EMPA-KIDNEY Body Composition Measurement Substudy

Study Title: A multicentre international randomized parallel group double-blind placebo-controlled clinical trial of EMPAgliflozin once daily to assess cardio-renal outcomes in patients with chronic KIDNEY disease

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Summary

This document provides the rationale and design of an EMPA-KIDNEY substudy to measure body composition in a subset of the 5000 EMPA-KIDNEY participants using bioimpedenace spectroscopy. The substudy does not alter the main protocol in any respect.

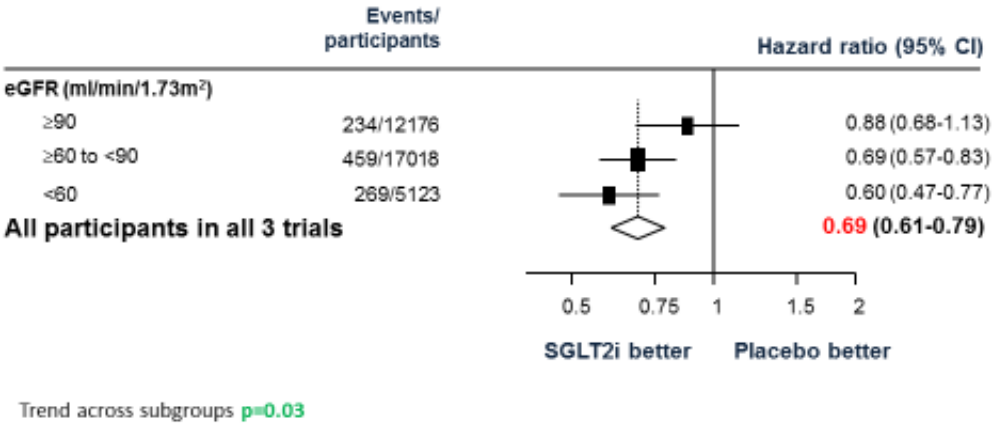
Background

In the EMPA-REG OUTCOME trial, empagliflozin 10-25mg was shown to reduce the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke by 14% compared to placebo (hazard ratio [HR] 0.86, 0.74-0.99) in 7020 people with type 2 diabetes mellitus (T2DM) and prior atherosclerotic cardiovascular disease.[1] This effect was in large part the result of a highly significant 38% (HR 0.62, 0.49-0.77) reduction in cardiovascular death. The pre-specified secondary outcome of hospitalization for heart failure was reduced by 35% (HR 0.65, 0.50-0.85).[1] Exploration of EMPA-REG OUTCOME data has suggested that the increase in haematocrit caused by empagliflozin, a possible surrogate for reductions in plasma volume, was the intermediate clinical parameter with the largest mediating effect on the reduction in cardiovascular death.[2] These observations may have particular relevance in people with chronic kidney disease (CKD) who have disturbed salt and water homeostasis which may cause chronic fluid overload which in turn contributes to the observed excess of structural heart disease and heart failure.[3]

In EMPA-REG OUTCOME, allocation to empagliflozin led to a sustained loss of weight (of about 2Kg from a mean of 86Kg) and a 2cm reduction in waist circumference (from a mean of 105cm).[1] How much of this weight change reflected reduction in total body water versus adipose tissue is unknown. A previous trial suggested that, after 2 years, weight loss resulting from SGLT-2 inhibition in people with T2DM appears almost completely attributable to reduced adipose tissue (measured using dual energy X-ray absorptiometry).[5] Lower kidney function substantially reduces glycosuric effects of SGLT-2 inhibition, and so reduced calorie loss at lower levels of kidney function may

attenuate any loss of adipose tissue. However, no attenuation of the weight-lowering effects of SGLT-2 inhibition was identified in those with CKD compared to those without (within the range of kidney function studied to date).[6-8] Furthermore, meta-analysis of three large placebo-controlled trials suggests effects of SGLT-2 inhibition on heart failure are at least as large among those with reduced kidney function.[9] Part of the preserved effect of SGLT-2 inhibition on body weight and heart failure in CKD may therefore result from reductions in excess extracellular water (ECW) being preserved in those with CKD, despite attenuated effects on glycosuria. This raises a hypothesis that the effects of empagliflozin on excess ECW and fat levels may be different in people with different levels of kidney function.

