

STATISTICAL ANALYSIS PLAN

Bioprofiling response to mineralocorticoid receptor antagonists
for the prevention of heart failure.

A proof of concept clinical trial within the EU FP7
Heart OMics in AGing (HOMAGE) programme

Co-Cl: Professors John Cleland and Faiez Zannad

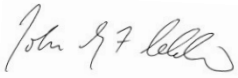

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Statistician: Tim Collier, Medical Statistics Dept., LSHTM, London, WC1E 7HT

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1. INTRODUCTION AND STUDY SYNOPSIS

The Heart OMics in AGing (HOMAGE) randomised controlled trial (RCT) is a Phase II proof of concept clinical trial within the EU FP 7 HOMAGE programme, with the aim of bioprofiling response to mineralocorticoid receptor antagonists for the prevention of heart failure.

1.1 Administrative details

Co-principal investigators: Professor John Cleland, National Heart & Lung Institute, Imperial College, London, UK, and Professor Faiez Zannad, Centre d'investigation clinique CHU de Nancy, Inserm et Université de Lorraine, 54500 Vandoeuvre lès Nancy, France.

Study sponsor: ACS Biomarker, Paasheuvelweg 25, 1105 BP Amsterdam, the Netherlands

Data management: Professor Jan Staessen, katholieke universiteit leuven13 Oude Markt, 3000 Leuven, Belgium. E-mail : Jan.Staessen@med.kuleuven.be

Statistical center: Professor Stuart Pocock, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. E-mail: stuart.pocock@LSHTM.ac.uk

Biobank: Professor Stephane Heymans, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre+, P. Debyelaan 25, 6229 HX Maastricht, the Netherlands. E-mail: s.heyman@maastrichtuniversity.nl

Project coordination: Stéphanie Grojean, EDDH – European Drug Development Hub, 2 Rue du Doyen Jacques Parisot, 54500 Vandœuvre-lès-Nancy, France; E-mail: sgrojean@eddh.fr

1.2 Study rationale and hypothesis

Despite advances in care, prognosis remains poor once overt heart failure (HF) has developed. Therefore, prevention is an important frontier in HF management. Prevention is most efficient when directed toward patients at risk and when mechanistically targeted to those most likely to respond. An increase in myocardial and possibly vascular collagen content may be a major determinant of the transition to HF. In patients with hypertension and diabetes, two important risk-factors for HF, changes in blood markers of fibrosis occur before clinically overt HF develops. These markers are also related to prognosis.

We hypothesize that the mineralocorticoid receptor antagonist (MRA), spironolactone, may prevent HF by acting on extracellular matrix remodelling, especially in patients with active

fibrogenesis, identified by high Gal-3 levels. The benefit/risk ratio of spironolactone might be superior in patients with a higher compared to lower plasma concentrations of Gal-3.

1.3 Study design and population

The study follows a prospective randomized open label blinded evaluation (PROBE) parallel group design. All persons evaluating key tests, the clinical endpoints committee, and those conducting laboratory biomarkers tests will be kept blind to the treatment allocation.

The study will be conducted in approximately 800 patients at increased risk of developing heart failure in approximately 9 centers in the United Kingdom, France, Italy, Ireland, Germany and the Netherlands. Patients will be screened in primary and secondary care. Each center will have its own recruitment strategies under review of local ethics committees.

Inclusion criteria are as follows:

1. Written informed consent obtained prior to the initiation of any study procedures;
 2. Age >60 years
 3. Clinical risk factors, Either;
 - Coronary artery disease (MI, angioplasty or CABG)
- Or at least two of the following;
- Diabetes Mellitus requiring hypoglycaemic pharmacotherapy
 - Pharmacological treatment for hypertension
 - Microalbuminuria (defined as creatinine > 30mg/g or 3mg/mmol)
 - Abnormal ECG (LVH, QRS >120msec, pathological Q-waves)
4. Biological risk: NT- Pro-BNP values between 125 and 1,000 ng/L or BNP between 35 and 280pg/ml (consistent with ESC guidelines indicating risk of HF but helping to rule out prevalent HF or atrial fibrillation)

Exclusion criteria are described in full in the trial protocol.

All patients randomised before 31 December 2017 will be treated and followed up for a period of nine months. Patients randomised from 1 January 2018 until 30 June 2018 will be followed up for a period of between 3 and 6 months. The final patient visit will occur no later than 30 September 2018.

Patients randomised to the experimental group will receive spironolactone, titrated from 25 mg/day (or every other day in some cases) to 50 mg/day, adapted according to a pre-specified algorithm depending on occurrence/resolution of hyperkalaemia and/or

worsening renal function. Patients randomised to the control group will receive background therapy only. Background therapy may include any agent other than loop diuretics or potassium saving diuretics including mineral-corticoid antagonists (angiotensin converting enzyme inhibitors, angiotensin receptor blocker inhibitors, beta blockers and thiazide or thiazide-like diuretics).

