REnal and **C**ardiovascular **E**ffects of SGLT2 inhibition in combination with loop **D**iur**E**tics in diabetic participants with **C**hronic **H**eart **F**ailure

Statistical Analysis Plan

TRIAL FULL TITLE	**RE**nal and **C**ardiovascular **E**ffects of SGLT2 inhibition in combination with loop **D**iur**E**tics in diabetic participants with **C**hronic **H**eart **F**ailure: **RECEDE-CHF** trial
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2 Abbreviations and Definitions

AE	Adverse Event
ANOVA	Analysis of Variance
AR	Adverse Reaction
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
CHF	Chronic Heart Failure
CI	Chief Investigator
CRC	Clinical Research Centre
CRF	Case Report Form
CTIMP	Clinical Trials of Investigational Medicinal Product
DM	Diabetes Mellitus
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate

FBC	Full Blood Count
GCP	Good Clinical Practice
GP	General Practitioner
HbA1c	Glycated Haemoglobin
HF	Heart Failure
HIC	Health Informatics Centre
HR	Heart Rate
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
ISF	Investigator Site File
LFTs	Liver Function Tests
LPLV	Last Participant Last Visit
LVSD	Left Ventricular Systolic Dysfunction
MHRA	Medicines and Healthcare Products Regulatory Agency
NHS	National Health Service
NRES	National Research Ethics Service
NYHA	New York Heart Association
PI	Principal Investigator
PSF	Pharmacy Site File
QC	Quality Control
R&D	Research & Development
REC	Research Ethics Committee
RPT	Renal Physiological Test
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation

SDRN	Scottish Diabetes Research Network
SGLT2	Sodium-Glucose co-Transporter-2
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2D	Type 2 Diabetes
TASC	Tayside Medical Sciences Centre
TCTU	Tayside Clinical Trials Unit
TG	Tubulo-glomerular
TMF	Trial Master File
U&Es	Urea & Electrolytes
UAR	Unexpected Adverse Reaction
UoD	University of Dundee

3 Introduction

3.1 Preface

Sodium-Glucose co-Transporter-2 (SGLT2) inhibitors, the newest class of anti-diabetic drugs, may have additional beneficial cardio-renal effects in participants with concomitant Type 2 Diabetes Mellitus (DM) and Chronic Heart Failure (CHF). SGLT2 inhibitors have an osmotic diuretic effect by reducing sodium and glucose absorption in the proximal tubules of the nephrons. We hypothesize that these effects could potentially be beneficial in treatment of participants with HF who are prone to sodium and fluid retention.

3.2 Purpose of the analyses

The purpose of these analyses were to assess the efficacy and safety of empagliflozin 25 mg in comparison with the placebo and were included in the clinical study report.

4 Study Objectives and Endpoints

4.1 Study Objectives

The primary aim/objective was to assess whether empagliflozin (SGLT2 Inhibitor) can augment the diuretic effect of loop diuretics in diabetic participants with mild CHF with left ventricular systolic dysfunction (LVSD), as measured by urinary volume, when compared to placebo.

The secondary aims/objectives were to assess the effect of empagliflozin (SGLT2 inhibitor) on natriuresis when used with loop diuretics in diabetic patients with mild CHF with LVSD as measured by urinary sodium excretion, to measure the safety of add-on SGLT2 inhibitor therapy versus placebo on top of loop diuretics as measured by serum creatinine and estimated glomerular filtration rate (eGFR), to assess effects of empagliflozin on protein/creatinine ratio, albumin/creatinine ratio and on the renal biomarker, cystatin C.

4.2 Endpoints

The primary outcome for this trial was:

