Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. The « Heart OMics in AGing » (HOMAGE) trial.

Pierpaolo Pellicori[1], João Pedro Ferreira[2], Beatrice Mariottoni[3], Hans-Peter Brunner- La Rocca[4], Fozia Z Ahmed[5], Verdonschot Job[4], Tim Collier[6], Joe J Cuthbert[7], Johannes Petutschnigg[8]; Blerim Mujaj[9], Nicolas Girerd[2], Arantxa González [10], Andrew L Clark[7], Franco Cosmi[3], Jan A Staessen[9], Stephane Heymans[4,11,12], Roberto Latini[13], Patrick Rossignol[2], Faiez Zannad[2] , John GF Cleland[1].

[1] Robertson Institute of Biostatistics and Clinical Trials Unit, University of Glasgow.

[2] Université de Lorraine,INSERM, Centre d'Investigations Cliniques Plurithématique, INSERM 1433, CHRU de Nancy,Institut Lorrain du Coeur et des Vaisseaux, FCRIN INI-CRCT, Nancy,France.

[3]Department of Cardiology, Cortona Hospital, Arezzo, Italy.

[4]Department of Cardiology, Maastricht University Medical Center Maastricht, the Netherlands

[5]Manchester Heart Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, United Kingdom

[6] Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, United Kingdom

[7] Department of Academic Cardiology, Castle Hill Hospital, Hull York Medical School (at University of Hull), Kingston upon Hull, UK

[8]Charité Universitätsmedizin Berlin, Department of Cardiology, Berlin, Germany

DZHK (German Center of Cardiovascular Research), Partner Site Berlin, Germany

[9]Studies Coordinating Centre, Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium

[10] Program of Cardiovascular Diseases, CIMA Universidad de Navarra and IdiSNA, Pamplona, Spain; CIBERCV, Carlos III Institute of Health, Madrid, Spain

[11] Department of Cardiovascular Sciences, Centre for Molecular and Vascular Biology, KU Leuven, Belgium

[12] The Netherlands Heart Institute, Nl-HI, Utrecht, The Netherlands

[13] Department of Cardiovascular Medicine, Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Milan, Italy.

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Abstract (256 words)

Background: Asymptomatic patients with coronary artery disease (CAD), hypertension and/or type-2 diabetes (T2DM) are at greater risk of developing heart failure (HF). Fibrosis, leading to myocardial and vascular dysfunction, might be an important pathway of progression.

Aims: To investigate the effects of spironolactone on serum markers of collagen metabolism and on cardiovascular structure and function in people at risk of developing HF and potential interactions with a marker of fibrogenic activity, galectin-3.

Methods: A prospective, randomised, open-label, blinded end-point (PROBE) trial comparing spironolactone (up to 50mg/day) and standard care over 9 months in people with clinical risk factors for developing HF, including hypertension, CAD and T2DM, and elevated plasma concentrations of NT-pro-BNP (125 to 1,000 ng/L) or BNP (35 to 280 ng/L). Exclusion criteria included left ventricular ejection fraction (LVEF)<45%, atrial fibrillation, severe renal dysfunction or treatment with loop diuretics. The primary endpoint was the interaction between change in serum concentrations of procollagen type III N-terminal propeptide (PIIINP) and treatment with spironolactone according to median plasma concentrations of galectin-3 at baseline.

Results: For the 527 participants enrolled, median (inter-quartile range) age was 73 (69-79) years, 135 (26%) were women, 412 (78%) had hypertension, 377 (72%) CAD and 212 (40%) T2DM. At baseline, medians (IQR) were for LVEF 63 (58-67) %, for left atrial volume index 31 (26-37)mL/m^2, for plasma NT-proBNP 214 (137-356) ng/L, for serum PIIINP 3.9 (3.1-5.0) ng/mL and for galectin-3 16.1 (13.5-19.7) ng/mL.

Conclusions: The HOMAGE trial will provide insights on the effect of spironolactone on pathways that might drive progression to HF.

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Introduction

Despite advances in care, the prognosis of heart failure is generally poor once it is clinically obvious (1). Changes to lifestyle and treating modifiable risk factors, such as hypertension, hyperlipidaemia and diabetes, may have delayed the onset of heart failure but have not reduced the life-time risk. Only a few trials have specifically investigated interventions to delay or prevent the onset of heart failure (2). Population screening, using blood tests or cardiac imaging, for early identification or prevention of heart failure has not yet been widely adopted, but there is a growing body of evidence, especially for natriuretic peptides, that such an approach might be useful (3,4). Pharmacological approaches to prevention might be most efficient when targeted at those both at greater risk and most likely to respond to a specific intervention. Markers of activity of pathways driving disease progression might provide such mechanistic biotargets, thereby providing a precision- or personalised-medicine approach.

Natriuretic peptides are released by the heart in response to cardiovascular stress. About 10% of adults have increased plasma concentrations of natriuretic peptides (5), the proportion increasing with age and the development cardiovascular disease (6-9). Elevated plasma concentrations are associated with an increase in cardiovascular morbidity and mortality, including heart failure, whether or not the individual has clinically overt disease. Accordingly, low plasma concentrations of natriuretic peptides can be used to avoid unnecessary investigation and high concentrations used to target those in need of further investigation and treatment to delay or prevent the onset of clinically overt heart failure (4).

Fibrosis of the myocardium, vasculature and kidney and possibly other organs might be a key pathway of progression from asymptomatic cardiac dysfunction to heart failure (10) and therefore a therapeutic biotarget. Accordingly, markers of collagen synthesis and degradation might identify patients more likely to respond to an intervention targeting fibrosis.

Rationale for the trial

Serum concentrations of procollagen type I C-terminal propeptide (PICP) and procollagen type III N-terminal propeptide (PIIINP), indicating increased synthesis of type-1 and type-3 collagen, are increased in HF, regardless of LVEF, and are associated with a worse prognosis (11-13). Serum PICP correlates directly with plasma concentrations of NT-proBNP and left atrial volume suggesting it is influenced by the degree of congestion (14). Increased serum concentrations of PIIINP in people without established CV disease are also associated with a higher risk of developing heart failure and of death (15, 16). An increase in Type-I collagen predominates in the failing heart, contributing to increased myocardial stiffness (17). Serum concentrations of collagen type I C-terminal telopeptide (CITP), indicating increased degradation of Type-1 collagen, are also increased in heart failure (13) and associated with an adverse prognosis. Raised plasma concentrations of galectin-3, a marker of inflammation involved in aldosterone-mediated fibrogenesis (18, 19), also predict incident heart failure in the general population (20).

