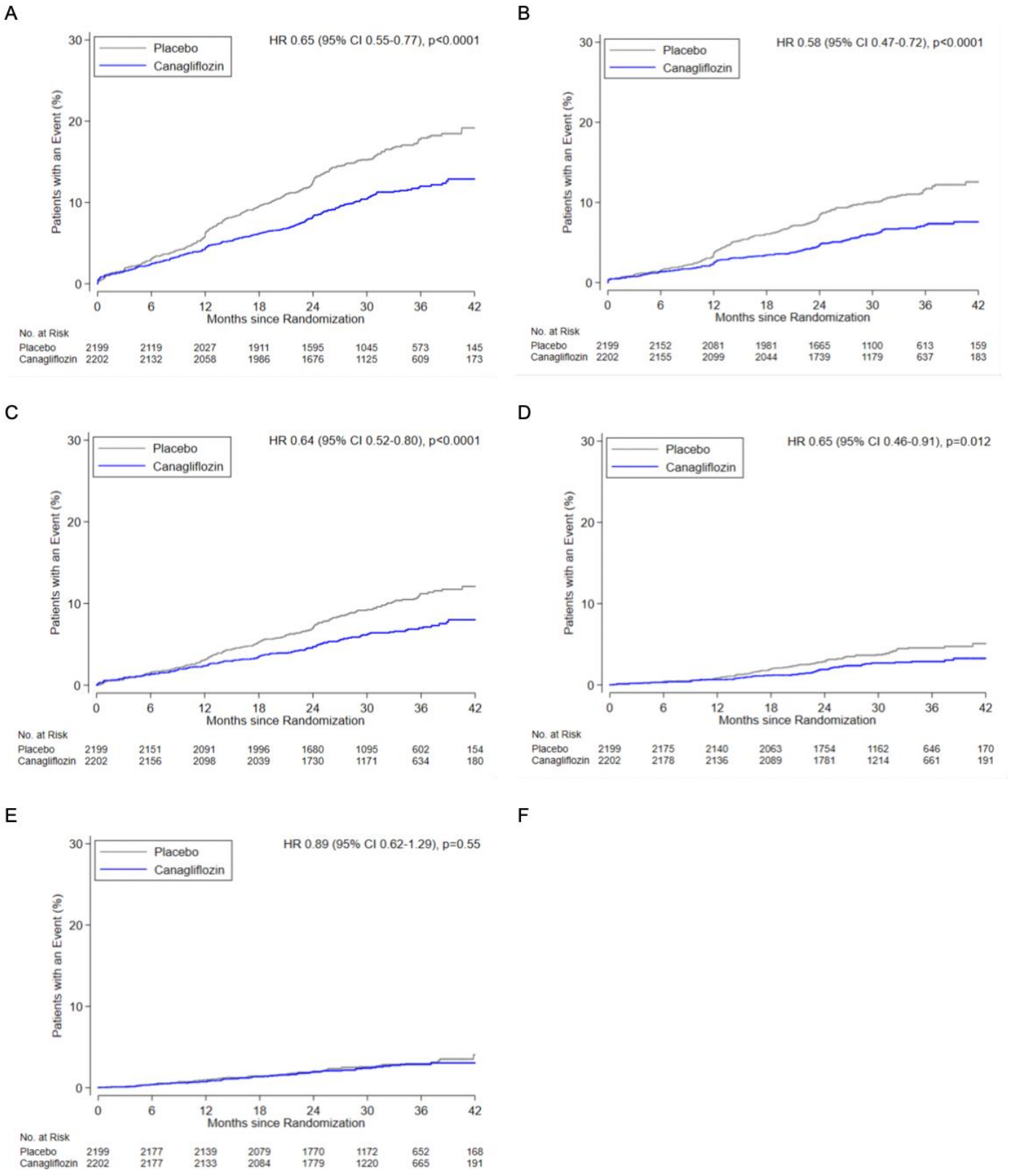


Figure 3: Forest plot of effects of canagliflozin versus placebo on anaemia or initiation of treatment for anaemia