- To assess the effect of empagliflozin versus placebo on urine output

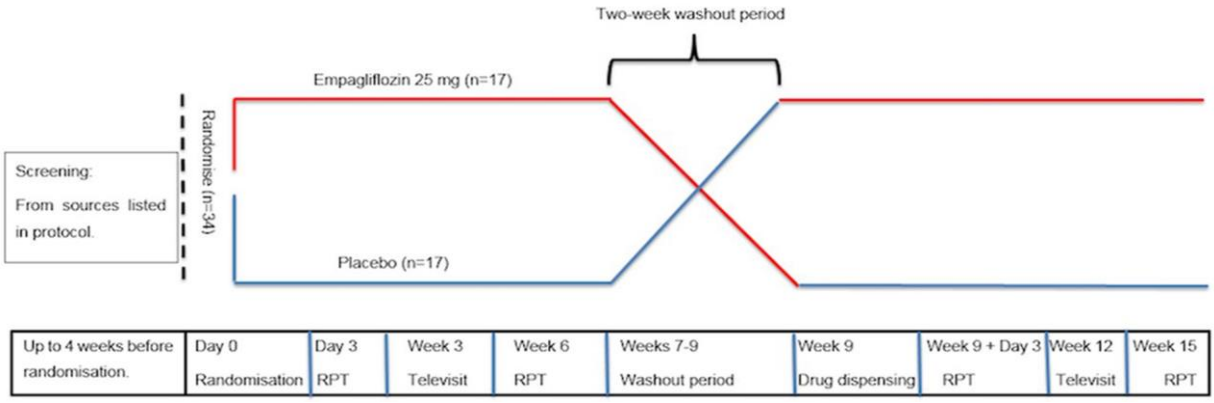
The secondary outcomes for this trial were:

- To assess the effect of empagliflozin versus placebo on urinary sodium excretion
- To assess the change in glomerular filtration rate (safety endpoint)
- To assess the change in creatinine (safety endpoint)
- To assess the effect of empagliflozin on protein/creatinine ratio
- To assess the effect of empagliflozin on albumin/creatinine ratio
- To assess the effect of empagliflozin on the renal biomarker, cystatin C.

5 Study Methods

5.1 General Study Design and Plan

The RECEDE-CHF Trial is a single centre randomised, double-blind, placebo-controlled, crossover trial conducted in NHS Tayside to compare the SGLT2 inhibitor empagliflozin 25 mg, to placebo. Participants were randomised to treatment 1, empagliflozin 25 mg/placebo at week 1 for 6 weeks. Then following a two week washout period were randomised to treatment 2, placebo/empagliflzoin 25 mg for a further 6 weeks. Renal physiology test days (RPTs) were performed in the first week and the last week of both treatment arms.



5.2 Inclusion-Exclusion Criteria and General Study Population

Participants were eligible if they were:

- Aged 18 to 80 years with previously diagnosed Type 2 Diabetes Mellitus
- Diagnosed with NYHA Functional class II-III HF with prior echocardiographic evidence of LVSD
- On stable doses of furosemide, or alternative loop diuretic for at least one month.
- eGFR $\geq$ 45 ml/min
- Had stable HF symptoms for at least three months prior to consent

- Had stable HF therapy for at least three months prior to consent
- Had not been hospitalised for HF for at least three months prior to consent
- Women of childbearing potential* (WoCBP) must agree to take precautions to avoid pregnancy throughout the trial and for 4 weeks after intake of the last dose.

Participants were excluded if they:

- Had a diagnosis of chronic liver disease and/or liver enzymes that are twice the upper limit of normal
- Systolic BP of <95mmHg at screening visit
- HbA1c < 6.0%
- were on thiazide diuretics
- were receiving renal dialysis
- had previously had an episode of diabetic ketoacidosis
- had type 1 diabetes mellitus
- had malignancy (receiving active treatment) or other life threatening disease
- were pregnant or lactating women
- had difficulty in micturition e.g. severe prostate enlargement
- had allergy to any SGLT2 inhibitor or lactose or galactose intolerance
- had any past or current treatment with any SGLT2 inhibitor
- had participated in any other clinical interventional trial of an investigational medicinal product within 30 days
- were unable to give informed consent
- Any other reason considered by the physician to be inappropriate for inclusion

5.3 Randomisation and Blinding

Participants were randomised to either empagliflozin 25mg/placebo or placebo/empagliflozin 25 mg in a double blind fashion in this crossover study.

The double blind medication (empagliflozin or placebo) was prepared, packaged and labelled by Tayside Pharmaceuticals. Randomisation was carried out by Tayside Pharmaceuticals, using block randomisation using a validated randomisation programme, and will securely backup both the randomisation seed and the randomisation allocation. Tayside Pharmaceuticals use www.Randomization.com to generate the randomisation lists which they then apply when labelling and packing IMP. This is not specifically named in the Protocol or the Technical Agreement, however it is mentioned in TASC SOP 40 as an example of sources for Randomisation lists. The Clinical Trials Pharmacy, Ninewells Hospital was provided with a copy of the randomisation allocation for the purposes of 24h emergency unblinding.

5.4 Study Variables

The primary end point was analysed as the total volume of urine from the 24 hour urine collection at the end of treatment.