Figure 1: Effect of SGLT-2 inhibition versus placebo on hospitalization for heart failure, by baseline kidney function (meta-analysis EMPA-REG OUTCOME, CANVAS and DECLARE) [9]



Bioimpedance spectroscopy can assess different resistance patterns in the body which are affected by the amount of water present. Low frequency current exclusively passes extracellularly, whereas high frequencies can pass through all body water compartments. Comparing spectroscopy readings over a range of frequencies it is possible to derive total body water in Litres and separately the volume of ECW. From such measurements it is also possible to estimate normally hydrated adipose tissue and lean tissue mass, from which an index referred to as "Fluid Overload" (or overhydration) can be algorithmically calculated.[10] Sustained "Fluid Overload" measured by bioimpedance spectroscopy has been associated with increased risk of mortality among people on dialysis,[4] and some dialysis units are now using bioimpedance spectroscopy measurements clinically to guide patients' fluid management and dialysis prescription.

At each Follow-up Visit, EMPA-KIDNEY participants will have their weight measured and central plasma/serum blood samples collected. At Randomization, 2 & 18 months and Final Follow-up Visit, they will also have a measure of waist and hip circumference. A substudy using bioimpedance-

based body composition measurements will ensure uncertainty about the effects of empagliflozin on ECW, adipose tissue and particularly "Fluid Overload" will be assessed in a CKD population.

Aims

The primary aim of this substudy is to use bioimpedance spectroscopy to assess, in a subset of EMPA-KIDNEY participants, the effect of empagliflozin 10mg versus matching placebo on "Fluid Overload" at the 2 month and 18 month Follow-up Visits.

Secondary aims are to use bioimpedance spectroscopy to assess:

1. Whether any effects of empagliflozin 10mg versus matching placebo on "Fluid Overload" are modified by baseline factors, in particular by level of kidney function, glycosylated haemoglobin, body mass index, NT-proBNP, age, sex, RAS inhibitor use, and different diuretics
2. The effects of empagliflozin 10mg versus matching placebo early and later during follow-up on:
    - ECW
    - Intracellular water (ICW)
    - Adipose tissue mass indexed to weight (i.e. %)
    - Lean tissue mass indexed to weight (i.e. %)

Exploratory aims are to:

- Assess if changes in ECW, ICW, % adipose tissue mass, % lean tissue mass and "Fluid Overload" correlate with changes in blood pressure and relevant other biomarkers.

Sample size estimates

The study will start a vanguard phase in a small number of sites in which bioimpedance spectroscopy will be performed at Randomization, 2 months and 18 months of Follow-up Visits. This vanguard phase will be expanded to other sites once feasiblity of adding a bioimpedance spectroscopy measurement is demonstrated. Feasbility will be based on feedback from the participating sites, successful completion of the other protocol-specified procedures and logistical considerations. It is estimated that at least 400 (of the 5000) EMPA-KIDNEY participants with follow-up bioimpedance spectroscopy measurements will provide ample power (>90%, 2p=0.05) to detect at least a ±300mL difference in "Fluid Overload" (reference range in healthy adults is ± 1100mL with a standard deviation of 900mL) based on an independent 2-sided t-test (Table 1).

Table 1: Sample size calculations for a study with Randomization Visit measurements

Outcome	Effect size	Standard deviation	Required sample size
"Fluid Overload" (ref range: ±1.1L)	±300mL	900mL	382

Note: An estimate of the correlation between successive bioimpedance spectroscopy measurements would be required to calculate the reduction in sample size that could be achieved by using ANCOVA analyses, but no such longitudinal data has yet been collected in a CKD population.

If a bioimpedance spectroscopy measurement at the Randomization Visit is shown to be infeasible, the substudy will be modifed to exclude the measurement at the Randomization Visit and only be performed at the relatively less busy phases of the study (i.e. measurements will be restricted to the 2 and 18 month Follow-up Visits). In this design, the sample size would need to increase to 850. This is because the absence of a bioimpedance spectroscopy measurement at the Randomization Visit means any imbalances in "Fluid Overload" between treatment arms at baseline cannot be corrected for. These imbalances could result in either the treatment effect being overestimated or a smaller than expected difference in mean "Fluid Overload" at follow-up. However, with a sample size of 850, the probability of large baseline imbalances is small, making it unlikely that the treatment effect would be overstated by more than 100mL (Table 2). With a sample size of 850, there would be sufficient power to detect a reduced difference in mean "Fluid Overload" of ± 200 mL at follow-up. This calculation is based on an independent 2-sided t-test using data from a healthy population (Note: sample size estimates differ little if dialysis population data are used).

