PROJECT FINAL REPORT

Grant Agreement number: 305507

Project acronym: HOMAGE

Project title: Heart OMics in AGEing

Funding Scheme: Collaborative Project

Date of latest version of Annex I against which the assessment will be made:
05. July 2018

Period covered: from 01. February 2013 to 31. January 2019

Name, title and organisation of the scientific representative of the project's coordinator:
Prof. Faiez Zannad, Inserm

Project website address: www.homage-hf.eu

FINAL PUBLISHABLE SUMMARY REPORT

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1.1 Executive Summary

Despite major progress in heart failure (HF), HF mortality is still alarmingly high and little progress has been made to prevent and treat it. HOMAGE called for a disruptive strategic approach, combining knowledge based on i) underlying mechanisms, ii) omics bioprofiling, iii) and co-morbidities.

In summary, the objectives of the HOMAGE consortium were:

- Objective 1: Help the identification of patients at risk of developing HF before the onset of the symptoms
- Objective 2: Help the identification of sub-sets of patients who are more likely to respond to specifically targeted therapies (personalized medicine)
- Objective 3: Assess the predictive value of biomarker for other co-morbidities commonly associated to HF and ageing.

HOMAGE aimed to identify the most promising 'omics-based' BM profiles for the pre-symptomatic diagnosis and future prediction of HF in patients at risk. The predictive value of the BMs for other co-morbidities commonly associated with HF and ageing was investigated.

Furthermore, in a prospective trial, we investigated the potential for targeting preventive therapy at patients with the greatest likelihood of response and the lowest risk of adverse effects. The HOMAGE consortium was to deliver a randomized controlled trial delivering a mineralocorticoid antagonist (MRA) to older people who have cardiovascular disease and evidence of cardiac dysfunction who are at increased risk of developing HF. The trial investigated whether the MRA can reduce markers of myocardial fibrosis and whether bio-markers can prospectively identify responder-patients with a favourable benefit/risk profile.

This proof-of-concept study aimed to help design large future biomarker-guided, personalised medicine, outcome studies leading to treatments aiming at delaying or preventing the onset of heart failure. The information gained may also be applied to many other therapeutic areas, especially where prevention of disease is the target.

Our selection of innovative 'omics-based BMs was based on knowledge of biological pathways of the disease, which may facilitate identification of 'Biotargets' for future therapies. On the economic side, HOMAGE aimed to act as an economic catalyst for European SMEs in the field of cardiovascular and ageing BMs, estimated to peak annual turnovers of up to 800 M€.

1.2 Summary description of project context and objectives

The concept of the HOMAGE project is that, in **older people**, **'omics based biomarkers** (BMs) can detect **asymptomatic pathological processes** that predict who will develop of **Heart Failure** (HF) and other common serious cardiovascular (CV) conditions and characterize **distinct phenotype(s**) more likely to respond to targeted preventive therapy that could efficiently promote **active healthy ageing**.

Heart Failure is common in older people (about one in every five older people will develop it) and unlike other cardiovascular problems its prevalence is increasing. In the European Union, the average age of patients with HF is 76 years and it affects >5% of people over 65 years and ~20% of octogenarians. HF is one of the most common medical reasons for hospital admission, (up to 5% of emergency admissions amongst adults), is associated with damage and dysfunction in many other 'target' organs and complicates and is complicated by many other medical problems common in older

people, such as renal dysfunction, pulmonary disease, depression, cognitive decline, cerebrovascular disease and malnutrition. Despite advances in care the prognosis remains gloomy once overt HF has developed, especially in older patients. Delaying or preventing the onset of HF, which has had some success, may be a more effective approach to prolonging active life than trying to manage established HF. However, the annual incidence of HF is modest even in older people (<3% per year). Only very safe and inexpensive treatments are appropriate for universal treatment. Detection and targeted management of at-risk patients prior to the onset of symptoms is a logical next step, but requires proof. **HF and its related co-morbidities are amongst the biggest public health problems in the group of *"age- related diseases or disorders affecting the elderly."***

The validation of effective large-scale screening tools for the identification of patients at risk of HF (predictive markers) or at an early stage of HF (diagnostic markers) is a major unmet clinical need. Echocardiography is poorly suited for large scale routine screening. It has both low sensitivity and specificity and is unable to detect early disease processes such as inflammation, myocyte injury and extracellular matrix remodeling. Plasma concentrations of natriuretic peptides are superior to echocardiography in detecting early cardiac dysfunction but provide little information on aetiology. Research is needed to identify BMs that not only stratify risk but that also identify the underlying disease process that may also be for specific preventive therapies. Recognizing the heterogeneity of HF and dissecting it into different therapeutic groups would improve the targeting of interventions, which may improve response rates and avoid adverse effects in patients unlikely to benefit (personalized medicine). BMs have been used successfully in this way for cancer but not yet in HF. Several 'omics based BMs show promise for early HF identification and risk stratification, mainly from transcriptomic, miRNAomic, proteomic and metabolomic studies. Genome wide association studies in HF have borne little fruit as yet [11]. Using a systematic search of relevant databases advice from European (as well as some US) experts in this consortium, we have selected candidate **'omics** based BMs relevant to HF and its main co-morbidities in elderly people. These BMs have passed the discovery and verification phase. They have been published and/or patented by Small and Medium Enterprises (SME)s and/or academic groups (many of which are partners of our consortium) and they are readily measurable by available techniques. Although we make provision for testing a number of emerging BMs (hypothesis generation), we have deliberately focused on BMs involved in extracellular matrix (ECM) remodeling, inflammation, ageing and myocyte injury, which are all potentially major mechanisms of progression to HF that may be targeted by specific therapies, such as renin angiotensin system inhibitors and more specifically by mineralocorticoid receptor antagonists (MRAs) [12-14] (hypothesis-driven approach).