Visit	Visit 1* Screening	Visit 2* Baseline/ Randomisation	Visit 3	Visit 4 (Tele Visit)	Visit 5	Two week wash-out period	Visit 6	Visit 7	Visit 8 (Tele Visit)	Visit 9 ****	Visit 10 (Final visit)
	Up to 4 weeks pre visit 2	**Day 0**	**Day 3** (+/-2 days)	**Week 3** (+/- 3 days)	**Week 6** (+/- 3 days)		**Week 9** (+/- 3 days)	**Week 9 + 3 days** (+/- 2 days)	**Week 12** (+/- 3 days)	**Week 15** (+/- 3 days)	**Week 19** (+/- 7 days)
Height	X										
Weight	X	X	X		X		X	X		X	X
Vital Signs – blood pressure & pulse	X	X	X		X		X	X		X	
Safety Bloods	X	X	X		X		X	X		X	
HbA1c	X				X		X			X	
Research Bloods		X	X		X		X	X		X	
Urine sample	X	X	X		X		X	X		X	
24 hour urinary collection			X		X			X		X	
Renal Physiology Test			X		X			X		X	

Table 1: Trial schedule for RECEDE-CHF

6 Sample Size

Power calculations were based on our previous data in participants with CHF[1-5] as well as more contemporary data.[6] The sample size was based on the mean furosemide-induced urinary volume (SD=250). A 20% increase is expected in this parameter following SGLT2 inhibition. This increase in urinary volume is based on published percentage increases in urinary volume in T2D patients that range from 11%-33% depending on the dose of the SGLT2 inhibitor as shown by List and colleagues[7] in the available data with dapagliflozin (dapagliflozin 2.5 mg: 11% increase in 24-hr urine volume; dapagliflozin 5 mg: 22% 11% increase in 24-hr urine volume; dapagliflozin 10 mg: 24% 11% increase in 24-hr urine volume; dapagliflozin 20 mg: 27% 11% increase in 24-hr urine volume and dapagliflozin 50 mg: 33% 11% increase in 24-hr urine volume). There is no data of SGLT2 inhibition together with loop diuretics apart from a case report of a diuretic resistant HF patient in whom the fluid overload was successfully treated with the SGLT2 inhibitor, ipragliflozin. The authors reported a striking 50% increase in urinary volume (following treatment with 50 mg daily of oral ipragliflozin for 5 days).[8] Based on the above, it was determined that a 20% increase in furosemide induced increase in urinary volume was reasonable.

With an alpha 0.05 and power of 90%, 22 participants per arm are required, assuming 35% drop out. Since this trial is using an AB/BA crossover design, a total of 34 participants was required, as each participant was exposed to both arms of the trial. The rationale for the high dropout rate, was due to the high intensity renal physiological tests that will occur on 4 occasions for the participants, from 0830 to 1330 we feel that anything less than a 35% drop out rate would be conservative.

7 General Considerations

7.1 Timing of Analyses

The final analysis was performed when all the randomised subjects had completed visit 10 or dropped out prior to visit 10. The final analysis was performed on data transferred to the file RECEDE Master Spreadsheet having been documented as meeting the cleaning and approval requirements of SOP32 and after the finalisation and approval of this SAP document.

7.2 Analysis Populations

The full analysis population was an intention to treat analysis, including all subjects who were randomised and received any study drug after consent.

The safety population was all subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving any treatment.

Prior to breaking the blind, each subject's inclusion or exclusion status with regard to each analysis population was established. The exact process for assigning the statuses was defined and documented prior to breaking the blind along with any predefined reasons for eliminating a subject from a particular population.

7.3 Covariates and Subgroups

Covariates that may have an influence on the end points listed include gender, BMI, age and treatment order received i.e. placebo/empagliflozin versus empagliflozin/placebo. As the study is a crossover in design, the treatment order was included in the initial analysis as a covariate to ascertain if the intervention being investigated has a sustained effect on the primary outcome despite the 2 week washout period. These demographic subgroups were analysed for different treatments effects (for example comparison of effects by age, gender, BMI and treatment order). Where possible forest plot figures were used to communicate the relevant information about possible subgroup effects and interactions.

7.4 Missing Data

Missing values represent a potential source of bias in a clinical trial. Therefore every effort should be undertaken to fulfil all requirements of the protocol concerning the collection and management of data. The extent of the missing data was examined and the reason for drop-out ascertained. Multiple imputation was used to impute missing values where necessary and where assumptions for missing at random (MAR) data were met.