Altogether, these findings suggest that there is both increased synthesis and degradation of collagen in heart failure and that high collagen turnover is associated with an adverse outcome. Serum markers of collagen metabolism are also associated with an increase in myocardial extracellular matrix and therefore, presumably, collagen mass (11, 13, 21). It is likely that the pro-fibrotic state is systemic, with most collagen turnover occurring in organs such as bone-marrow and liver, but with the consequences expressed selectively by organs subject to dynamic change, such as the heart and blood vessels. Accordingly, serum markers reflecting collagen metabolism might be used to select patients more likely to benefit from anti-fibrotic interventions for the prevention of heart failure and might also be used as surrogate markers of a therapeutic effect.

Aldosterone plays an important role in regulating renal sodium and potassium exchange, plasma volume and systemic blood pressure. Experimental evidence also suggests that MRA have favourable effects on collagen metabolism (22). Randomised trials show that MRA are effective anti-hypertensive agents and reduce morbidity and mortality for patients with heart failure and a reduced (HFrEF) and, perhaps, mid-range (HFmrEF) or even preserved left ventricular ejection fraction (LVEF) (23-25). These benefits of MRA may be mediated through diuresis, potassium retention, autonomic effects and reduction in pre- and after-load. However, clinical trials in patients with either HFrEF or HFpEF also suggest that administration of an MRA often exerts changes in serum concentrations of markers of collagen turnover in a pattern favouring reduced synthesis (26). This may reflect both direct, systemic effects of MRA on collagen metabolism and the effects of MRA on pre- and after-load that may specifically prevent, retard or reverse myocardial, vascular and renal fibrosis.

Effect of Spironolactone on Serum Markers of Collagen Metabolism in Clinical Trials (Table 1)

The Randomized Aldactone Evaluation Study (RALES), a placebo-controlled trial of spironolactone in HFrEF, measured serum concentrations of collagen metabolism in a subgroup of 261 patients. Higher serum concentrations of PIIINP were associated with greater morbidity and mortality and, compared to placebo, treatment with spironolactone reduced serum PIIINP. Amongst patients with a serum PIIINP below median, spironolactone did not reduce death or the composite of hospitalisation and death but exerted a substantial effect on both endpoints amongst those with greater than median serum PIIINP (27). However, there were too few patients in to provide robust evidence of an interaction. Similar patterns were observed for PINP and PICP. Subsequently, other trials have variably confirmed (28, 29) or refuted (30) these results. Women and those with lower serum potassium concentrations, which might be associated with higher plasma concentrations of aldosterone, had a greater reduction in PIIINP following treatment with

eplerenone in one trial (31). Smaller trials of HFpEF have also shown conflicting results (14, 32, 33). Trials of patients with obesity and/ or hypertension have more consistently shown reductions in both PIIINP and PICP, as well as improvement in Doppler indices of diastolic LV dysfunction. (34-36). Overall, the trials suggest that MRA lead to more consistent reductions in serum PIIINP than PICP, markers of synthesis, with little effect on CITP, a marker of collagen degradation.

The above observations provide the rationale for the « Heart OMics in AGing » (HOMAGE) trial, investigating whether spironolactone favourably alters markers of extra-cellular matrix remodelling for patients at increased risk of developing heart failure. The trial (clinicaltrials.gov: NCT02556450, EudraCT-No.: 2015-000413-48) was funded by the European Commission under the Health Cooperation Work Programme of the 7th Framework Programme, under the Grant Agreement n. 305507.

Design of the trial

HOMAGE is a multicentre, proof of concept, prospective, randomised, open-label, blinded end-point (PROBE) trial in patients at high risk of developing heart failure, comparing spironolactone titrated to a target dose of 50mg/day compared to no additional treatment. The primary endpoint is the interaction between changes in serum concentrations of PIIINP from baseline to nine months and baseline plasma concentrations of galectin-3 in participants assigned to spironolactone or not. The trial was conducted in ten centres in six European Countries: France (Nancy, Corbeil-Essonnes and Toulouse), Italy (Cortona), United Kingdom (Kingston upon Hull, Manchester and Glasgow), The Netherlands (Maastricht), Germany (Berlin) and Ireland (Dublin). The protocol was approved by the institutional review board or the independent

ethics committee for each institution before trial initiation. All patients provided written informed consent before participating in the trial.

Inclusion and Exclusion Criteria (Table 2)

People aged older than 65 years (later amended to >60 years) with established coronary artery disease or at least two criteria indicating CV disease (T2DM, hypertension, microalbuminuria, or an abnormal ECG) were invited to participate. Patients with a pre-existing diagnosis of heart failure or an LVEF <45% or currently treated with a loop diuretic were excluded. Patients with atrial fibrillation or severe renal dysfunction were excluded, as these conditions may cause increased plasma concentrations of natriuretic peptides without other evidence of CV disease. MRA are contraindicated in severe renal dysfunction or hyperkalaemia (>5.0mmol/L) and therefore such patients were excluded.

At screening, after giving informed consent in writing, a blood test was taken and plasma concentrations of NT-proBNP or BNP measured in local-site laboratories. Plasma concentrations of NT-pro-BNP had to be in the 'window' between 125 and 1,000 ng/L or BNP between 35 and 280 ng/ml. The lower boundary for plasma natriuretic peptide concentration was based on ESC guidelines for the exclusion of heart failure in the presence of symptoms and signs suggesting such a diagnosis (37). The upper boundary of the 'window' was introduced in order to exclude patients who had a high probability of serious, undiagnosed cardiac or renal disease who required further investigation and treatment rather than participation in this trial.

Although people with an LVEF <45% or with moderate or severe valve disease were excluded, those with left ventricular hypertrophy or diastolic left ventricular dysfunction were not. Accordingly, patients enrolled in HOMAGE could have cardiac dysfunction on imaging and increased plasma concentrations of natriuretic peptides, thereby fulfilling two of the three

diagnostic criteria for heart failure according to ESC guidelines, and for stage B heart failure in the ACC/AHA guidelines (37, 38).

Randomization and Blinding

Patients were randomised in a 1:1 ratio to spironolactone or control using random permuted blocks stratified by centre. Randomisation lists for each centre were created by the trial coordinating centre in Leuven, using the statistical software SAS 9.4. Randomisation was carried out via a web-based management system. All persons evaluating key tests, the clinical endpoints committee, and central laboratories were kept blind to treatment allocation.

Trial Assessments (Figure 1)

Patients were screened in primary and secondary care. All patients provided informed consent in writing before undergoing any research related procedures. The first patient was randomised in February 2016. The plan was to randomise 800 patients by the end of 2017 and follow all patients for nine months, completing the trial by a grant-deadline of November 2018. Due to slow recruitment rate, the trial steering committee extended the enrolment period until June 2018 and reduced the minimum period of follow-up to 3 months. Eventually, 877 patients were screened, of whom 527 were randomised. (Figure 2)

Major visits: baseline, one month and end of trial.