The **objectives** of the HOMAGE project are as follows

- To identify **'omics based** BMs that reflect specific pathological pathways (early diagnosis) leading to HF and other serious CV conditions that are also potential targets for therapy (stratification for personalised medicine)
- To **validate the predictive value of these BMs for the development of HF and commonly associated co-morbid conditions**
- **To demonstrate the feasibility of an** 'omics **BM-based approach to select patients for whom treatment will prevent or delay the onset of HF.**

Beyond the minimum requirement of the call ***("BMs should be potentially usable indicators for at least one of the following: prediction, diagnosis, prognosis, response to therapy")*** and in order to achieve meaningful progress that may benefit patients, we propose to validate BMs that can stratify patients by pathological disease activity in the pre-symptomatic and thus act as a predictor of the

onset of symptoms, an early diagnostic marker, a method of predicting outcome and a means of predicting the response to therapy and potentially avoiding unnecessary side effects. This last objective is especially important in frail elderly patients who are prone to adverse events, but in whom effective strategies to predict and reduce such events have not been realized.

1.3 Main S&T results

A- COHORTS STUDIES

A large clinical database, based on (1) population studies, (2) cross-sectional and prospective studies of patients with overt disease or at risk or (3) patients enrolled in randomized clinical trials, have been constructed to validate the association of 'omics'-based biomarkers with the risk of developing heart failure and co-morbidities. Eligible studies for inclusion received ethical approval, have baseline information on cardiovascular risk factors available and if the subsequent follow-up includes fatal and nonfatal outcomes, including heart failure. Population studies, patient cohorts and randomized controlled trials are eligible for inclusion in the common database. Currently, the HOMAGE database includes 43133 subjects, from 20 studies in 8 European countries. Data from healthy subjects were obtained from 3 population studies in France, Belgium and Italy (n=7124). The database also consists of patients with heart failure (n=3690), from 3 cohorts in UK and Spain. Eight cohorts in patients with cardiovascular risk factors (n=5829) from France, Austria, the Netherlands and Italy were also included. Finally, data from 6 randomized controlled trials (n=26490) in heart failure patients, hypertensive patients and patients with high cardiovascular risk, from Austria, Ireland, the Netherlands, and UK, were included in the HOMAGE database. At this moment, follow-up data are available in 12 of the 20 studies (n=38144).Overall partners have collected and supplied information on >45,000 patients regrouped within population cohorts (n=3; 7,134 individuals), Heart failure patients cohorts (n=3; 3,690 individuals), at CV-risk patient cohorts (n=8; 5,829 patients) and RCTs (n=6; 26,490 patients) with up to 10 years follow-up.

A common HOMAGE data dictionary has been established. A SOP was developed on guidelines for common database management and analysis. It proposes a definition of events within the cohorts contributed to HOMAGE. A detailed description of the database and corresponding biosamples is available. The database is extensively described in the design paper. The definition of new onset heart failure ("Cases") and of control patients with no new onset heart failure ("Controls"), was defined.
Two definitions were chosen by the consortium to select the "new onset HF" cases and corresponding controls in retrospective studies:
- "**hard definition of hospitalization for heart failure**" that lead to the selection of 982 cases and 1969 controls. The selection of cases and controls on the hardest point definition is completed.
- "**echocardiography based HF definition**". The case and control selection is ongoing within the FLEMENGHO and STANISLAS cohorts

The first step in selecting new onset HF cases and controls was to define the cohorts with follow-up information on morbidity and mortalityallowing the use of the "hard definition of hospitalization for HF". HF patient cohorts were not eligible, since the goal was to base case/control selection on incident heart failure. We identified 9 cohorts with follow-up and information on HF events. Four cohorts had less than 20 cases of incident HF and therefore were not considered. One cohort included patients with suspected heart failure at baseline and since more than two third of these patients had a history of heart failure or were diagnosed with HF at baseline, this cohort was not

included (HULL lifelab cohort). The remaining 4 cohorts were: ASCOT, Health ABC, PREDICTOR and PROSPER.

Based on the second echocardiographic based definition of HF, persons with diastolic dysfunction (=cases) have also been pre-selected.

Eligible cohorts, with a baseline examination and a baseline blood sample and with extensive information on follow-up echocardiography include FLEMENGHO and STANISLAS (cases and control under selection and to be adjusted depending on the biosamples availability that is currently being examined).
A table summarizing the clinical data and biosamples was prepared to give clear overview of the sample availability (for biomarker analysis in WP5). The volume and quality (serum, plasma, urine, freeze/thaw cycles, storage conditions) of the samples were referenced within a table.

A-1 Biobanking

In summary, we have collected over 35.000 samples in our central Biobank in Maastricht from existing and ongoing cohorts from partners in both Europe and United States, and samples from the ongoing HOMAGE prospective trial. Finally, we have aliquoted and shipped over 13.000 samples to partners within the consortium.

A-2 Biomarkers analysis

The initial proposed list of target biomarkers has been discussed by consortium members at consortium meetings and via telephone conferences. The volume of samples in the integrated HOMAGE cohort was insufficient to complete analysis of all targets and there were insufficient PBMC samples for analysis of transcriptomic targets. The initial list of biomarkers has been refined to produce a priority list consisting of transcriptomic, miRNA, proteomic and metabolomics targets.
Alternative strategies for investigation of transcriptomic targets have been performed due to the lack of available PBMC samples within existing cohort samples. Initial investigations using the IBLOMAVED cohort have been performed and analysis of mRNA from isolated white blood cells completed. ACS Biomarker and MHH have collaborated to share microRNA samples and reduce the volume of samples required. ACS also assessed the optimal miRNA detection method. There is currently no global consensus on the best way to isolate and detect circulating microRNAs and we believe that HOMAGE provided the perfect platform to initiate the development of such a gold standard. By doing this we will facilitate the process of implementing microRNAs to a clinical platform. The feasibility of combining serum/plasma biomarkers in a single multiplexed assay has been investigated as an alternative to measuring biomarkers with existing ELISA.