8 Summary of Study Data

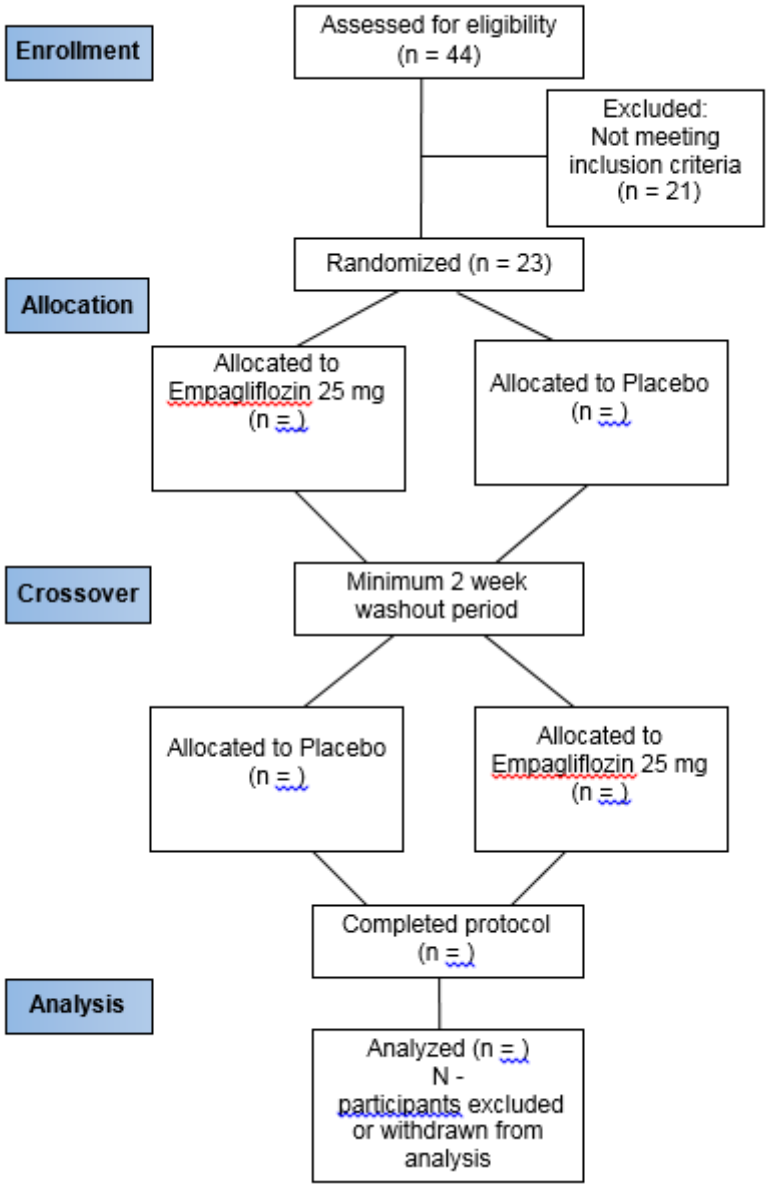
All continuous variables were summarised using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels were reported for all categorical measures. In general, all data were listed, sorted by site, treatment and subject, and when appropriate by visit number within subject. All summary tables were structured with a column for each treatment in the order (Intervention, Control) and was annotated with the total population size relevant to that table/treatment, including any missing observations.

Baseline characteristics for patients are: age, gender, past medical history, BMI, smoking history, blood pressure, HbA1c, eGFR, medication classes, severity of heart failure as identified by the ejection fraction (mild, moderate or severe), and medication classes.

Concurrent illnesses and medical conditions were recorded and listed but not analysed.

Patient medication was recorded at baseline and at each study visit. Anti-diabetic, diuretic and anti-hypertensive drug classes were reported.

8.1 Subject Disposition



From the CRF it was established how many subjects reached what stage of the trial, how many dropped out and for what reasons (death, toxicity, treatment failure, withdrew consent). It was therefore identified the number screened, randomised, completed treatment 1, completed treatment 2, and ultimately how many completed the study.

8.2 Protocol Deviations

Specific protocol deviations that could impact the analysis of the trial include those entered into the study even though the subject did not satisfy the entry criteria, the development of withdrawal criteria during the study but were not withdrawn, or those who received the wrong treatment or incorrect dose.