Table 2: Sample size calculations for a study without Randomization Visit measurements

Sample size	Assumed possible baseline imbalance in Fluid Overload (mL)	Probability of a baseline imbalance at least this size due to chance (1-sided)	Difference between groups at follow-up (mL) after subtracting possible baseline imbalance from assumed treatment effect of 300 mL	Power to detect reduced difference in follow-up values at 2p=0.05
850	0 50 100 150 200	Not applicable 12.6% 1.1% 0.03% 0.0002%	300 250 200 150 100	>99% 98% 90% 68% 37%

Data Analysis Plan

The primary analysis will estimate the differences in "Fluid Overload" between treatment groups across all time points, regardless of whether a participant received all, some or none of their allocated treatment (i.e. intention-to-treat analyses). Secondary outcomes include ECW, ICW, % adipose tissue mass, and % lean tissue mass“. Differences in "Fluid Overload” and the secondary outcomes between treatment groups overall, and separately at 2 and 18 months, will be calculated using linear regression adjusted (or stratified) for the elements included in the minimization

algorithm. The primary analysis will focus on a weighted average of the values at the two time points (with weights proportional to the amount of time between visits). Missing measurements will be imputed. Results from the imputed analyses will be compared with those from equivalent "complete-case" analyses, but primary emphasis will be placed on the results after multiple imputation. More complete details of statistical methods, including definitions of subgroups, methods of imputation, approaches to adjustments and weighting of averages will be set out in a separate full Data Analysis Plan which will be consistent with the main study Data Analysis Plan.

Flowchart of Substudy Activities

INVITATION
• Invite potential participants shortly before or at the time of the Randomization Visit • Written informed consent is sought from willing individuals at the first visit when a bioimpedance spectroscopy measurement is offered
RANDOMIZATION VISIT AND AT 2 & 18 MONTHS OF FOLLOW-UP
• A bioimpedance spectroscopy measurement is added to the protocol-specified study follow-up visit procedures

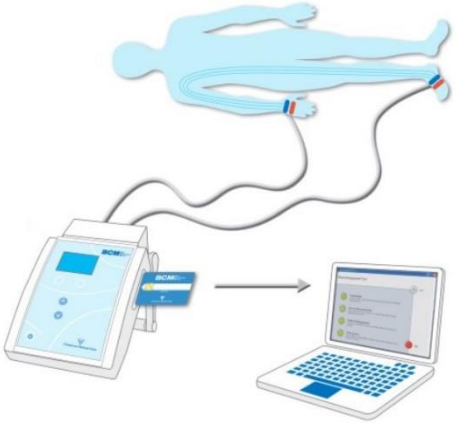
Design

Eligibility: In selected regions, EMPA-KIDNEY Local Clinical Centres (LCCs) with a Fresenius Medical Care Body Composition Monitor (BCM) machine will be invited to join this optional substudy. All those participants at these LCCs who have yet to attend the relevant scheduled study visit are eligible for invitation. There are no exclusion criteria.

Invitation and methods: Potential participants will be invited to join this substudy at before or around the time of their Randomization Visit. At the relevant visit, clinic staff will explain the substudy to potential participants using the Participant Information Leaflet and Consent Form. Consenting participants will have a measure of bioimpedance made in addition to the protocol-specified follow-up procedures. Bioimpedance measurements take about 2 minutes to record and pose no risk to health (although it is conceivable the 4 self-adhesive pads could rarely cause a skin reaction).

Body Composition Measurement

Training materials on how to perform Body Composition Measurements will be provided. The measurement requires



four disposable self-adhesive electrode pads (2x on a wrist and the other 2x on an ankle) to be attached to a portable machine whilst a participant is lying supine. Bioimpedance spectroscopy readings are made automatically across about 50 frequencies over a range of 5-1000 kHz. The measurements take about 2 minutes to make. Data are then automatically transferrable onto a Storage Card which is linked securely to the participant by means of a unique Storage Card ID entered onto the relevant study visit form on trial's web-based data entry system (i.e. Storage Cards containing results are pseudonymised). The Storage Card will be stored securely before being transferred securely to the Central Coordinating Office in Oxford for downloading into the study database. Data may be transferred securely to specialists in bioimpedance for Quality Control review.

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