A-3 Proteomics

ELISA validation for proteomic targets Ser208 phosphorylated troponin T was tested. Initially Western blot screening identified antibodies specific for phosphorylated troponin T but these antibodies failed to bind native phosphorylated epitope conformation in ELISA. Consequently additional strategies were employed to resolve this issue by: 1) generating of monoclonal antibodies by genetic immunization; 2) screening hybridoma by ELISA to identify specific clones that detect phosphomimetic troponin T using Ser208(TnT-Glu208).positive control and mutated troponin T (TnT-Ala208), negative control; 3) selecting hybridoma secreting IgG. Furthermore, 600 hybridoma for binding to the target epitope TnT Ser208 were tested and 4 hybridoma secreting IgG-specific cell lines have been selected and confirmed following amplification. 4 hybridoma are being cultured to produce 10 mg of monoclonal antibodies ; this enabled the development of an ELISA. The biomarker

CD146 was validated as a biomarker of congestion. CD146 was identified through a proteomic assay and was included in HOMAGE as a potential biomarker of interest.
From February 2015, the consortium started to adapt the work plan of WP5 aiming at an optimal strategy for proteomic analysis of serum/plasma samples that maximizes the number of proteomic biomarkers measured within a minimum bio-sample volume.
After the primordial TC in February 2015, P01a-Inserm was in charge to check out the existing multiplexing platforms and to study the feasibility according to the list of selected candidate biomarkers within HOMAGE. P01a-Inserm thus has worked on bibliographic research to list multiplexing companies. The following companies were contacted: **OLINK, MERCK-MILLIPORE, SPARTACUS BIOMED,** FIRALIS, BIO-RAD, R&D SYTEMS, MYCARTIS, SYRIUS, MESOSCALE, CBL, PARATOPES, PLUSMORE, ISOGENIA, BECKMAN COULTER

P01a-Inserm asked companies to fill a "Term of reference" document including:
- the adequacy between HOMAGE biomarker list and the biomarkers effectively measurable
- the volume required to do so and
- a quote for either consumables alone or consumable, biomarker measurement and analysis together.

Rapidly, some companies declined the possibility of developing a customized HOMAGE multiplex assay in the restricted volume available and timeline. Eventually, a few companies engaged in depth discussion and negotiation (company's name written in bold) with P01a-Inserm and Olink technology was chosen and the SME TATAA was added as a partner to perform all proteomic analysis with the Olink technology.

A-4Collagen markers

P08-FIMA identified the ratio between the CITP and matrix metalloproteinase-1 (MMP-1) as a potential biomarker of myocardial collagen cross-linking in chronic HF patients of hypertensive etiology. FIMA further showed that high sensitivity cardiac troponin T is associated with the dilatation of the left atria, left ventricular hypertrophy and asymptomatic left ventricular diastolic dysfunction in subjects presenting risk factors for the development of HF providing in combination with NT-proBNP incremental diagnostic utility over conventional risk factors. In addition, P08-FIMA found that while galectin-3 levels are increased both in the myocardium and the circulation of heart failure (HF) patients of hypertensive etiology, there is no association with myocardial fibrosis or circulating biomarkers of fibrosis in these patients requiring further investigation of its pathophysiological role. P08-FIMA also found increased levels of cystatin C in chronic HF patients with preserved ejection fraction (HFPEF) of hypertensive etiology, even in those with normal renal function, suggesting that it may play a role as HFPEF biomarker. Cystatin C levels were associated with elevated filling pressure, biomarkers of fibrosis, osteopontin and the tissue inhibitor of metalloproteinases-1 (TIMP-1). Finally, P08-FIMA showed that GLP-1 may act as an anti-oxidant agent and that lower circulating levels of the incretin GLP-1 were associated with cardiac remodelling and the incidence of cardiovascular events in patients with type II diabetes mellitus. GLP-1 determination might thus be useful to identify cardiomyopathy and benefit especially of an incretin-based therapy. In a study led by P14-KUL in collaboration with P18-MOS and P08-FIMA we evaluated diastolic left ventricular (LV) function in relation to urinary and serum collagen biomarkers in a general population (Zhang ZY et al., PLos One 2016;11:e0167582). By sequencing urinary peptides, 70 urinary collagen fragments were identified. Biomarkers of collagen synthesis and degradation were evaluated in the serum of these subjects: the carboxyterminal propeptide of procollagen type 1 (PICP) and the amino-terminal propeptide of procollagen type III (PIIINP) were measured as markers of collagen type I and III

synthesis, respectively. The carboxy-terminal telopeptide of collagen type I (CITP) and the tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) were quantified related to collagen degradation. In categorical analyses, diastolic LV dysfunction was associated with higher levels of urinary collagen I (uCI) fragments, lower levels of urinary collagen III (uCIII) degradation products, and higher levels of CITP and TIMP-1. Of interest, PICP and TIMP-1 increased in relation to uCI, whereas these serum markers decreased with uCIII. Moreover, diastolic LV dysfunction was associated with higher levels of TIMP-1. In summary, in a general population, the non-invasively assessed diastolic LV function correlated inversely with uCI and serum markers of collagen type I metabolism, but positively with uCIII. These observations generalise previous studies in patients to randomly recruited people, in whom diastolic LV function ranged from normal to subclinical impairment.
Furthermore, circulating biomarkers related to different aspects related to myocardial remodelling in patients from the ASCOT-SPIRO cohort (n=728 patients) were quantified. In particular P08-FIMA have measured the amino-terminal pro brain natriuretic peptide (NT-proBNP) as a marker of cardiac stress and high sensitivity troponin T (hs-TnT) as an early marker of cardiomyocyte damage. On the other hand P08-FIMA have evaluated several biomarkers related to cardiac fibrosis: the carboxy-terminal propeptide of procollagen type 1 (PICP) and the amino-terminal propeptide of procollagen type III (PIIINP) as markers collagen type I and III synthesis, respectively. The carboxy-terminal telopeptide of collagen type I (CITP) corrected by the matrix metalloproteinase 1 (MMP-1; CITP:MMP-1) as an index of collagen cross-linking, since increased crosslinking of CITP increases its resistance to degradation by MMP-1. P08-FIMA measured plasma PICP concentration in phase 1a (n=1211) samples from the HOMAGE study 1a.

