ERADICATE Data Analysis Plan

ERtugliflozin triAl in DIabetes with preserved or reduced ejeCtion FrAcTion mEchanistic evaluation in Heart Failure: "ERADICATE-HF"

Clinicaltrialgov: NCT03416270

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Objectives

1. Primary objective:

The primary objective is to determine if acute and chronic ertugliflozin use causes a persistent proximal renal tubular natriuretic effect compared to placebo. **Acute** means changes from baseline to Visit 4 (1 week), and **chronic** means changes from baseline to Visit 8 (12 weeks).

2. Secondary objectives:

2.1. We will assess whether the proximal renal tubular natriuretic effect of ertugliflozin leads to volume contraction compared to placebo. To this end, we will determine the association between acute and chronic changes to fractional sodium handling and acute and chronic changes in volume.
2.2. We will assess whether volume contraction with ertugliflozin leads to a decline in hormones that are activated in heart failure (HF) patients, without activating the sympathetic nervous system (SNS) compared to placebo. To this end, we will determine the association between acute and chronic changes to volume and acute and chronic changes in hormone levels.
2.3. We will determine the impact of ertugliflozin on renal function. Specifically, the acute and chronic change in markers of renal hemodynamic function will be measured and compared between ertugliflozin and placebo.
2.4. We will determine if ertugliflozin causes a blood pressure lowering effect. Specifically, we will evaluate the acute and chronic change in cardiovascular hemodynamic assessments with ertugliflozin compared to placebo.
2.5. We will assess the acute and chronic changes to SNS activation with ertugliflozin use compared to placebo.
2.6. We will assess the acute and chronic effects of ertugliflozin on the level of urinary natriuretic modulators normalized to creatinine compared to placebo.
2.7. We will characterize the safety of ertugliflozin use compared to placebo.

3. Exploratory objectives:

3.1. We will assess whether ertugliflozin causes acute and chronic changes in measures of system volume compared to placebo.
3.2. We will assess whether ertugliflozin causes acute and chronic changes in measures of systemic water and fluid balance compared to placebo.
3.3. We will assess the acute and chronic change of all blood and urine biomarker levels with ertugliflozin compared to placebo.
3.4. We will determine whether changes in neurohormones are associated with changes in renal function. To this end, we will determine the association between acute and chronic changes in absolute measures of renal function and the acute and chronic changes in neurohormones with ertugliflozin compared to placebo.

3.5. We will determine whether changes in neuro-hemodynamic markers associated with HF patients are associated with changes in arterial stiffness. To this end, we will determine the association between acute and chronic changes in arterial stiffness and the corresponding acute and chronic changes in neuro-hemodynamic markers with ertugliflozin compared to placebo.
3.6. We will determine whether changes in neurohormones that are activated in HF patients are associated with changes in blood pressure. To this end, we will determine the association between acute and chronic changes in blood pressure and the acute chronic changes in neurohormones with ertugliflozin compared to placebo.
3.7. We will assess the acute and chronic effects of ertugliflozin on the level of oxidative stress markers compared to placebo.
3.8. We will assess the acute and chronic changes with ertugliflozin on urine and plasma chemistry marker levels and metabolic parameters compared to placebo.

Outcomes

1. Outcomes for the primary objective:

1.1. The primary outcome is measured by the acute and chronic change in lithium clearance from baseline with ertugliflozin vs placebo.
$FE(Li^+) = 100\ X\ ([(urine\ Li^+)\ X\ (creatinine\ plasma)]/[(plasma\ Li^+)\ X\ (creatinine\ urine)]$

1.2. We will also measure the primary objective on these secondary outcomes:
a. *Total overall (proximal and distal) tubular sodium handling*
$FE(Na^+) = 100\ X\ ([(urine\ Na^+)\ X\ (creatinine\ plasma)]/[(plasma\ Na^+)\ X\ (creatinine\ urine)]$
b. *Distal sodium handling*: $FE(Na^+) – FE(Li^+)$