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the HOMAGE trial is to investigate whether spironolactone can favourably alter extra-cellular matrix remodelling, assessed by changes in the fibrosis biomarker Procollagen Type III N-Terminal Peptide (PIIINP), in patients at increased risk of developing heart failure and whether this effect is greater in patients with increased plasma concentrations of Gal-3.

The primary outcome measure is change in serum concentrations of PIIINP (RI assay, central lab) from baseline to nine months or final visit (3-6 months in patients randomized after 1st January 2018). Three to nine months is thought to be a period sufficient to influence cardiac fibrosis for which PIIINP is a widely accepted marker. Sensitivity analyses will be carried out for the primary endpoint including only patients who achieved the 9 month visit. Further sensitivity analyses will be carried using PIIINP results at the 1 month visit.

2.2 Secondary Objectives

To investigate whether, over three to nine months, spironolactone can induce favourable changes in:

1. Serum or plasma concentrations of other biomarkers of extracellular matrix turnover: PICP (synthesis), ICTP (degradation) and Gal-3;
2. Cardiac remodelling, assessed by echocardiography, including left atrial volume, left ventricular mass and Doppler measures of right and left ventricular function (E' , E/A , E/E'), tricuspid regurgitation velocity (if measurable), tricuspid annular plane systolic excursion (TAPSE) and NT-proBNP;
3. Vascular function (substudy C) assessed by pulse-wave analysis (BPLab-Germany) before and after nitrates (patients may opt out of nitrates test);
4. Exercise capacity assessed by a shuttle walk test Cardiorespiratory monitoring during and after the shuttle walk test (substudy D) using a chest band (EQUIVITAL-UK) that monitors heart rate and respiration or an auditory electrode (AUDICOR-Germany) that measures heart and respiratory sounds before and immediately after exercise.

To investigate whether, over three to nine months, other biomarkers (i.e. other than Gal-3), including markers of inflammation and markers of cardiovascular disease, are predictive of treatment response for the outcomes PIIINP, PICP and ICTP. These will be regarded as secondary, exploratory analyses and will be interpreted in light of multiple testing. Treatment response at one month will also be investigated.

To investigate whether spironolactone alters the rate of:

5. The clinical composite endpoint of development of HF or AF, non-fatal MI or stroke or CV death;
6. Worsening renal function (decline in eGFR >20%);
7. Hyperkalemia or hypokalaemia (serum potassium >5.5 or <3.5mmol/L);
8. Gynaecomastia and/or breast pain;
9. Hypotension, falls and fractures.

3. STUDY DESIGN

3.1 Study design

The HOMAGE trial follows a prospective randomized open label blinded evaluation (PROBE) parallel group design.

3.2 Sample Size

Sample size calculations determined that 800 patients were required to detect an interaction term of 0.79µg/l in PIIINP with a two sided significance level of 5% and 90% power. A residual standard deviation of PIIINP of 1.73µg/l was assumed based on results from Kosmala M et al. JACC 2011; 4:1240.

The sample size was calculated using the formula for testing interactions in analysis of variance - see Lachenbruch P, (1988) Statistics in Medicine, (7) 467-469. The interaction term represents the difference in the effect of Spironolactone on PIIINP in patients with or without an elevated Gal-3 (below/above median value).

The sample size of 800 patients will provide sufficient statistical power for exploratory analyses of the main secondary endpoints listed above. For example, the trial will have 92%-power to find significant, at the 5%-alpha error level, a difference of 15 µg/l for PICP and 78%-power to find significant a difference of 1.1 m/s for E/E' ratio.

Due to difficulty with patient recruitment updated sample size calculations were carried out in March 2018. It was determined that 500 patients would provide 80% power to detect an

interaction of 0.87µg/l in PIIINP with a two sided significance level of 5% (or 90% power to detect an interaction of 1.0µg/l in PIIINP). Gains in statistical power may be gained attained through the method of analysis, in particular through treating GAL-3 as a continuous variable and using methods for repeated measurements.

3.3 Randomization and Blinding

Patients were randomised in a 1:1 ratio to Spironolactone or control using random permuted blocks stratified by center. Randomisation lists for each center were created by the Study Coordinating Center, KU Leuven, using the statistical software SAS 9.3. Randomisation was carried out via a web-based management system located at KU Leuven. A full randomisation list will be held securely at KU Leuven until data base lock is achieved.

All persons evaluating key tests, the clinical endpoints committee, and those conducting laboratory biomarkers tests were kept blind to the treatment allocation.