A-5 Urinary peptidomics

By PLS-DA analysis of baseline urine peptidomic data of the FLEMENGHO cohort, P14-KUL, in close collaboration with P18-MOS, identified additional collagen fragments as urinary peptide biomarkers correlating with serum markers of collagen I synthesis (PICP) and breakdown (collagen type I carboxy-terminal telopeptide, CITP), tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1). Supporting the depiction of pathologically relevant myocardial fibrosis in urine, these new urinary peptide biomarkers also correlated with echocardiography parameters in diastolic left ventricular dysfunction (manuscript submitted). Additionally, analysis of baseline urine peptidomic profiles and follow-up clinical data again in close cooperation with P14-KUL revealed prognostic utility of HF1 for cardiovascular and cardiac outcomes including non-fatal and fatal heart failure over and beyond traditional risk factors. The baseline peptidomic data of the FLEMENGHO cohort were also (i) included in the discovery and validation of a diagnostic panel of 103 urinary proteome biomarkers specific for heart failure with reduced ejection fraction (HFrEF) by P18-MOS (manuscript submitted) and (ii) utilized for independent validation of a prognostic heart failure classifier recently established by P18-MOS based on 96 urinary peptide biomarkers to predict the progression from asymptomatic preclinical left ventricular cardiac dysfunction (systolic and diastolic) to symptomatic heart failure (manuscript in preparation).
P18-MOS already demonstrated in previous reports the robustness of the CE-MS platform and its clinical utility in the management of heart failure. Several classifiers for cardiovascular diseases have previously been developed including HF1 (comprising 85 peptides) and HF2 (comprising 671 peptides) for the diagnosis of asymptomatic diastolic left ventricular dysfunction, HFrEF103 (including 103 peptides) and HFrEF-CKD (including 107 peptides) for the diagnosis of HF with reduced ejection fraction, the prognostic classifier LVHFP (96 peptides), CAD238 (238 peptides) for the diagnosis of coronary artery diseases and the prognostic classifier for acute coronary syndromes ACSP75 (75 peptides). Additional efforts were made to validate these classifiers in the independent validation

cohort composed of 429 samples from STANISLAS (study 1a'). Samples have been analysed (in total N= 429) by CE-MS and scored with above mentioned classifiers.

A-6 Metabolomics

UG presented two techniques for metabolomics measurements, NMR and mass spectrometry. NMR technology is more suitable to the requirements within HOMAGE. Metabolomic measurements was performed, employing mass spectroscopy in several cohorts and interventional studies (GECOH Study, Styrian Hypertension Trial and EPATH Trial) e.g a metabolomic steroid hormone profile and an amino acid profile have been determined in the Styrian Hypertension Trial. The method was used to measure the arginine metabolism/pathway in the EPATH Trial. The method was also used to perform NMR metabolomic/lipidomic analysis of PROSPER samples. The trial included 5804 elderly individuals with cardiovascular risk factors. Approximately 200 study participants experienced an incident heart failure event; analysis will focus on these subjects. NMR metabolomics was performed though an established collaboration between the UG team and their collaboration partners for metabolomics in Finland. All samples have been analysed and results are available to the Glasgow Team. The association between 1H-NMR spectroscopy derived metabolite and lipoprotein measures and incident HF hospitalisation (HFH) in the elderly, and examined whether such measurements improve HFH prediction. 5,348 serum samples from **PRO**spective **S**tudy of **P**ravastin in the **E**lderly at **R**isk (PROSPER) had 136 primary measures of metabolite concentration, lipoprotein subparticle concentration (and lipid content) and extracted lipid concentration measured by 1H-NMR. 182 out of 5,348 participants were hospitalised for HF over a mean follow up period of 2.7 years. 14 1H-NMR measures were found to be significantly different in those who were later hospitalised for HF after correction for false discovery rate (Benjamini Hochberg method) at $p<0.004$. These included creatinine, phenylalanine and various HDL measures. In Cox models, 10 of these measures were associated with risk of HFH after adjustment for clinical risk factors and NT-proBNP. Of these 10, only two, *phenylalanine*, (hazard ratio (HR) 1.31 (95%CI 1.12, 1.54; p=0.001)) and *total cholesterol* in medium high-density lipoprotein particles (M-HDL-TC) (HR 0.85 (95%CI 0.73, 0.98; p=0.012) were retained in the model. Compared to a model with established risk factors and NTproBNP only, this model improved prediction, with the continuous NRI for 3 year risk being 27.5% (24.8% to 30.1%; $p<0.001$) for non-cases and 31.3% (16.5% to 46.1%; $p<0.001$) overall.

A-7 miRNA

P04-ASC and P13-MHH jointly worked on Standard Operational Protocols for all stages involved in miRNA biomarker measurements. Due to the limited amount of sample volume that is available for biomarker measurements, P04-ASC and P13-MHH establish a protocol that enabled both partners to measure their candidate markers using their own preferred detection method. Consequently a miRNA isolation/detection scheme was employed to identify which method was the most robust and also provided the highest correlation between the two groups (Annex 2). In brief: both groups isolated miRNAs from 200ul of plasma using both TRIZOL LS (Invitrogen) and the miRNeasy kit (Qiagen). RNA was shared between the two groups and subjected to the miRNA detection methods routinely used in both labs. Data analysis indicated that miRNeasy kit generated robust and comparable results, regardless of the detection method employed. Based on this trial, SOPs for miRNA isolation, detection and transport were established (included in Annex 2.2 (ACS) and Annex 2.3 (MHH)).

P01a-Inserm has asked partners focusing on miRNA analysis to reduce the requested sample volumes for miRNA measurement from 200 µl to 150 µl. In a face-to face meeting dedicated to WP5 issues in Brussels (see below), it was agreed by P04-ACS and P13-MHH that:

- 150ul of plasma were sufficient to perform miRNA measurement

- miRNA will be not measured in ASCOT study cohort as only serum is available and thus a comparison of the miRNA data with data from cohorts having only plasma samples would not be possible.
- miRNA extractions will be performed by P13-MHH and resulting material will be shared with P04-ACS.

In tight collaboration with P04-ASC, P13-MHH has nonetheless developed a reliably and reproducible assay for the measurements of miRNAs and plasma and serum. Plasma samples have been crosschecked for validation in both sides; e.g. P04-ASC has isolated RNA and shipped samples to P13-MHH for miRNA measurements and vice versa. Furthermore, a SOP has been developed by P04-ASC and P13-MHH for miRNA isolation and measurement procedures. P13-MHH has received about 500 test samples for validation of an applicable miRNA-test system together. This has been successfully established together with a SOP.