2. Outcomes for the secondary objectives:

2.1. Relationship between proximal renal tubular natriuretic effects and volume reduction: proximal renal tubular natriuretic effects will be evaluated by lithium clearance FE(Li+); volume change will be measured using the following two outcomes:
a. Plasma volume (PV)
i. Measured by indocyanine green (for site 2 and 3)
ii. Estimated percent change by Strauss estimation
$([\{Hb_{baseline}/Hb_{end}\}\times\{(100-Ht_{end})/(100-Ht_{baseline})\}]-1)\times 100$
b. Extracellular fluid (ECF)
i. Measured by bioimpedance spectroscopy
2.2. Relationship between volume and hormones: Volume will be measured by PV and ECF. Hormones will be measured by:
a. Hormones activated in HF patients (NT-proANP, BNP)
b. Renin-angiotensin-aldosterone system (RAAS) markers (serum aldosterone, renin, PRA, ANGII, AGT, ACE2, ACE)

c. SNS markers (norepinephrine, epinephrine, heart rate, heart rate variability (SDNN and RMSSD)

2.3. Renal hemodynamic function will be measured using the following groups of outcomes:
   a. Absolute measured values
      i. *mGFR*
      ii. *ERPF (for site 1 and 2)*
      iii. *Hematocrit*
   b. Creatinine-derived values
      i. *24-hour Crt (mmol/day)*
      ii. *Creatinine clearance (ml/min)*
   c. Gomez-derived values *(for site 1 and 2)*
      i. *RBF = RPF/(1-HCT)*
      ii. *RVR = MAP/RBF [Mean arterial pressure = MAP]*
      iii. *Filtration fraction = GFR/RPF*
      iv. $PGLO=(GFR/KFG)+10mmHg+[5\times(TP/FF\times ln(1/1-FF)-2)]$
      v. $R_A=[(MAP-PGLO)/RBF]\times 1{,}328$
      vi. $R_E=[GFR/(KFG\times(RBF-GFR))]\times 1{,}328$

2.4. Blood-pressure lowering effect will be measured using the following group of outcomes:
   a. blood pressure (systolic [SBP] and diastolic [DBP])
   b. NICOM parameters
   c. echocardiographic measures
   d. systemic vascular resistance
   e. arterial stiffness
      i. radial, aortic and carotid augmentation index (AIx)
      ii. carotid-radial pulse-wave velocity (PWV)
      iii. carotid-femoral PWV

2.5. SNS activation will be measured by norepinephrine, epinephrine, heart rate and heart rate variability (RMSSD and SDNN).

2.6. Changes to urinary natriuretic modulators will be assessed by the level of ACE-2, adenosine, ACE activity, NT-proANP and ANGII, normalized to urine creatinine.

2.7. Safety will be assessed by the number of hypoglycemic episodes and serious adverse events.

3. Outcomes for the exploratory objectives:

3.1. Volume contraction will be assessed by:
   a. Plasma volume measured by ICG (site 2 and 3)
   b. Plasma volume measured by Strauss estimation
   c. Extracellular body of water measured by bioimpedance spectroscopy

3.2. Water and fluid levels will be assessed by:
   a. Absolute (L) and relative (%) total body water (TBW)
   b. Absolute (L) and relative (%) intracellular fluid (ICF)
   c. Absolute (kg) and relative (%) fat-free mass (FFM)
   d. Absolute (kg) and relative (%) fat mass (FM)
   e. BMI

3.3. The blood and urine biomarkers are:
   a. Blood: Hemoglobin, hematocrit, creatinine, total bilirubin, albumin, bicarbonate, NT-proANP, BNP, PGF2a, cGMP, nitric oxide (nitrite, nitrate, total nitrite)
   b. Urine: NT-proANP, sodium, RAAS markers – normalized to urine creatinine
      i. 24-hour urine clearance markers (protein, creatinine, UACR, urea, potassium, sodium)

3.4. Relationship between neurohormones and renal function. Hormones will be measured by NT-proANP, BNP, and RAAS markers (serum aldosterone, renin, PRA, ANGII, AGT, ACE2, ACE). Absolute measure of renal function outcomes is measured by:
   a. mGFR
   b. ERPF
   c. hematocrit

3.5. Relationship between arterial stiffness and neuro-hemodynamic markers. Markers will be measured by SBP, DBP, MAP, NT-proANP, BNP, SNS markers (epinephrine, norepinephrine) and RAAS markers (serum aldosterone, renin, PRA, ANGII, AGT, ACE2, ACE). Arterial stiffness outcomes will be measured by:
   a. Radial, aortic and carotid AIx
   b. Carotid-radial PWV
   c. Carotid-femoral PWV

3.6. Relationship between neurohormones and blood pressure. Hormones will be measured by NT-proANP, BNP, and RAAS markers (serum aldosterone, renin, PRA, ANGII, AGT, ACE2, ACE). SBP, DBP, and MAP will be the outcome.