Three major visits are planned, at baseline (randomisation), one month and nine months (modified to a minimum of 3 months for patients enrolled after January 2018). The one-month visit was designed to investigate short-term effects of spironolactone that might be attributed to direct effects on fluid and electrolyte balance and the later visit to investigate longer-term effects on cardiovascular structure and function.

At these visits, patients completed quality of life (EQ5D) and symptom questionnaires (appendix) and blood for local and central laboratory analysis is collected, and an ECG and echocardiogram done. Echocardiograms were analysed, blind to allocation, in a core lab located in Nancy (France). Participants also did incremental shuttle walk-tests (ISWT), using a 10m course (the 'shuttle) marked by two cones. The walking speed is determined by bleeps played from a compact disc: after every minute, walking speed is increased. There are up to 12 levels of speed and, potentially, 102 shuttles. Changes of 50-70 metres (5-7 shuttles) are considered clinically meaningful (39). Blood pressure was measured at the end of ISWT. During and after the ISWT, in a subgroup of participants, heart and respiratory rate were measured with a chest-worn sensor (Equivital Inc, New York, USA) or heart sounds measured via acoustic cardiography (Audicor, Inovise Medical, Inc., Portland, USA).

Minor Follow-up visits: 7 days, three and six months of follow-up.

The protocol required at least three additional visits, on Day 7 and at 3 and 6 months, when serum potassium and creatinine is measured in order to adjust the dose of spironolactone (Table 3) and to ascertain adverse events.

Assays

Assays were done in a central laboratory. Plasma concentrations of galectin-3 were measured using an enzyme-linked immunosorbent assay (BG Medicine). Serum PIIINP and CITP were measured using a radio-immunoassay (Orion Diagnostica) and serum PICP using an enzyme immune-assay (Quidel Corp). Plasma NT-proBNP was measured using the Roche Elecsys assay (Roche Diagnostics GmbH, Mannheim, Germany).

Trial End-points

Primary Endpoint

The hypothesis was that the effects of spironolactone on serum concentrations of collagen markers would be greater in patients with increased plasma concentrations of galectin-3, a marker of aldosterone-mediated, fibrotic activity. The primary endpoint was the interaction between baseline plasma concentration of galectin-3 and the effect of randomised treatment (MRA or control) on changes, from baseline to nine months, in serum concentrations of PIIINP.

Secondary endpoints

Secondary endpoints will be assessed both at one and nine months and will include other biomarkers of extracellular matrix turnover, PICP (synthesis) and CITP (degradation). We will also assess the effects of spironolactone on weight, serum electrolytes, blood pressure, renal function, symptoms, quality of life, exercise capacity and cardiac function, assessed by echocardiography, and plasma concentrations of NT-proBNP and galectin-3. In addition, new onset atrial fibrillation, heart failure and other serious adverse events will be assessed.

Sample Size Calculation

The trial sample size was determined based on the ability to detect a difference of 0.79μg/l in the change in PIIINP amongst participants assigned to spironolactone or control with a baseline plasma galectin-3 above or below the median value using a two-sided significance level of 5% and 90% power and a standard deviation estimated at 1.73μg/l (35). The original calculation required 800 participants, with >700 having evaluable serum PIIINP data but, as only 527 patients were randomised, the trial has less power than originally conceived. Nonetheless, a trial of 500 patients provides 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). Thus, the trial has sufficient power to provide estimates of the likely effect-size and interaction effects between 0.87 and 1.0μg/l. Further statistical power may be gained through the method of

analysis in the final analysis plan, in particular by treating galectin-3 as a continuous variable and using methods for measurements from several time-points. No interim efficacy analyses were planned or done.

Statistical Analysis

Statistical analyses will be carried out using Stata ® version 15.1. Baseline characteristics are presented as median and inter-quartile range for continuous variables and frequencies and percentages for categorical. The primary efficacy analyses will be carried out on the Full Analysis Set, defined as all randomized patients who received at least one dose of the trial medication. Analysis will be conducted according to the intention to treat principle.

Analysis of the primary end-point 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, a binary variable to indicate galectin-3 above or below the median and baseline PIIINP. An interaction term will be included to evaluate the impact of spironolactone in those with galectin-3 above median. 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. Sensitivity analyses will be carried out using multiple imputation with chained equations to impute missing baseline and/or final visit values. As this is a proof of concept trial, additional exploratory analyses may be carried out for the primary outcome, if appropriate, as pre-specified in a statistical analysis plan. There are no planned adjustments for multiple comparisons due to the exploratory nature of this trial.

Baseline Characteristics

The median (IQR) age of participants in the HOMAGE trial was 73 (69-79) years and a high proportion were men (74%), perhaps reflecting the use of CAD as a screening strategy (72%).

Most participants (78%) had treated hypertension, but the median systolic blood pressure at entry was still 140mmHg and 25% had a systolic blood pressure exceeding 154mmHg. Most patients had stage II chronic kidney disease (eGFR 60-89 ml/min/1.73m^2) and 40% had T2DM (Table 4) Median LVEF was normal at 63% (IQR 58-67%) and median plasma concentrations of NT-proBNP were 214 (IQR: 137-356) ng/L (Table 5). Left atrial volume and the ratio of E/E' were increased and left ventricular mass was at the upper limit of normal. On a symptom questionnaire (appendix), the median score in response to the question "do you get breathless on moderate exertion (eg:- walking quickly, climbing 2-3 flights of stairs)" the median score was 5 (2 - 6) indicating that most participants found this amount of exertion troublesome. Baseline serum concentrations of collagen markers were similar to those observed in previous trials of hypertension and heart failure.

Potential impact

As life expectancy increases, so will the prevalence of heart failure (40). Preventing or delaying the onset of heart failure could prolong active life and potentially reduce healthcare costs. Some treatments for hypertension can delay or prevent the development of heart failure in older people, but not all anti-hypertensive agents are similarly beneficial (41-43). Spironolactone has been used to treat patients since 1959. Its side effects are well-known, reversible and, at the doses used in this trial, usually not severe. It is a highly effective treatment for resistant hypertension (44), and is known to reduce morbidity and mortality for HFrEF. MRA also appear to have favourable effects on myocardial fibrosis, which might be a key pathway for the development of heart failure in general, and perhaps specifically HFpEF.