3.4 Study Assessments

The study assessment schedule is detailed in appendix 1.

4. STATISTICAL ANALYSIS

4.1 General

Statistical analyses will be carried out using Stata[®] version 15.1. The primary efficacy analyses will be carried out on the Full Analysis Set using the Intention to Treat principle. Missing data will be identified and an effort will be made where possible to obtain the missing values from the original medical records. Data will be checked for outliers and, where possible, outlying values will be validated against the original medical records.

4.2 Interim Analyses

No interim efficacy analyses will be carried out. An independent Data and Safety Monitoring Committee will review safety data throughout the trial and advice on trial modifications or premature stopping for safety.

4.3 Time-Points For Analysis

The original primary efficacy analysis requires measurement of serum levels of PIIINP at baseline and at 9 months. Patients randomised after 1 January 2018 will only achieve a maximum final visit of between 3 and 6 months. For the primary analysis, we will use

samples from the final trial visit provided the visit occurred at least 3 months after randomisation. Other visits are described in the schedule in appendix 1.

4.4 Analysis sets

The Full Analysis Set (FAS) will consist of all randomized patients who receive at least one dose of the study medication. This will be the primary efficacy population. Following the intention-to-treat (ITT) principle, patients will be analysed according to the treatment to which they were assigned at randomization.

The Safety Set (SS) will consist of all patients that received at least one dose of the study drug and had at least one post-baseline safety assessment. Patients will be analysed according to the treatment received.

The Per Protocol Set (PPS) will consist of all randomized subjects without any major protocol violation. Major protocol deviations will be defined prior to un-blinding. Results based on the PPS population will be used to support the primary analysis results. Each analysis population will be defined before unblinding.

4.5 Screening and Baseline Characteristics

The baseline characteristics of patients who were screened, but who were not included in the trial (either refused or ineligible) will be summarized along with reasons for refusal or ineligibility. It is recognised that only a limited set of baseline characteristics will be collected for these patients. Baseline characteristics will be described for all included and randomised patients by randomised group. Categorical variables will be summarised using frequencies and percentages, and continuous variables using mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. The number and percentage of patients with missing data will be reported for each variable.

4.6 Patient Disposition, Adherence and Follow-up

The number and percentage of patients screened, randomised and achieving each visit will be reported. Adherence to randomised treatment will be summarized. The number, percentage and type of protocol deviations and violations will be reported. The number of patients withdrawing prematurely from the study or lost to follow-up will be summarised and reported along with the reasons for withdrawal or loss to follow-up.

4.7 Primary Endpoint Analysis

The analysis of the primary end-point PIIINP (change from baseline to the final visit) will be carried out using analysis of covariance (ANCOVA). A linear regression model will be fitted,

including a binary variable to indicate the treatment group (placebo/spironolactone), a binary variable to indicate the baseline Gal-3 group (below/above median) and baseline PIIINP. An interaction term will be included to evaluate the additional impact of spironolactone in the elevated Gal-3 group. Residual analysis will be used to examine the fit of the model to the assumptions of linear regression and data may be transformed to meet the assumptions of linear regression.

As this is a proof of concept trial additional exploratory analyses will be carried out for the primary outcome. In particular Gal-3 will be considered categorised into thirds i.e. cut at the tertiles of the distribution, and treated as a continuous variable. Both of these methods may provide gains in power to detect an interaction compared to treating Gal-3 as elevated or not based on the median value.

The ANCOVA model analysing change in PIIINP requires PIIINP to be available at both the baseline and final visit. If a patient is missing PIIINP at either baseline or final visit they are automatically dropped from the analysis. Sensitivity analyses will be carried out using multiple imputation with chained equations to impute missing baseline and/or final visit values.

An alternative approach to the standard ANCOVA analysis for clinical trials with a single follow-up measure of the outcome plus a baseline measure, was proposed by White and Thompson [2005]. This method includes the baseline measure as an additional outcome, constrained to have the same mean in both groups and allowed to be correlated with the follow-up measurement. In the absence of missing data this model produces identical effect estimates to the ANCOVA model and almost identical standard errors. In the presence of missing baseline data this model has the advantage of being able to include patients with a follow-up measurement of the outcome but who are missing a baseline measure. This method may therefore allow gains in statistical power in the presence of missing data.

4.8 Secondary Endpoints

Secondary endpoints will be analysed using ANCOVA for continuous endpoints or multivariable logistic regression or multivariable Cox regression in the case of dichotomous and time to event endpoints respectively.

4.9 Methods for Handling Missing Data

Every effort will be made to ensure that missing data is kept to a minimum. Data collection will be monitored throughout the conduct of the trial to identify any missing data and efforts will be made to return to the original medical records to obtain the data where possible.