3.7. Urine oxidative stress markers normalized to urine creatinine, including 8-isoprostane, 8-OHdG, cGMP, nitric oxide (endogenous nitrite, nitrate, total nitrite), PGF2a, as well as plasma oxidative stress markers, such as cGMP, nitric oxide, and PGF2a.

3.8. Plasma/urine chemistry markers and metabolic parameters are:
   a. Metabolic (weight, waist measurement, HbA1c)
   b. Urine chemistry (UACR, urea, protein, potassium)
   c. Plasma chemistry:
      i. Sodium, potassium, urea, urate, magnesium, phosphate, chloride, calcium
      ii. AST, ALT, ALP, LDL, HDL, cholesterol, triglyceride
      iii. Glucose, total protein

Statistical Analysis Plan (SAP)

0. Summary of baseline demographic and clinical characteristics separated by ertugliflozin group and placebo group will be generated. Significance testing between-group will be performed. The following variables will be included:
   - *Age, sex, weight, waist circumference, BMI, HbA1c, hemoglobin, fasting blood glucose*
   - *race (white/black/Hispanic/Asian/other)*
   - *SBP, DBP, HR*
   - *Mean eGFR*
   - *UACR (median and IQR)*
   - *Concomitant medication (ACEi, ARB, Diuretic, statin)*

1. ***Analysis for the primary objective:***

1.1. To assess ertugliflozin-related acute and chronic changes in the primary outcome FE(Li+) compared to placebo, linear mixed-effects models will be used. Two separate models will be fit. For acute change, the vector of outcomes will include the baseline and 1-week values (i.e. Visit 3 and Visit 4 values). For chronic changes, the vector of outcomes will include the baseline and 12-week values (i.e. Visit 3 and Visit 8 values). The covariates will include the visit and treatment-by-visit interaction; due to randomization, a covariate for the main effect of treatment will not be included. The treatment-by-time coefficient, its 95% confidence interval, and its p-value will be used to assess the effect of treatment.

1.2. We will repeat this analysis with:
   a. FE(Na+) as an outcome
   b. FE(Na+) – FE(Li+) as an outcome

1.3. In exploratory analysis to determine the overall effect of ertugliflozin across all time-points, we will repeat the above analysis with one model where the vector of outcomes will include all data collected at baseline, 1-week, and 12-week values.

2. ***Analysis for secondary objective:***

2.1. Linear regression will be performed with change in volume as the dependent variable and change in FE(Li+) as the independent variable, with treatment as a covariate. Two separate models will be fit for acute and chronic changes. Acute changes will use the baseline and 1-week values for both the dependent and independent variable values; chronic changes will use baseline and 12-week values.

2.2. The same models described in the analysis of secondary objective 2.1 will be completed, with changes in PV or ECF as independent predictors, for neurohormones as the dependent variables/outcomes.

2.3. – 2.6. The same models described in the analysis of primary objective 1.1 and 1.3 will be completed for each of the different outcomes.

2.7. The numbers of hypoglycemic events and serious adverse events will be reported for both the placebo group and ertugliflozin group. No statistical comparisons will be performed.

3. ***Analysis for exploratory objective:***

3.1.– 3.3. The same models described in the analysis of primary objective 1.1 and 1.3 will be performed for each of the different outcomes.

3.4. The same models described in the analysis of secondary objective 2.1 will be completed, with changes in neurohormones as independent predictors, for changes in absolute measure of renal function as the dependent variables/outcomes.

3.5. The same models described in the analysis of secondary objective 2.1 will be completed, with changes in neuro-hemodynamic markers as independent predictors and changes in arterial stiffness as the different dependent variables/outcomes.

3.6. The same models described in the analysis of secondary objective 2.1 will be completed, with changes in neurohormones as independent predictors and changes to blood pressure as dependent variable/outcome.
3.7.– 3.8. The same models described in the analysis of primary objective 1.1 and 1.3 will be completed for each of the different outcomes.

Analysis population: The intention to treat population will be used for all analyses. There are no planned subgroup analyses for the primary outcome.

Hypothesis testing: For hypothesis testing, a 5% significance level will be used (two-sided). No interim analyses will be performed. For secondary objectives, nominal p-values will be reported.

General model assumptions and model diagnostic procedures: For the linear mixed-effects models with visit and treatment-by-visit interaction as covariates, a participant-level random intercept will be included. Restricted-maximum-likelihood will be used for estimation and the missing-at-random framework will be assumed. Plots of marginal and conditional residuals will be used for model diagnostics and assessment. Similar diagnostic procedures will be used for the linear regression models.