Targeting specific mechanistic pathways involved in the progression to heart failure may increase the efficiency, safety and cost-effectiveness of pharmacological interventions for the prevention of heart failure. Patients with a biomarker profile suggesting increased fibrotic

activity might receive greater benefit from an agent directed at fibrosis, such as an MRA. Targeting treatment at those who have more to gain could also avoid treating those with a less favourable benefit/risk profile.

The HOMAGE trial enrolled people with few symptoms to suggest heart failure but with moderately elevated plasma concentrations of natriuretic peptides and a high prevalence of atrial dilation and left ventricular hypertrophy suggesting diastolic dysfunction. Indeed, patients enrolled in HOMAGE had higher plasma concentrations of NT-proBNP and greater left atrial dilatation than those enrolled in the ALDO-DHF (45) (Table 5), a trial of spironolactone in patients with symptomatic HFpEF. The patients in HOMAGE could be considered to have a "pre-HFpEF" syndrome. However, participants did report, on average, troublesome breathlessness on moderate exertion and therefore others might consider the patients already to have HFpEF. However, plasma concentrations of NT-proBNP were somewhat lower than in large clinical outcome trials of HFpEF, such as PEP-CHF (46), PARAGON-HF (47) or TOPCAT (48) (Table 5), which generally required patients to have symptoms of congestion treated with diuretics, prior hospitalisations for heart failure, echocardiographic evidence of LV disease or left atrial dilation in addition to high plasma concentrations of natriuretic peptides.

Trials of HFpEF have failed to show conclusive benefit, especially when those with HFmrEF are excluded. It is unclear whether women and men with HFpEF respond differently to treatments. In PARAGON-HF women may have responded better (47). In TOPCAT, the overall effect was similar for men and women, but this masked a greater benefit for men with an LVEF <50% but a higher risk if LVEF was >65%, whereas woman obtained little or no benefit throughout the range of measured LVEF (25). Patients with advanced disease may be less likely to respond to interventions. In a post-hoc analysis of the I-PRESERVE trial (49), for patients with HFpEF who had NT-proBNP below the median value (339ng/L), irbesartan reduced mortality by 25% and

heart failure hospitalisation or death by 40% (both statistically significant) but had no benefit for patients with higher NT-proBNP values who were at greater risk. This suggests that whilst patients with less severe diastolic dysfunction may be at lower risk that are also more amenable to treatment. Further credence to this hypothesis comes from the HYVET trial, which showed that a combination of indapamide and perindopril administered to elderly patients with poorly controlled hypertension had a striking reduction in heart failure events and mortality (41). Many of these patients must have had left ventricular hypertrophy, dilated left atria, raised natriuretic peptides and, had they been assessed carefully, symptoms and signs of heart failure; in other words HFpEF. These considerations suggest that an effective method for the management of HFpEF might be early detection and treatment to delay or prevent progression to the more severe manifestations of this condition. Those with advanced disease may be recalcitrant to therapy.

Conclusions

HOMAGE is a proof-of-concept trial that should be a catalyst for further research on biomarkers that might also be biotargets that indicate activation of specific, modifiable pathological pathways. This research approach may help deliver precision-medicine for personalised care. Further trials are required to ensure that theory translates into practice.

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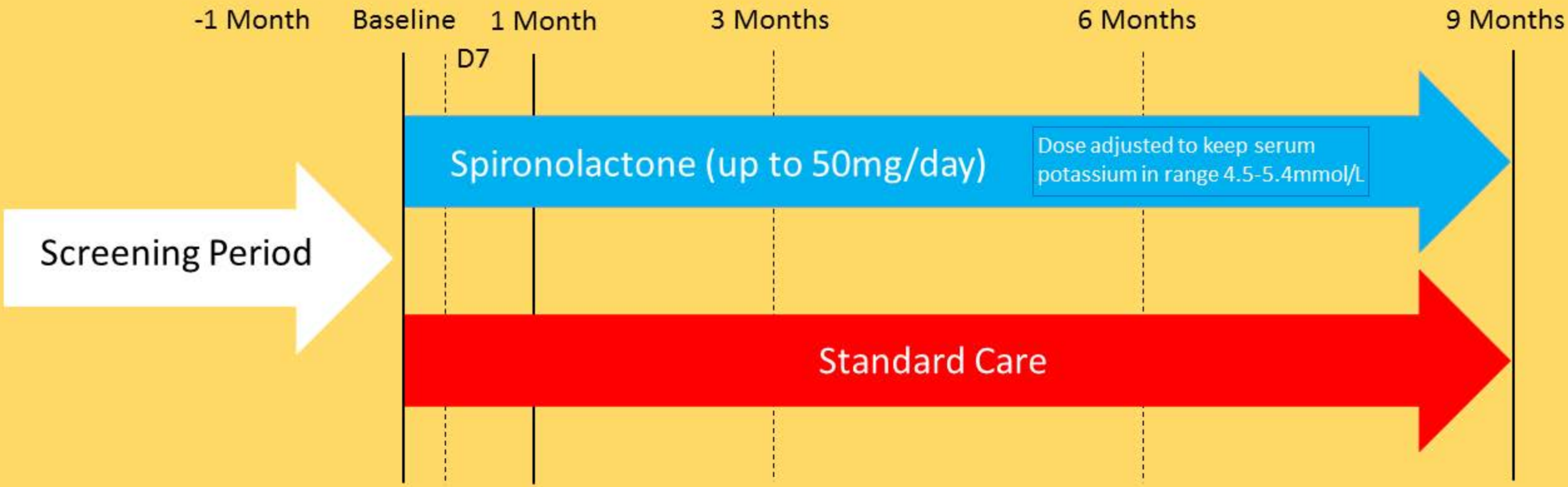
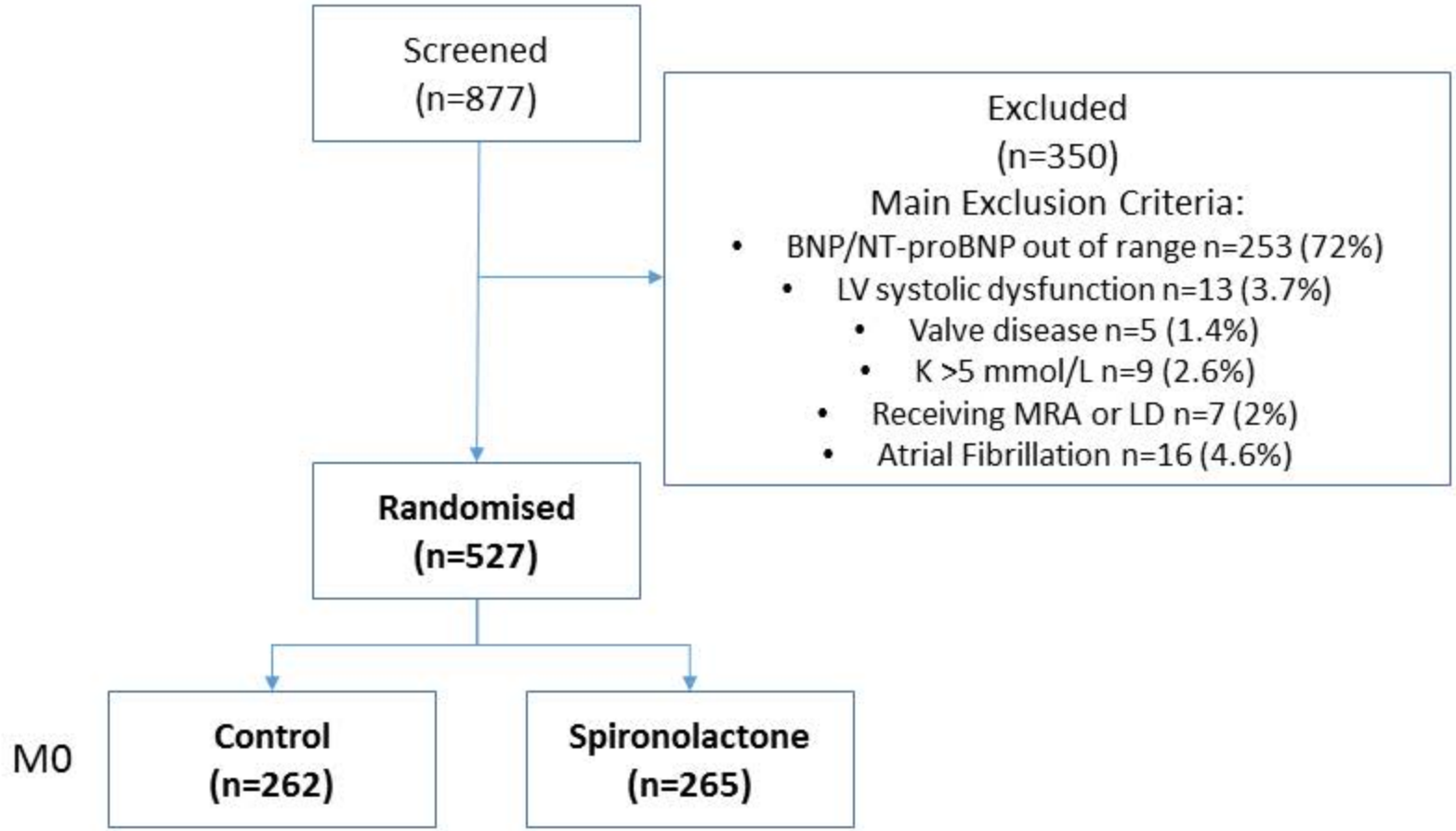