P04-ACS measured a series of miRNAs in samples that were selected for the Phase 1a measurement phase (screening). The goal of Phase 1a is the identification of promising prognostic biomarkers (BM) for heart failure from among the many candidate BMs measured in the Phase 1a assays. The miRNAs measured by P04- ACS were selected by the consortium based on their role in heart failure and fibrosis. The following miRNAs were measured: miR-126, miR-208a, miR-499, miR-1254, miR1306-5p, miR-423-5p, miR 26b-5p, miR-320a, miR-21-5p miR-22-3p. On top of these, miR-486-5p and cel-miR-39 were added.

These miRNAs are used to correct for technical efficiency (cel-miR-39) and for normalization (miR-486-5p). cDNA synthesis of isolated miRNAs was performed using the qScript technology from Quanta Biosciences. After diluting the cDNA, miRNAs were measured using quantitative qPCR using a universal reverse primer (quanta biosciences) and a miRNA specific forward primer. Roche LightCycler 480 SYBR Green I Master Mix was used as fluorescent qPCR technology. All measurements were performed in triplicate on a LightCycler 480.

After the qPCR run we analyzed and handled the data as follows:

Step 1:

A manual analysis of the melt curves was performed to ensure a single PCR product has been formed. A bad melt curve, such as showing double peaks, a Tm shift or deviations within the melt curves of the triplicate measurement will receive different coding compared to good melt curves.

Step 2:

The amplification curve data was exported from the Lightcycler.ixo file with LC480Conversion (free software from AMC Heart Failure Research Center). This step ensures that the raw data will be in a format compatible with the LinRegPCR analysis program (free software from AMC Heart Failure Research Center).

Step 3:

The amplification curves were analyzed using LinRegPCR. Data with abnormal amplification curves were removed from the data-set error codes LinReg). “LinRegPCR is a program for the analysis of quantitative RTPCR data resulting from monitoring the PCR reaction with SYBR green. The program determines a baseline fluorescence and does a baseline subtraction. Then a window-of-linearity is set and PCR efficiencies per sample are calculated. With the mean PCR efficiency per amplicon, the Cq value per sample and the fluorescence threshold set to determine the N0, the starting concentration per sample, expressed in arbitrary fluorescence units, is calculated. (See Ramakers et al. NeuroSci Lett 2003, Ruijter et al Nucleid Acids Research 2009)”. http://www.hartfaalcentrum.nl/index.php?main=files&sub=LinRegPCR In short the application of LinRegPCR has been shown to reduce the variability as well as the bias in qPCR results.

Step 4:
The Cq and N0 per sample were further analyzed with a novel recently developed algorithm, accepted for publication in RNA. In short, the algorithm combines the information from the melt curve analysis, the Cq and N0 values to distinguish artefacts from valid measurements and undetectables. This algorithm results in robust data based on triplicate measurements
Step 5:
P04-ASCapplied an overall sample quality control step in which they compared all miR signals for a single sample with special attention to the quality control spike-in Cel-miR-39. The spike-in RNA was added during RNA isolation and can be applied to monitor RNA isolation efficiencies as well as qPCR efficiency. An undetectable Cel-miR39 is indicative of a bad sample quality. Indeed several samples were identified where Cel39 was undetectable, for these samples also other miRs were undetectable or invalid. All samples with an undetectable Cel39 were removed from the data sets.
This data needs to be excluded and cannot be imputed in case Cel39 will be applied as a technical normalizer of the qPCR data.

A-8 Transcriptomics

A protocol was established and validated as follows: total RNA was extracted using Qiacube automat (Qiagen), mRNA quality was evaluated by capillary electrophoresis (Experion, Biorad), sample RNA concentration was monitored using Ribogreen (Molecular Probes) and a standard curve. RNA was retro-transcribed and the cDNA level monitored by real-time qPCR using Biomark Apparatus (Fluidigm) with profiling on 96x96 chip arrays and oligonucleotides probe validation using SYBR green technology. P01c-Inserm has extracted and analysed a set of 140 WBC samples from diabetic patients at high risk for HF. NGF, ALK, TMEM, UBN1, FBXW7, SLC43A2 and FECH gene expression were tested in these samples. P01c-Inserm observed that FECH was significantly increased in WBC from diabetic patients with systolic dysfunction. Extraction of additional WBC and test of other transcriptomics biomarkers are under way.

A-9 Bioinformatics analysis

For HFH study, complete bioinformatics analyses have been done on predictors. With the identification of 39 OLINK predictors for HFH from Statistics part (P15-LHTSM), we further classified these biomarkers based on GO Ontology, and searched for underline links among these predictors with knowledge-based network analysis, which pointed to TP53 as an important player for these predictors. We also performed Random forest classification analysis to select predictors for HFH. This supervised machine learning outcome is well in line with uni-biomarker analysis outcome, thus confirmed the top predictors. We tried to understand the prediction structure of top predictors with Classification and regression trees (CART) by Recursive partitioning. The final top 5 predictors model implies that when BNP is high, HFH is mainly driven by growth factors, heart and vascular system; while BNP is low, HF is mainly driven by apoptosis and inflammatory pathways. We tried non-supervised k-means classification to link clinical parameters with OLINK biomarkers. However, the preliminary results didn’t show promising groups.

For echo-defined HF study, we reported problems in clinical data, which now is still under check. The statistics analysis is still ongoing. Thus, we only tried to explore present data, with the similar methods as for HFH study, such as Random forest analysis, decision tree, knowledge-based network analysis, and kmeans classification, also with classical regression models. In this well-defined cohort study, biomarkers seem to be more powerful. The tops are more associated with metabolism disturbance than in HFH study.