	N of participants with an event/total (%)		Participants with an event per 1000 patient-years			
	Canagliflozin	Placebo	Canagliflozin	Placebo	Hazard ratio (95% CI)	*P* value
Anaemia or initiation of treatment for anaemia	230/2202 (10·4)	343/2199 (15·6)	42·2	64·9	0·65 (0·55–0·77)	<0·0001
Anaemia	134/2202 (6·1)	224/2199 (10·2)	23·9	41·1	0·58 (0·47–0·72)	<0·0001
Initiation of iron preparation	136/2202 (6·2)	207/2199 (9·4)	24·4	37·9	0·64 (0·52–0·80)	<0·0001
Initiation of erythropoiesis stimulating agent	56/2202 (2·5)	85/2199 (3·9)	9·8	15·1	0·65 (0·46–0·91)	0·012
Blood transfusion	54/2202 (2·5)	60/2199 (2·7)	9·5	10·6	0·89 (0·62–1·29)	0·55

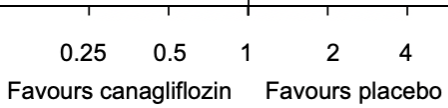


Figure 4: Forest plot of effects of canagliflozin versus placebo on anaemia or initiation of treatment

		N of participants with an event/total (%)		Participants with an event per 1000 patient-years		Hazard ratio (95% CI)	P for interaction
		Canagliflozin	Placebo	Canagliflozin	Placebo		
Age (years)	<65	116/1193	187/1151	39·7	68·3	0·58 (0·46–0·73)	0·18
	≥65	114/1009	156/1048	45·1	61·3	0·73 (0·57–0·93)	
Sex	Male	137/1440	225/1467	37·8	63·5	0·60 (0·48–0·74)	0·24
	Female	93/762	118/732	51·0	67·8	0·74 (0·56–0·97)	
Anaemia	No	78/1430	120/1366	21·0	34·7	0·59 (0·45–0·79)	0·32
	Yes	152/769	222/830	88·2	122·1	0·72 (0·59–0·89)	
Haemoglobin (g/L)	<100	24/59	25/58	240·1	258·4	0·93 (0·53–1·63)	0·40
	100 to <120	95/462	140/513	92·0	122·9	0·74 (0·57–0·97)	
	120 to <130	50/438	87/449	46·8	83·2	0·56 (0·40–0·79)	
	≥130	61/1240	90/1176	18·8	30·1	0·61 (0·44–0·85)	
Haematocrit	<median	167/928	250/1010	76·6	109·3	0·60 (0·43–0·82)	0·41
	≥median	61/1257	92/1181	18·9	30·9	0·71 (0·58–0·86)	
Plasma volume	<median	94/1053	140/1027	36·4	58·3	0·65 (0·50–0·84)	0·88
	≥median	133/1126	201/1161	47·3	70·4	0·66 (0·53–0·82)	
Iron preparation	No	211/2079	315/2089	40·9	62·5	0·65 (0·55–0·78)	0·81
	Yes	19/123	28/110	65·3	114·1	0·62 (0·34–1·11)	
Erythropoiesis stimulating agent	No	229/2184	341/2184	42·3	65·0	0·65 (0·55–0·77)	0·83
	Yes	1/18	2/15	26·1	54·2	0·57 (0·05–6·52)	
Race or ethnic group	White	143/1487	183/1444	37·9	51·3	0·74 (0·59–0·92)	0·22
	Black	19/112	23/112	68·3	85·4	0·74 (0·40–1·37)	
	Asian	48/425	93/452	47·8	90·4	0·53 (0·38–0·76)	
	Other	20/178	44/191	50·6	105·6	0·47 (0·28–0·80)	
Smoker	No	199/1861	282/1901	43·2	61·5	0·70 (0·58–0·84)	0·029
	Yes	31/341	61/298	37·0	87·4	0·42 (0·27–0·65)	
History of hypertension	No	9/71	12/70	54·8	78·0	0·64 (0·27–1·53)	0·96
	Yes	221/2131	331/2129	41·8	64·5	0·65 (0·55–0·77)	
History of cardiovascular disease	No	99/1089	164/1092	36·3	62·0	0·59 (0·46–0·76)	0·30
	Yes	131/1113	179/1107	48·1	67·8	0·70 (0·56–0·88)	
Duration of diabetes (years)	<15	88/1062	147/1010	33·7	60·5	0·56 (0·43–0·72)	0·13
	≥15	142/1140	196/1189	50·1	68·7	0·72 (0·58–0·90)	
Body-mass index (kg/m^2)	<30	117/998	175/1028	49·0	73·2	0·68 (0·54–0·86)	0·63
	≥30	112/1198	167/1168	36·7	57·8	0·62 (0·49–0·79)	
Systolic blood pressure (mmHg)	≤140	111/1205	161/1189	36·7	55·8	0·66 (0·52–0·85)	0·81
	>140	119/997	182/1010	49·1	75·9	0·64 (0·50–0·80)	
Glycated haemoglobin (% [mmol/mol])	<8 (<64)	119/1027	167/1029	46·6	66·9	0·70 (0·55–0·88)	0·42
	≥8 (≥64)	111/1174	176/1169	38·3	63·1	0·61 (0·48–0·77)	
Total protein (g/L)	<60	13/53	21/47	112·8	241·0	0·52 (0·26–1·06)	0·42
	≥60	217/2148	322/2152	40·7	61·9	0·66 (0·55–0·78)	
Serum albumin (g/L)	<35	36/190	69/216	83·8	158·1	0·55 (0·36–0·82)	0·28
	≥35	194/2010	274/1983	38·7	33·6	0·68 (0·57–0·82)	
Estimated glomerular filtration rate ($mL/min/1.73\ m^2$)	30-<45	103/657	148/656	65·4	98·0	0·67 (0·52–0·86)	0·92
	45-<60	65/640	102/639	40·8	66·8	0·62 (0·45–0·84)	
	60-<90	62/905	93/904	27·2	41·4	0·65 (0·47–0·90)	
Urinary albumin:creatinine ratio (mg/g)	<1000	88/1185	126/1163	29·1	43·4	0·67 (0·51–0·88)	0·77
	≥1000	142/1017	217/1036	58·6	91·2	0·64 (0·52–0·79)	
Diuretic	No	96/1176	173/1168	32·9	62·3	0·52 (0·41–0·67)	0·018
	Yes	134/1026	170/1031	53·0	67·8	0·79 (0·63–0·99)	