Major visits

- I/E criteria (screening)
- Randomisation (baseline)
- Clinical history (baseline)
- Demographics (baseline)
- ECG & Echo
- Bloods & Urine (Safety and research)
- ISWT
- Symptoms questionnaire
- Heart and respiratory rate (optional)
- Vascular function (optional)
- Heart sounds (optional)
- Dose adjustments
- Adverse events

Minor visits

- Bloods(safety)
- Dose adjustments
- Adverse events


Screened
(n=877)
Excluded
(n=350)
Main Exclusion Criteria:
• BNP/NT-proBNP out of range n=253 (72%)
• LV systolic dysfunction n=13 (3.7%)
• Valve disease n=5 (1.4%)
• K >5 mmol/L n=9 (2.6%)
• Receiving MRA or LD n=7 (2%)
• Atrial Fibrillation n=16 (4.6%)
Randomised
(n=527)
M0
Control
(n=262)
Spironolactone
(n=265)

Trial	RALES[27]		AREA-in-CHF[30]		EPHESUS[28]		PARADIGM-HF[55]	
Population	HFrEF		HFrEF		AMI complicated by HFrEF		HFrEF	
Treatment	Spironolactone vs. Placebo		Canrenone vs. Placebo		Eplerenone vs. Placebo		Sac./Val. vs. Enalapril (E)	
N=	261		382		476		2067	
Follow-up	6 months		6 months (12m for echo)		Up to 9 months		8 months	
	Controls	MRA	Controls	MRA	Controls	MRA	Enalapril	Sac/Val
PIIINP - µg/L - pre	4.9 (2.5)	4.9 (2.7)	5.4 (4.0-7.4)	5.3 (3.9-6.9)	4.2 (1.5)	4.2 (1.7)	4.6 (3.6-5.9)	
PIIINP - µg/L – post	5.0 (2.8)	4.0 (1.9)	Δ:+5.0%	Δ:+3.7%	4.5 (6M)	4.0 (6M)~	-1.7%	-4.5%
	Δ: 0.11	Δ:-0.87*~			4.3 (9M)	3.7 (9M)~	Sac/Val vs E: -3% (p=0.086)	
PINP - µg/L - pre	51 (57)	41 (23)	NR	NR	35 (17)	33 (14)	36 (27–48)	
PINP - µg/L - post	57 (76)	36 (18)	NR	NR	44 (6M)	38 (6M)~	+0.6%	-6.2%
	Δ:6	Δ:-5*~			44 (9M)	36 (9M)~	Sac/Val vs E: -6% (p<0.001)	
CITP - µg/L - pre	NR	NR	NR	NR	6.7 (4.0)	6.4 (3.4)	NR	NR
CITP - µg/L - post	NR	NR	NR	NR	5.3 (6M)	5.0 (6M)	NR	NR
					5.3 (6M)	4.6 (9M)	NR	NR
PICP - µg/L - pre	129 (47)	139 (117)	NR	NR	NR	NR	NR	NR
PICP - µg/L - post	142 (79)	131 (113)	NR	NR	NR	NR	NR	NR
	Δ:13	Δ:-8						
LAD – cm – pre	NR	NR	4.1 (0.6)	4.2 (0.8)	NR	NR	NR	NR
LAD – cm – post	NR	NR	4.1 (0.7)	4.1 (0.8)~	NR	NR	NR	NR
LAVI – ml/m^2	NR	NR	NR	NR	NR	NR	NR	NR
Doppler	NR	NR	NR	NR	NR	NR	NR	NR