B-MAIN RESULTS

B-1 Heart 'omics' in AGEing (HOMAGE): design, research objectives and characteristics of the common database

Heart failure is common in older people and its prevalence is increasing. The Heart 'omics' in AGEing (HOMAGE) project aims to provide a biomarker approach that will improve the early diagnosis of heart failure. A large clinical database, based on (1) prospective population studies or (2) cross-sectional, prospective studies or randomized controlled trials (RCTs) of patients at risk for or with overt cardiovascular disease will be constructed to determine most promising 'omics'-based biomarkers to identify the risk of developing heart failure and/or comorbidities. Population studies, patient cohorts and RCTs are eligible for inclusion in the common database, if they received ethical approval to obtain and share data and have baseline information on cardiovascular risk factors. Currently, the HOMAGE database includes 43,065 subjects, from 20 studies in eight European countries, including healthy subjects from three population studies in France, Belgium and Italy (n = 7,124), patients with heart failure (n = 4,312) from four cohorts in the UK, Spain and Switzerland and patients at high risk for cardiovascular disease (n = 31,629) in 13 cohorts. It is anticipated that more partners will join the consortium and enlarge the pooled data. After the initiation of HOMAGE, external collaborations were established and contributed important additional databases, such as from new clinical trials with diabetes post-acute coronary syndrome patients (EXAMINE), Obesity cohort (SOS) and US cohorts (Framingham and ARIC). A largeer merged database will be a useful resource with which to identify candidate biomarkers that play a role in the mechanism underlying the onset and progression of heart failure.

B-2 Priority Study

Analysis of the PRIORITY data indicates that this is such a different population to HOMAGE that there is virtually no overlap. The patients with CKD in PRIORITY appear to have much less advanced cardiovascular dysfunction than patients in HOMAGE.

The PRIORITY trial has now completed recruitment. As of September 2016 there were: Patients screened 2259; Screening failures 443; High risk patients 216; Low risk patients 1551. The 216 High Risk Patients entered the RCT (Spironolactone 25 mg vs placebo) and will be followed up until the end of the funding period (currently February 2018 – discussions about an application for extension are underway). All included patients will be followed up for the primary renal endpoint but also for secondary cardiovascular endpoints including development of heart failure and hospitalisation for heart failure. However, it is clear that patients even in the high-risk group of PRIORITY are at much lower cardiovascular risk compared to patients in HOMAGE. Few have an NTproBNP >125ng/L.

B-3 Risk for Incident Heart Failure: A Subject-Level Meta-Analysis From the Heart "OMics" in AGEing (HOMAGE) Study.

To address the need for personalized prevention, we conducted a subject-level meta-analysis within the framework of the Heart "OMics" in AGEing (HOMAGE) study to develop a risk prediction model for heart failure (HF) based on routinely available clinical measurements.

Three studies with elderly persons (Health Aging and Body Composition [Health ABC], *Valutazione della PREvalenza di DIsfunzione Cardiaca asinTOmatica e di scompenso cardiaco* [PREDICTOR], and Prospective Study of Pravastatin in the Elderly at Risk [PROSPER]) were included to develop the HF risk function, while a fourth study (Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]) was used as a validation cohort. Time-to-event analysis was conducted using the Cox proportional hazard model. Incident HF was defined as HF hospitalization. The Cox regression model was evaluated for

its discriminatory performance (area under the receiver operating characteristic curve) and calibration (Grønnesby-Borgan χ^2 statistic). During a follow-up of 3.5 years, 470 of 10 236 elderly persons (mean age, 74.5 years; 51.3% women) developed HF. Higher age, BMI, systolic blood pressure, heart rate, serum creatinine, smoking, diabetes mellitus, history of coronary artery disease, and use of antihypertensive medication were associated with increased HF risk. The area under the receiver operating characteristic curve of the model was 0.71, with a good calibration (χ^2 7.9, *P*=0.54). A web-based calculator was developed to allow easy calculations of the HF risk.

Simple measurements allow reliable estimation of the short-term HF risk in populations and patients. The risk model may aid in risk stratification and future HF prevention strategies.

B-3 Potential spironolactone effects on collagen metabolism biomarkers in patients with uncontrolled blood pressure.

An increase in myocardial collagen content may contribute to the development of heart failure; this might be inhibited or reversed by mineralocorticoid receptor antagonists (MRAs). We investigated changes in serum concentrations of the collagen synthesis biomarkers N-terminal propeptide of procollagen type III (PIIINP) (primary outcome) and C-terminal propeptide of procollagen type I (PICP) (secondary outcome) after non-randomised initiation of spironolactone as add-on therapy among patients with resistant hypertension enrolled in the 'Anglo-Scandinavian Cardiac Outcomes' trial (ASCOT).

An age/sex matching plus propensity-scored logistic regression model incorporating variables related to the outcome and spironolactone treatment was created to compare patients treated with spironolactone for a 9-month period versus matched controls. A within-person analysis comparing changes in serum biomarker concentrations in the 9 months before versus after spironolactone treatment was also performed.

Patients included in the between-person analysis (n=146) were well matched: the mean age was 63±7 years and 11% were woman. Serum concentrations of PIIINP and PICP rose in 'controls' and fell during spironolactone treatment (adjusted means +0.52 (-0.05 to 1.09) vs -0.41 (-0.97 to 0.16) ng/mL, p=0.031 for PIIINP and +4.54(-1.77 to 10.9) vs -6.36 (-12.5 to -0.21) ng/mL, p=0.023 for PICP). For the within-person analysis (n=173), spironolactone treatment was also associated with a reduction in PICP (beta estimate=-11.82(-17.53 to -6.10) ng/mL, p<0.001) but not in PIIINP levels.

Treatment with spironolactone was associated with a reduction in serum biomarkers of collagen synthesis independently of blood pressure in patients with hypertension, suggesting that spironolactone might exert favorable effects on myocardial collagen synthesis and fibrosis. Whether this effect might contribute to slowing the progression to heart failure is worth investigating.

B-4 Proteomic Bioprofiles and Mechanistic Pathways of Progression to Heart Failure: the HOMAGE (Heart OMics in AGEing) study

Background: Identifying the mechanistic pathways potentially associated with incident HF may provide a basis for novel preventive strategies.
Aims: To identify proteomic biomarkers and the potential underlying mechanistic pathways that may be associated with incident HF defined as first hospitalization for HF.
Methods: A nested-matched case-control design was used with cases (incident HF) and controls (without HF) selected from 3 cohorts (>20,000 individuals). Controls were matched on cohort, follow-up time, age, and sex. Two independent sample sets (a “discovery” set, with 300 cases and 600 controls and a “replication” set with 315 cases and 315 controls) were used to discover and replicate the findings. 252 circulating proteins in the plasma were studied.