Supplementary appendix to: Effects of canagliflozin on anaemia in patients with type 2 diabetes and chronic kidney disease: a post-hoc analysis of the randomised, double-blind, multicentre CREDENCE trial

Supplement table 1: Detailed information about anaemia events

Supplement table 2: Associations of the occurrence of the primary anaemia outcome and kidney, cardiovascular, and safety events

Supplement figure 1: Forest plot of effects of canagliflozin versus placebo on (A) new-onset or worsening anaemia and (B) correction of anaemia

Supplemental table 1: Detailed information about anaemia events

	Total	Canagliflozin	Placebo
Anaemia events	358	134	224
Preferred term reported by investigators, n (%)			
Anaemia	286 (79·9)	107 (79·9)	179 (79·9)
Iron deficiency anaemia	26 (7·3)	9 (6·7)	17 (7·6)
Nephrogenic anaemia	22 (6·2)	9 (6·7)	13 (5·8)
Haemorrhagic anaemia	6 (1·7)	2 (1·5)	4 (1·8)
Pancytopenia	5 (1·4)	1 (0·8)	4 (1·8)
Bicytopenia	2 (0·6)	1 (0·8)	1 (0·5)
Anaemia vitamin B12 deficiency	2 (0·6)	2 (1·5)	0 (0)
Anaemia folate deficiency	1 (0·3)	1 (0·8)	0 (0)
Normocytic anaemia	3 (0·8)	0 (0)	3 (1·3)
Normochromic normocytic anaemia	3 (0·8)	1 (0·8)	2 (0·9)
Hypochromic anaemia	1 (0·3)	1 (0·8)	0 (0)
Microcytic anaemia	1 (0·3)	0 (0)	1 (0·5)
Drug action, n (%)			
Drug interrupted	27 (7·5)	10 (7·5)	17 (7·6)
Drug withdrawn	4 (1·1)	1 (0·8)	3 (1·3)
Dose not changed	229 (64.0)	82 (61·2)	147 (65·6)
Not applicable	97 (27·1)	40 (29·9)	57 (25·5)
Unknown	1 (0·3)	1 (0·8)	0 (0)
Serious anaemia events*, n (%)	38 (10·6)	17 (12·7)	21 (9·4)
Reasons for serious adverse events, n (%)			
Required hospitalization	35 (92·1)	14 (82·4)	21 (100·0)
Prolong hospitalization	3 (7·9)	3 (17·7)	0 (0)
Persistence or significant disability	2 (5·3)	0 (0)	2 (9·5)
Life threatening	3 (7·9)	2 (11·8)	1 (4·8)
Death	0 (0)	0 (0)	0 (0)

* Numbers do not add up since more than one reason could be reported per patient.