Trial	PARAMOUNT [53,54]		NCT00505336[32]		RAAM-PEF[33]		ALDO-DHF[14,45]	
Population	HFpEF		HFpEF		HFpEF		HFpEF	
Treatment	Valsartan vs Sac/Val		Eplerenone vs. Placebo		Eplerenone vs. Placebo		Spironolactone vs. Placebo	
N=	301		44		44		381	
Follow-up	36 weeks		Up to 12 months (M)		Up to 26 weeks (W)		1 year	
	Valsartan	Sac/Val	Controls	MRA	Controls	MRA	Controls	MRA
PIIINP - µg/L - pre	5.6 (4.3–6.8)	5.5 (4.4–7.2)	5.0 (2.0)	6.2 (3.3)	6.3 (5.2-8.9)	6.0 (4.3-10.1)	NR	NR
PIIINP - µg/L – post	5.4 (4.1–7.0)	5.3 (4.2–7.2)	4.7 (3.3) (6M)	5.1 (2.1) (6M)*	6.2 (5.0-9.6) (W14)	4.7 (3.8-8.9) (W14)*~	NR	NR
			7.3 (4.0) (12M) *~	5.7 (3.1) (12M)	5.9 (4.3-8.7) (W26)	5.8 (4.2-11.5) (W26)	NR	NR
PINP - µg/L - pre	NR	NR	40 (17)	42 (21)	41 (29-60)	39 (26-66)	NR	NR
PINP - µg/L - post	NR	NR	31 (16)* (6M)	32 (17)* (6M)	45 (35-61)(W14)*	31 (25-56) (W14)~	NR	NR
	NR	NR	48 (20)* (12M)	43 (14) (12M)	43 (35-61) (W26)	30 (22-52)(W26)*~	NR	NR
CITP - µg/L - pre	NR	NR	5.8 (2.9)	6.7 (3.7)	6.0 (4.0-9.6)	5.8 (4.2-11.5)	NR	NR
CITP - µg/L - post	NR	NR	5.7 (2.6) (6M)	6.8 (2.8) (6M)	5.7 (4.7-9.3) (W14)	6.4 (3.8-9.1)	NR	NR
	NR	NR	6.6 (4.2) (6M)	6.1 (3.4) (12M)	6.5 (4.8-8.7) (W26)	5.5 (4.3-8.9)~		
PICP - µg/L - pre	NR	NR	400 (107)[#]	415 (133)[#]	949 (652-1365)[#]	913 (708-1107)[#]	108 (88-129)	104 (81-129)
PICP - µg/L - post	NR	NR	383 (99) (6M)[#]	399 (152) (6M)[#]	1081 (691-1325) (W14)[#]	793 (670-1086) (W14)[#]	Δ 0.95 (0.91 to 0.99) (p=0.050)	
	NR	NR	402 (104) (12M)[#]	425 (109) (12M)[#]	959 (781-1291) (W26)[#]	821 (673-1195) (W26)[#]		
LAD – cm – pre	3.7 (0.5)	3.7 (0.5)	NR	NR	NR	NR	NR	NR

LAD – cm – post	Δ: -0.08	Δ:-0.15*	NR	NR	NR	NR	NR	NR
LAVI – ml/m^{2} - pre	36.3 (14.7)	35.2 (12.3)	45 (12)	50 (17)	80 (24)**	73 (26)	27.8 (26.9-28.6)	27.4 (26.6-28.2)
LAVI – ml/m^{2} - post	Δ:0.3	Δ:-2.6*	44 (14) (6M)	49 (20) (6M)	74 (23) (26W)	64 (23) (26W)*	Δ -0.33 (-1.48 to 0.82) (p=0.58)	
			53 (23) (12M)	52 (19) (12M)				
Doppler	No changes		E' decreased 6/12M*	E' decreased 12M*	MRA improved E/E'~		MRA improved septal E' and LVMi~	
Trial	REMINDER[29]		ASCOT[36]		ACTRN12609000655246[34]		NA[35]	
Population	STEMI without HF		Hypertension		Obesity		Metabolic syndrome	
Treatment	Eplerenone vs. Placebo		Spironolactone		Spironolactone vs. Placebo		Spironolactone vs. Placebo	
N=	526		146		113		80	
Follow-up	6 months		9 months		6 months		6 months	
	Controls	MRA	Matched Cohort	MRA	Controls	MRA	Controls	MRA
PIIINP - µg/L - pre	4.0 (3.3-4.6)	3.9 (3.2-4.7)	4.5 (3.7–6.3)	5.1 (3.6–6.4)	5.2(2.0)	5.2(2.0)	5.3 (1.9)	5.8 (2.0)
PIIINP - µg/L – post	Δ 0.5±1.4	Δ 0.2±1.5	Δ: 0.5	Δ:–0.4~	5.4(2.9)	4.7 (1.9)*~	5.2 (2.0)	4.9 (1.7)*~
PINP - µg/L - pre	29 (22-42)	32 (23-44)	NR	NR	NR	NR	NR	NR
PINP - µg/L - post	Δ 2.1±15.4	Δ -1.5±14.6~	NR	NR	NR	NR	NR	NR
CITP - µg/L - pre	3.8 (2.9-4.6)	3.7 (3.0-4.5)	5.9 (3.3–9.1)	7.1 (4.4–8.9)	NR	NR	NR	NR
CITP - µg/L - post	Δ 0.2±1.4	Δ 0.13±1.46	Δ: −1.2	Δ: −0.0	NR	NR	NR	NR
PICP - µg/L - pre	NR	NR	76 (63–90)	80 (66–102)	184 (75)	175 (76)	150 (44)	142 (33)
PICP - µg/L - post	NR	NR	Δ: 5	Δ :−6~	190 (79)	146 (71)*~	146 (47)	122 (23)*~
	NR	NR						
LAD – cm – pre	NR	NR	NR	NR	4.3(0.4)	4.4(0.5)	4.3 (0.4)	4.4 (0.3)
LAD – cm – post	NR	NR	NR	NR	4.3(0.4)	4.2(0.4)*~	4.3 (0.5)	4.3 (0.3)*
LAVI – ml/m^{2}	NR	NR	NR	NR	NR	NR	NR	NR
Doppler	NR	NR	NR	NR	MRA improved E/E' & GLS'*~		MRA improved E/E' & GLS'*~	

Table 1. RCTs of mineralo-corticoid receptor antagonists (MRA) investigating effects on serum collagen-derived markers, in patients with Heart Failure and Reduced (HFrEF; top panel) or Preserved (HFpEF; mid panel) Left Ventricular Ejection Fraction, and in those at high risk of developing heart failure (lower panel). Data for two trials of sacubitril/valsartan are also shown. Data are reported as median (Interquartile range) or mean (Standard deviation), when available. *Indicates significant changes during follow-up within same arm ($p<0.05$). ~Indicates significant changes during follow-up between the two arms ($p<0.05$). Δ: change from baseline, or between arms; cells with significant differences are also shaded for ease of reading; ** not indexed; # these values are extremely high, and higher than what has been reported in other studies, which is likely to reflect differences in kits and methods used to measure biomarker circulating levels.