Results: 38 proteins and 4 main underlying network clusters underlying incident HF were independently identified in both sets: 1) inflammation and apoptosis, indicated by the expression of the TNF-family members; 2) extracellular matrix remodelling, angiogenesis and growth, indicated by the expression of proteins associated with collagen metabolism, endothelial function and vascular homeostasis; 3) blood pressure regulation, indicated by the expression of natriuretic peptides and proteins related to the renin angiotensin aldosterone system; and 4) metabolism, associated with cholesterol and atherosclerosis.
Conclusion: Clusters of biomarkers associated with mechanistic pathways leading to HF were identified linking inflammation, apoptosis, vascular function, matrix remodelling, blood pressure control and metabolism. These findings provide important insight on the pathophysiological mechanisms leading to HF.

B-5 Identification of biomarkers and mechanistic pathways associated with diastolic dysfunction-Insights from the HOMAGE consortium

Background Diastolic dysfunction (DD) is a major contributor to heart failure (HF) and affects 26 million people worldwide. The ageing of the population and increased BMI is increasing the prevalence of diastolic dysfunction, considered as a first stage of heart failure. Current treatments target symptoms but do not treat DD. The HOMAGE consortium aimed at identifying biomarkers associated with DD to identify underlying mechanistic pathways.
Methods and results From the HOMAGE database, the Flemengho and Stanislas cohorts were selected. 305 cases of prevalent diastolic dysfunction (88 Flemengho, 217 Stanislas) were matched 1:1 on cohort, age and sex. DD was defined by (e' septal<8 and/or e' lateral<10) and (E/A≥0.8 and/or deceleration time ≤200 and/or E/e'≥9). Blood biomarkers were measured with the Olink technology.
The cases and control were clinically comparable and echocardiographic parameters were different only for DD markers. After adjustment for clinical factors 34 biomarkers were associated with DD. Several analysis showed that galectin 4 (gal-4), carbonic anhydrase 5a (Ca5a), interleukin 4 receptor α (IL4Ra) and CD6 were consistently identified as strong markers of DD. Bioinformatics analysis showed that inflammation, protein glycosylation and matrix pathways were the most enriched.
Conclusions We identify 34 plasma biomarkers strongly associated with DD. In those early stages, BNP and NTproBNP were not associated with DD and thus, reinforce for the need of new biomarkers to detect the disease early. Our findings are consistent with the complexity of DD and our study opens new mechanistic target pathways to understand DD and identify new potential treatments.

B-6 Renal function estimation and Cockcroft–Gault formulas for predicting cardiovascular mortality in populationbased, cardiovascular risk, heart failure and post-myocardial infarction cohorts: The Heart 'OMics' in AGEing (HOMAGE) and the high-risk myocardial infarction database initiatives

Renal impairment is a major risk factor for mortality in various populations. Three formulas are frequently used to assess both glomerular filtration rate (eGFR) or creatinine clearance (CrCl) and mortality prediction: body surface area adjusted-Cockcroft–Gault (CG-BSA), Modification of Diet in Renal Disease Study (MDRD4), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The CKD-EPI is the most accurate eGFR estimator as compared to a "gold-standard"; however, which of the latter is the best formula to assess prognosis remains to be clarified. This study aimed to compare the prognostic value of these formulas in predicting the risk of cardiovascular mortality (CVM) in population-based, cardiovascular risk, heart failure (HF) and post-myocardial infarction (MI) cohorts.
Two previously published cohorts of pooled patient data derived from the partners involved in the HOMAGE-consortium and from four clinical trials – CAPRICORN, EPHESUS, OPTIMAAL and

VALIANT – the high risk MI initiative, were used. A total of 54,111 patients were included in the present analysis: 2644 from population based cohorts; 20,895 from cardiovascular risk cohorts; 1801 from heart failure cohorts; and 28,771 from postmyocardial infarction cohorts. Participants were patients enrolled in the respective cohorts and trials. The primary outcome was CVM.
All formulas were strongly and independently associated with CVM. Lower eGFR/CrCl was associated with increasing CVM rates for values below 60 mL/min/m2. Categorical renal function stages diverged in a more pronounced manner with the CG-BSA formula in all populations (higher $\chi 2$ values), with lower stages showing stronger associations. The discriminative improvement driven by the CG-BSA formula was superior to that of MDRD4 and CKD-EPI, but remained low overall (increase in C-index ranging from 0.5 to 2%) while not statistically significant in population-based cohorts. The integrated discrimination improvement and net reclassification improvement were higher ($P < 0.05$) for the CG-BSA formula compared to MDRD4 and CKD-EPI in CV risk, HF and post-MI cohorts, but not in population-based cohorts. The CKD-EPI formula was superior overall to MDRD4. The CG-BSA formula was slightly more accurate in predicting CVM in CV risk, HF, and post-MI cohorts (but not in population-based cohorts). However, the CG-BSA discriminative improvement was globally low compared to MDRD4 and especially CKD-EPI, the latter offering the best compromise between renal function estimation and CVM prediction.

C- ANCILLARY STUDIES

As HOMAGE was getting presented by the partners, we gained interest by several groups to collaborate and extend the efforts of HOMAGE. These collaborations, performed within HOMAGE, presented below, are promising and will allow to complete our understanding of the disease and study population with different phenotype and co-morbidities:

(1) HERMES: Heart Failure Molecular Epidemiology for therapeutic targets, is an international collaboration to investigate the genetic architecture of heart failure. The HERMES consortium comprises 42 population-based studies and clinical trials across Europe and North America and includes over 30,000 heart failure cases and over 200,000 controls. With HERMES we looked at the correlation between genetic mutations and biomarkers association (link gene-protein). A paper entitled “Genome-wide association study provides new insights into the genetic architecture and pathogenesis of heart failure” with significant contribution from HOMAGE is now submitted to Nature Genetics. The results cannot be made public before publication

(2) Framingham and ARIC cohorts: 2 large American cohorts to externally replicate findings in HOMAGE but also performed meta-analysis and discovery for new proteins. The preliminary results are very promising with a significant number of HOMAGE biomarkers replicated in these cohorts. Final results are ongoing further statistical analyses. Additional analyses will examine whether there might be specific signatures of progression to heart failure in i) ethnic subgroups, ii) patients with diabetes, and iii) Obese patients. Finally, given that echocardiography data are available in these cohorts, analyses are underway examining i) signatures associated with diastolic dysfunction and ii) whether there are distinct proteomic bioprofiles of progression to HF with reduced ejection fraction, as compared to progression to HF with preserved ejection fraction

(3) SOS study: The Swedish Obese Subjects (SOS) study is the first long-term, prospective, controlled trial to provide information on the effects of bariatric surgery on the incidence of objective endpoints (diabetes, cardiovascular disease events, cancer and overall mortality). HOMAGE have gained access to examine the proteomic profiles of progression to HF related to obesity,in this specific important risk population.