8.3 Demographic and Baseline Variables

Demographic variables that were recorded at the screening visit prior to randomisation were:

- Age
- Gender
- Concurrent Illnesses and Medical Conditions
- BMI
- Smoking history
- Alcohol intake (units/week)
- Severity of heart failure as identified by the ejection fraction (mild, moderate or severe) on echocardiogram
- Medication classes

All baseline variables that were recorded at the screening visit prior to randomisation include:

- blood pressure
- pulse
- weight
- BMI

- HbA1c
- eGFR
- Serum creatinine
- Urine Protein/Creatinine Ratio
- Urine Albumin/Creatinine Ratio

8.4 Prior and Concurrent Medications

Patient medication was recorded at baseline and at each study visit. Anti-diabetic, diuretic and anti-hypertensive drug classes were reported.

8.5 Treatment Compliance

The calculation of adherence was performed and reported as a percentage, were the number of tablets issued minus the number of drugs returned, was then divided by the number of drugs that should have been taken. This was performed by the PI at visit 3 and visit 7, and by the Pharmacy department at visit 5 and visit 9.

Adherence of 80-120% will be deemed as being compliant. Where an issue of non-compliance was evident, a sensitivity analysis was performed and adherence was included as a covariant.

9 Efficacy Analyses

Efficacy analysis was performed on the intention to treat population as defined in section 7.2.

Data for continuous outcome measures were assessed for normality prior to analysis. Transformation of the outcome variables was used where necessary were these were not normally distributed. Outcome measures were assessed by 2 way Analysis of Variance (ANOVA). Initial comparison was between treatment groups (empagliflozin

vs placebo) at the final visit of each treatment arms (visit 5 and visit 9) adjusted for baseline measures (visit 2 and visit 6) of the outcomes.

Where data was not normally distributed and could not be transformed into a normal distribution, data were analysed using non-parametric methods. Data for categorical outcomes measures were assessed by logistic regression in the same way as described for continuous outcome measures.

All efficacy variables were listed by subject within study centre. Data were summarised by treatment group. Number, Mean, Standard Deviation, Minimum and Maximum will summarise continuous efficacy variables, whereas number and percent will summarise categorical efficacy variables.

9.1 Primary Efficacy Analysis

The primary outcome measure was analysed in the same manner as described above and presented as the between treatment group difference in change in urine volume between the baseline and final visits.

9.2 Secondary Efficacy Analyses

All secondary outcomes were analysed in the same manner as the primary outcomes. Results were presented as:

- between treatment group difference in urinary sodium excretion, from the 24 hour urinary collection
- between treatment group difference in estimated glomerular filtration rate (safety endpoint)
- between treatment group difference in serum creatinine (safety endpoint)
- between treatment group difference on protein/creatinine ratio
- between treatment group difference on albumin/creatinine ratio

- between treatment group difference on the renal biomarker, cystatin C.

In analysing the results from the Renal Physiology test days, these will be analysed via a 2 way repeated measures ANOVA for both urinary volume and urinary sodium.

10 Safety Analyses

An all patient group analysis was performed for safety variables (glomerular filtration rate and serum creatinine).

10.1 Adverse Events

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions of adverse events was ignored; the denominator was the total population size. The adverse events will be coded using the MedDRA coding system, version 21.1 dated September 2018.

Treatment emergent adverse events are those events that occur after the baseline assessment. Those subjects with adverse events that worsen post-treatment will also be included. The incidence of AEs that are judged to be related to the treatment was reported.

10.2 Deaths, Serious Adverse Events and other Significant Adverse Events

Serious Adverse Events (SAE) was reported with all other AEs. However, they were reviewed for the trial report on a case by case basis by the PI.

10.3 Pregnancies

The study did not perform any pregnancy tests on the subjects randomised as all female randomised subjects signed in their informed consent form that they were not of child bearing potential i.e. post-menopausal.

10.4 Clinical Laboratory Evaluations

Laboratory tests was summarised using descriptive statistics, which will show the change in laboratory values from baseline to each subsequent visit.

10.5 Other Safety Measures

Vital signs was summarised using descriptive statistics and will show the change in vital signs from baseline to each subsequent visit.

11 Reporting Conventions

P-values $\geq$0.001 was reported to 3 decimal places; p-values less than 0.001 was reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, was reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) was reported to 3 significant figures.

12 Technical Details

All analysis was performed using IBM SPSS Statistics 22 for Windows or R Ver 3.4.1 for Windows. All data, analysis programs and output was kept on the University server and backed up according to the internal IT SOPs. Analysis programs was required to run without errors or warnings.

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