Abbreviations used: Procollagen type I C-terminal propeptide – PICP; Procollagen type III N-terminal propeptide – PIIINP; Collagen type I C-terminal telopeptide – CITP; N-terminal propeptide of collagen I – PINP. Left atrium – LA; Left atrial volume indexed – LAVI; Not reported – NR; Global longitudinal strain – GLS.

Inclusion criteria	Exclusion criteria
1.Ability to provide written informed consent prior to any study procedure 2. Age >65years *(modified to >60 years during the trial)* 3. Clinical risk factors for developing heart failure, either: **A.** Coronary artery disease (h/o myocardial infarction, angioplasty or coronary artery bypass) Or **B.** At least two of the following: • Diabetes Mellitus requiring Hypoglycaemic Pharmacotherapy • Receiving pharmacological treatment for Hypertension • Microalbuminuria • Abnormal ECG (left ventricular hypertrophy, QRS >120msec, abnormal Q-waves) 4. Biological risk: NT-pro-BNP values between 125 and 1,000 ng/L or BNP values between 35 and 280 pg/ml	1. Recent wound healing/inflammation: • Surgical procedure, coronary, cerebral or peripheral vascular events or infection in the prior 3 months • Cancer (life limiting or less than 2 years in remission) • Autoimmune disease • Hepatic Disease 2. Pre-existing diagnosis of clinical HF 3. Moderate/severe LV systolic ventricular dysfunction, i.e. LVEF <45% 4. Moderate or severe valve disease (investigators opinion) 5. Corrected eGFR< 30ml/min/1.73m2, using the MDRD four-variable equation 6. Serum potassium >5.0 mmol/L and serum sodium <125 mmol/L 7. Treatment with an MRA or a loop diuretic (furosemide, bumetanide, ethacrynic acid or torasemide) in the previous three months 8. Potassium supplements or potassium-sparing diuretic at time of enrolment. 9. Atrial fibrillation within one month prior to inclusion 10. History of hypersensitivity to spironolactone or to any of its excipients 11.Patients who require treatment with prohibited medications according to the summary of product characteristics with the exception of ACE inhibitors or angiotensin receptor blockers – although not their combination 12. Patients unable to give written informed consent 13. Participation in another interventional trial in the preceding month 14. Ability to walk is, in the investigators opinion, clearly limited by joint disease or other locomotor problems or lung diseases rather than by cardiorespiratory fitness

Table 2. Main inclusion and exclusion criteria.

Serum potassium (mmol/l)	**Action**	**Dose adjustment**
<4.5	Increase	25 mg OD to 50 mg OD 25 mg EOD to 25 mg OD
4.5 – 5.4	Maintain	No dose adjustment
5.5 – 5.9	Decrease	50 mg OD to 25 mg OD 25 mg OD to 25 mg EOD 25 mg EOD to withhold
≥ 6.0	Withhold	N/A

Table 3. Dose adjustments after initiation of spironolactone treatment. Abbreviations used**:** EOD: Every Other Day; OD: Once daily. After withholding spironolactone due to high serum potassium, it can be re-started at a dose of 25 mg EOD when potassium levels have fallen below 4.5 mmol/L. Spironolactone must be discontinued if serum creatinine ≥220 μmol/L (or eGFR ≤30 ml/min/1.73m^2). Treatment may be restarted at the investigator's *discretion once serum* creatinine is <220 μmol/L and eGFR >30 ml/min/1.73m^2.

	Not Randomised	Randomised
	N=350	**N=527**
Demographics and Lifestyle		
Age-years	70(67-75)	73(69-79)
Female-N.(%)	70 (20)	135 (26)
Caucasian-N.(%)	346 (99)	518 (98)
Current smoker-N.(%)	39(11)	44(8)
Inclusion Criteria		
CAD-N.(%)	249(71)	377(72)
Diabetes-N.(%)	131(37)	212(40)
Hypertension-N.(%)	280(80)	412(78)
Micro albuminuria-N.(%)	79(23)	108(21)
Abnormal ECG*-N.(%)	83(24)	176(33)
Exclusion Criteria		
BNP/NT-ProBNP -N.(%)	253(72)	
LV systolic dysfunction-N.(%)	13(4)	
Valve disease-N.(%)	5(1)	
eGFR<30ml/min-N.(%)	2(<1)	
Serum potassium >5.0 mmol/L-N.(%)	9(3)	
MRA or loop diuretic treatment-N.(%)	7(2)	
Potassium supplements-N.(%)	3(<1)	
Atrial fibrillation-N.(%)	16(5)	
Confounding Medical Condition – N (%)	13(4)	
Other – N (%)	29(8)	
Medical History		
Myocardial Infarction-N.(%)	136(39)	214(41)
PCI-N.(%)	176(50)	265(50)
CABG-N.(%)	72(21)	136(26)
Stroke/TIA-N.(%)	14(4)	28(5)
COPD-N.(%)	23(7)	33(6)
Pacemaker-N.(%)	12(3)	22(4)
Medications		
Thiazide-N.(%)	50(14)	85(16)
Beta Blocker-N.(%)	203(58)	365(69)
ACE-I-N.(%)	175(50)	275(52)
ARB-N.(%)	89(25)	145(28)
CCB-N.(%)	58(17)	108(21)
Statin/Lipid lowering drug-N.(%)	280(80)	431(82)
Anticoagulant-N.(%)	27(8)	29(6)
Aspirin-N.(%)	210(60)	373(71)
Other Antiplatelet -N.(%)	58(17)	99(19)
Anthropometrics		
Weight-kg	84 (75-95)	82 (72-92)
BMI-kg/m^2	28.5 (26.2-31.3)	28.1 (25.4-31.6)

Waist-Hip ratio	0.98 (0.94-1.02)	0.98 (0.94-1.02)
Symptoms & Vital Signs		
Systolic Blood Pressure-mmHg	141 (130-153)	140 (128-154)
Diastolic Blood Pressure-mmHg	80 (74-87)	78 (71-85)
Heart Rate-bpm	64 (58-71)	61 (55-67)
NYHA I-N.(%)	279(80)	433(82)
NYHA II-N.(%)	56(16)	76(14)
NYHA III-N.(%)	6(2)	9(2)
NYHA – Not done-N.(%)	9(3)	9(2)
Blood Results		
eGFR- ml/min/1.73m^2	77 (65-91)	72 (62-85)
Haemoglobin-g/dl	14.4 (13.4-15.2)	14.0 (13.1-14.9)
Creatinine-mg/dl	0.9 (0.8-1.1)	1.0 (0.8-1.1)
Sodium-mmol/l	139 (137-141)	140 (138-141)
Potassium-mmol/l	4.3 (4.0-4.5)	4.3 (4.1-4.6)
EQ5D & HOMAGE symptoms questionnaire		
Visual Analogue Score	80 (65-90)	80 (70-90)
Breathlessness on moderate exercise	3 (2-6)	5 (2-6)
Swollen ankles	0 (0-1)	0 (0-2)
Tiredness	2 (0-5)	3 (0-5)