Supplemental table 2: Associations of the occurrence of the primary anaemia outcome and kidney, cardiovascular, and safety events

	N of participants with an event/total (%)		Participants with an event per 1000 patient-years		Multivarible adjusted hazard ratio (95% CI)	P value
	Yes	No	Yes	No		
Kidney and cardiovascular events						
Doubling of serum creatinine, end-stage kidney disease, renal death, or cardiovascular death	118/498 (23·7)	394/3828 (10·3)	183·1	40·0	3·37 (2·68–4·25)	<0·0001
Doubling of serum creatinine, end-stage kidney disease, or renal death	90/498 (18·1)	215/3828 (5·6)	139·8	21·8	4·72 (3·56–6·26)	<0·0001
Cardiovascular death, myocardial infarction, or stroke	68/525 (13·0)	373/3828 (9·7)	100·4	38·3	2·06 (1·54–2·74)	<0·0001
Cardiovascular death or hospitalization for heart failure	73/529 (13·8)	318/3510 (8·3)	106·3	32·3	2·65 (1·99–3·51)	<0·0001
Fatal or nonfatal stroke	16/556 (2·8)	113/3828 (3·0)	21·8	11·5	1·91 (1·07–3·40)	0·029
Safety events						
Any adverse events other than anaemia	467/573 (81·5)	3135/3828 (81·9)	2202·7	854·6	1·47 (1·32–1·63)	<0·0001
Any serious adverse events other than anaemia	288/573 (50·3)	1280/3828 (33·4)	570·1	153·9	2·95 (2·56–3·40)	<0·0001
Any renal related adverse events	151/573 (26·4)	538/3828 (14·1)	244·0	57·5	2·86 (2·33–3·50)	<0·0001
Acute kidney injury	47/573 (8·2)	143/3828 (3·7)	65·8	14·6	3·20 (2·21–4·63)	<0·0001

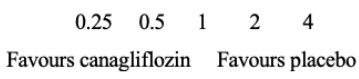
In participants who experienced the primary anaemia outcome, the time to the first occurrence of kidney, cardiovascular, and safety events after the anaemia outcome was assessed while in those who did not experience the anaemia outcome, the time from baseline was assessed. Kidney and cardiovascular events were excluded if they occurred before the anaemia outcome.

Hazard ratios (95% CI) were calculated with Cox proportional hazard regression and adjusted for baseline covariates including age, sex, race, current smoking, history of hypertension, history of heart failure, duration of diabetes, history of cardiovascular disease, body-mass index, systolic blood pressure, haemoglobin, glycated haemoglobin, eGFR, HDL cholesterol, LDL cholesterol, log-transformed triglycerides, diuretic use, randomised treatment (canagliflozin or placebo), and log-transformed urinary albumin-to-creatinine ratio.

Supplemental figure 1: Forest plot of effects of canagliflozin versus placebo on (A) new-onset or worsening anaemia and (B) correction of anaemia

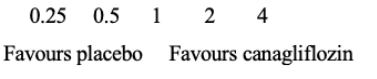
A

	N of participants with an event/total (%)		Participants with an event per 1000 patient-years		Hazard ratio (95% CI)	*P* value
	Canagliflozin	Placebo	Canagliflozin	Placebo		
New-onset anaemia (Hb<13 in men/<12 in women)	281/1042 (21·2)	471/1250 (37·7)	84·2	165·6	0·48 (0·41–0·55)	<0·0001
Worsening anaemia (Hb<10)	112/2024 (5·5)	215/2002 (10·7)	19·2	37·8	0·50 (0·39–0·64)	<0·0001



B

	N of participants with an event/total (%)		Participants with an event per 1000 patient-years		Hazard ratio (95% CI)	*P* value
	Canagliflozin	Placebo	Canagliflozin	Placebo		
Correction of anaemia (Hb≥13 in men/≥12 in women)	370/713 (51·9)	195/771 (25·3)	275·6	109·8	2·59 (2·18–3·08)	<0·0001



New-onset anaemia was assessed in the participants without anaemia or baseline use of anti-anaemic medications.
Worsening anaemia was assessed in the participants with baseline Hb ≥10 and no baseline use of anti-anaemic medications.
Correction of anaemia was assessed in the participants with anaemia.