Where it is not possible and the data are missing, appropriate multiple imputation methods will be used depending on the scale and pattern of the missing data. Patterns and levels of missing data will be reported. Results from analysis including multiply imputed missing values will be treated as a sensitivity analysis.

4.10 Multiple Comparisons

There are no planned adjustments to the Type 1 error for multiple comparisons.

REFERENCES

1. Kosmala W, Przewlocka-Kosmala M, Szczepanik-Osadnik H, Mysiak A, O'Moore-Sullivan T, Marwick TH. A randomized study of the beneficial effects of aldosterone antagonism on LV function, structure, and fibrosis markers in metabolic syndrome. *JACC Cardiovasc Imaging*. 2011 Dec;4(12):1239-49.
2. Lachenbruch PA: A note on sample size computation for testing interactions. *Stat Med* 1988, 7(4):467-469.
3. White IR, Thompson SG. Adjusting for partially missing baseline measurements in randomised controlled trials. *Stat Med*. 2005; 24(7):993-1007

APPENDIX 1: VISIT SCHEDULE

The visit schedule is shown in the table on the following page.

Key to visit schedule:

- (A) At M1 the dose of spironolactone will be increased according to the algorithm specified in the protocol.
- (B) Patients deemed at risk of hypoglycaemia are exempt from the need to fast.
- (C) If screening and baseline visits are combined, this sample will be taken after all screening parameters are assessed. NTProBNP should be taken in the morning to confirm eligibility before central laboratory samples are taken. The fasting condition isn't requested in this case.
- (D) Exam must be done either at screening or at baseline prior to randomization
- (E) If the test is not contra-indicated (please see appendix 4)
- (F) once 1 month after the end of study treatment and once at the end of research (after last patient, last visit)
- (G) Visits may be performed remotely
- (H) Assessment is optional if the patient does NOT report: symptoms suggesting hypotension or heart failure, start of diuretics, start of new or addition anti-coagulants
- (I) These visits are not applicable for patients in the three month follow up
- (J) For patients in the three month follow up, this will be visit at Three months.

Blood sample for local biological assessment:

- 1: Haemoglobin, Sodium, Potassium, Urea, Creatinine, Total Cholesterol, HbA1c, NT-ProBNP or BNP
- 2: Sodium, Potassium, Urea, Creatinine,
- 3: Haemoglobin, Sodium, Potassium, Urea, Creatinine

Urinary sample for local biological assessment:

- 4: Urine albumin/creatinine ratio where this is available as part of routine care (eg:0 for assessment of patients with diabetes)

Blood sample for central biological assessment:

- 5: Blood sample (50mls) taken, spun and plasma taken into 500uL aliquots and stored, preferably at -80°C, for later central analyses.
- 6: One paxgene tube collected for genetic analyses only if patient agreed

Urinary sample for central biological assessment:

- 7 : Urine samples stored in 500uL aliquots preferably at -80°C, for later central analyses.
- Total research blood volume 120mls over 10 months.

Visit Schedule	Time for Test (min)	Screening M -1	Inclusion M0 +/- 7d	Visit Day 7 +/-3d (G)	Visit M1 +/-	Visit M2 +/- 15d	Visit M3 +/- 15d (G)	Visits M6 +/- 15d (G)	Final Visit M9 +/- 15d	Follow up visit by phone
Informed consent	30	x								
Verification of inclusion and exclusion criteria	10	x	x							
Demographics, Life Style and general characteristics	5	x								
Medical history	5	x	x (update)	x(update)	x(update)		x(update)	x(update)	x(update)	
Concomitant medication	5	x	x	x	x		x	x	x	
Questionnaires on Quality of Life and Symptoms	10	x	x						x	
Clinical examination	5	x	x	X (H)	x		X (H)	X (H)	x	
ECG	10	X (D)	X (D)						x	
Blood sample for local biological assessment	5	x (1)	x (2)	x (2)	x (3)	X (2)	x (2)	x (2)	x (3)	
Urinary sample for local biological assessment (optional)	0	x (4)							x (4)	
Blood sample for central biobank	8		x (5) fasting (B) (C) includes paxgene						x (5) fasting(B) includes paxgene	
Urine sample for central biobank	5		x (7) fasting(B) (C)						x (7) fasting(B)	
Echocardiography	30	x (D)	X (D)						x	
Shuttle walking Test (SWT)	15		x(E)						x(E)	
Randomisation			x							
Dispensing of Spironolactone (A)	10		x	x	x		x	x		
Assessment of morbidity and mortality				x	x		x	x	x	x (F)
Time for Tests (minutes)		Minimum 75minutes	~115	30	30	5	30	30	~115	