Table 4. Baseline characteristics of participants who were randomised or not in the HOMAGE trial. Abbreviations used: CAD – Coronary artery Disease; PCI - Percutaneous Coronary Intervention; CABG – Coronary Artery Bypass Grafting; TIA - Transient Ischaemic Attack; COPD - Chronic Obstructive Pulmonary Disease; ACE-I -Angiotensin-converting enzyme inhibitors; ARB - Angiotensin receptor blockers ; CCB - Calcium channel blockers; MRA- mineralocorticoid receptor antagonist; eGFR - Estimated glomerular filtration rate; BMI- Body Mass Index; NYHA - New York Heart Association; LV – Left Ventricular; EQ5D - EuroQol-5D. * Includes: Left Ventricular Hypertrophy, QRS >120msec, pathological Q-waves

	Reference Population[50]	ALDO-DHF[45]	ASCOT-MRA[36]	HOMAGE*	I-PRESERVE[51]	CHARMES[52]	PEP-CHF[46]	PARAMOUNT[53,54]	PARAGON-HF[47~]	TOPCAT[48^]
Population	No DD / DD	HFpEF	Hypertension	At risk of HF	HFpEF	HFpEF	HFpEF‡	HFpEF	HFpEF	HFpEF
n =	600 / 182	422	173	527	4,133	109Ⅱ	375	149 to Sac/Val	4,822	3,445
Age – Years	46 / 64	67 (8)	64 (8)	73 (69-79)	72	65	~77	71	73 (8)	69 (61-76)
Men (%)	50 /43	48	80	74	40	65	45	43	48	48
h/o CAD (%)	NR	40	Excluded	72	48	76	25”	21	43	59
h/o HTN (%)	30 / 81	92	100	78	88	69	73	95	96	91
h/o T2DM (%)	1 / 3	17	46	40	27	26	21	41	43	32
h/o AF (%)	**NR**	5	NR	**Excluded**	29	16	20	27	32	35
SBP –mmHg	125 / 142	135 (18)	167 (19)	140 (128-154)	136	134 (19)	~138	136	131 (15)	130 (120-140)
Diuretic (%)	5 / 25****	54**	41 (Thz)	**LD Excluded**	52	67**	53 (LD)	100	96#	82**
eGFR ml/min/1.73m^2	83 / 72	79 (19)	70 (62-77)	72 (62-85)	72	NR	52@	67 (19)	63 (19)	68 (20)
NT-proBNP – ng/L	39 (17-75)Δ	158 (83-299)	176 (87–354)	214 (137-356)	339 (133-964)	376 ± 638ng/LⅡⅡ	409 (176-1,035)	828 (460-1341)	885 (863-908)	~950***
Echocardiography										
LVEF - %	NR	67 (8)	NA	63 (58-67)	64 (9)	~50	63	58	58 (8)	60 (56-64)
LAVI – mL/m^2	22 / 27	28 (8)	NR	31 (26-37)	NR	~40	NR	35	NR	28 (21-35)
LA Diameter - mm	NR	NR	NR	46 (41-50)	NR	NR	45 (41-48)	37	NR	42 (38-47)
E/E’	6.4 / 9.3	12.8 (4.0)	NR	9.4 (7.5-11.8)	10 (5)	NR	NR	12.4	NR	10.5 (L)/14.7 (S)
LVMI – g/m^2	88 / 105	109 (28)	NR	95 (81-113)	164 (48)@	~235@	NR	78	NR	108 (90-128)
Collagen Biomarkers										
PIIINP – µg/L	4.5 / 5.6	NR	4.9 (3.7–6.3)	3.9 (3.1-5.0)	4.3 (3.7-5.4)	NR	NR	5.6 (4.3–6.9)	NR	Uncertain
PICP – µg/L	100 / 103	111 (92-136)	78 (66–100)	81 (67-98)	NR	NR	NR	NR	NR	130 (94-170)
CITP – µg/L	5.3 / 6.3	NR	6.4 (4.2–8.8)	3.8 (2.9-5.1)	NR	NR	NR	NR	NR	Uncertain
Gal-3 – µg/L	10.9 (9.0-13.1)Δ	12.5 (3.8)	NR	16.1 (13.5-19.7)	NR	NR	NR	17.8 (14.1–22.8)	NR	19.2 (15.2-23.5)

Table 5. Selection of trials, ranked by plasma NT-proBNP concentration conducted in patients with hypertension, at high risk of developing heart failure (HF), or with heart failure and preserved ejection fraction (HFpEF) showing clinical and echo data and, where available, serum collagen markers at baseline. Flemish population data is shown for reference. * At screening; **Did not distinguish between different classes of diuretics; ***Available for a subset only,Ⅱ - excludes patients with moderate or severe diastolic grade 38% of whom had a h/o AF; ⅡⅡ - mean and standard deviation of patients reported to have normal or mild diastolic dysfunction and predominantly in sinus rhythm; # of which 26% were mineralocorticoid receptor antagonists; ~ required recent heart failure hospitalisation (within 9 months from screening) or elevated NT-proBNP (>300 ng/L if sinus rhythm, > 900 ng/L if atrial fibrillation) as inclusion criteria; ^ required recent heart failure

hospitalisation (within 12 months from screening) or elevated BNP (≥100 ng/L) or NT-proBNP (≥360 ng/L) as inclusion criteria; “ history of myocardial infarction; @ creatinine clearance (Cockcroft-Gault). ‡ - only cohort with NT-proBNP measured

Δ = data taken from the PREVEND study in Netherlands population mean age 50 years (56).**** LD (%): 0.2/2.2; @not indexed.

Abbreviations used: NR = not reported; h/o – history of; CAD – Coronary Artery Disease; HTN – Hypertension; T2DM – Type II Diabetes Mellitus; AF – Atrial Fibrillation; SBP – Systolic Blood Pressure; eGFR -Estimated glomerular filtration rate; LVEF – Left Ventricular Ejection Fraction; Thz = thiazide or thiazide-like diuretic; LD = loop diuretic

Some data has been reported in abstract from for TOPCAT but vales for PIIINP and CITP appear highly inconsistent with ranges in published literature and some of the changes reported implausible.