(4) EXAMINE: Cardiovascular Outcomes Study of Alogliptin in Patients with Type 2 Diabetes and Acute Coronary Syndrome; NCT00968708: is a trial database of 5,380 patients with diabetes post-acute coronary syndrome, at risk of developing HF. In 5,066 patients we performed a CVDII Olink® panel measurement (n=92 BM) + Troponin I. Early results show that there are different associations of specific biomarkers with respective cardiovascular events. Signatures associated with the risk of HF are partially different from signatures associated with cononary events or stroke events. In addition results suggest that mechanistic pathways of progression to HF in patients with diabetes might be distinct from those underlying progression to HF in patients with other risk factors than diabetes. Sophisticated network analyses are ongoing. And comparison to results across the other cohorts available is underway (BIOSTAT, HOMAGE, FRAMINGHAM, ARIC, SOS), examining especially subgroups of interest e.g. obesity, CKD, etc.

D- MAIN RESULTS OF THE CLINICAL TRIAL: The Effect of Spironolactone on Cardiac, Vascular and Renal Function and Markers of Fibrosis in Patients at High-Risk of Developing Heart Failure: The Heart "OMics" in AGEing (HOMAGE) Randomised Trial

Despite advances in care, prognosis remains poor once overt heart failure has developed. Prevention of heart failure might be an effective strategy to increase longevity, increase quality-adjusted and reduce disability-adjusted life-years.

Prevention is most efficient when directed at patients who are both at risk and likely to respond to the intervention in question. An increase in myocardial and possibly vascular collagen content (fibrosis) may be an important determinant of disease progression from cardiac dysfunction to heart failure. In patients with hypertension and diabetes, two important risk-factors for heart failure, increases in blood markers of collagen turnover, which are thought to reflect fibrosis, occur before clinically overt heart failure develops. These markers are also related to prognosis after heart failure develops.

In the general population, plasma concentrations of Galectin-3 (Gal-3), a potential marker of activation of pro-fibrotic pathways, is associated with cardiovascular (CV) risk factors, and predicts development of heart failure. In animal models, Gal-3 is a key mediator of aldosterone-induced cardiovascular and renal fibrosis and dysfunction.

We hypothesised that the mineralocorticoid receptor antagonist (MRA), spironolactone, would favourably influence extracellular matrix remodelling, especially in patients with active fibrogenesis, identified by high plasma concentrations of Gal-3.

The main objective of the HOMAGE trial was to investigate whether spironolactone could 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. However, MRA might also have other favourable effects on cardiovascular and renal function that could delay or prevent the onset of heart failure including an increase in sodium and reduction in potassium excretion that are, in turn, associated with a reduction in extra-cellular and increase in intra-cellular water. These fluid shifts may reduce intra-cardiac volumes and blood pressure. MRA may also reduce sympathetic activity. All of these factors may combine to delay the progression from cardiac

dysfunction to heart failure. The HOMAGE randomised trial was also designed to assess the other potential mechanisms for prevention of heart failure.

Trial Design and Oversight

HOMAGE was a prospective, randomised, open-label, blinded-endpoint (PROBE), multicenter trial comparing the addition of spironolactone titrated to a maximum dose of 50mg/day to a control group that received no additional therapy using . The rationale and trial design have been published (Jacobs L et al 2014) and the protocol and statistical analysis plan are available at https://clinicaltrials.gov/ct2/show/NCT02556450. The study was approved by all relevant ethics committees and regulatory bodies. All patients provided written informed consent prior to study-specific procedures. The Executive Committee developed and amended the protocol, oversaw the conduct of the trial, and interpreted the results. A Clinical Endpoints Committee adjudicated all hospitalisations and deaths blind to treatment assigned. An independent Data Monitoring Committee regularly reviewed safety data.

Study Population

Patients of either sex, aged 65 Years and older who were either known to have or were at high risk of coronary artery disease were screened for inclusion. Patients who had a plasma concentration of amino-terminal pro-B-type natriuretic peptide (NT-proBNP) in the 'window' between 125 and 1,000ng/L (BNP 35-280ng/L), measured in local-site laboratories, were eligible for randomisation provided none of the exclusion criteria were met. This natriuretic peptide 'window' was chosen in order not only to exclude patients without substantial cardiac dysfunction who would have little to gain from treatment but also patients with advanced disease requiring further investigation and in whom withholding additional treatment might not be ethically possible. The main exclusion criteria were absence of sinus rhythm, a glomerular filtration rate less than 30mL/minute/1.73m^2, a left ventricular ejection fraction less than 45%, a serum potassium of greater than 5.0mmol/L, a diagnosis of heart failure or receiving treatment with loop diuretics. Background therapy could include any conventional treatment for coronary artery disease or hypertension other than loop diuretics or potassium saving diuretics, including mineralo-corticoid antagonists. Angiotensin converting enzyme inhibitors or angiotensin receptor blocker inhibitors (but not their combination), beta blockers and thiazide or thiazide-like diuretics were permitted as were treatments for concomitant conditions, such as diabetes mellitus.

Procedures

Patients participating in the randomised trial had baseline information collected ncluding demographic details, medical history and medications, symptom and quality of life questionnaires, height, weight and vital signs including lying and standing blood pressure. A 12-lead electrocardiogram, transthoracic echocardiogram and shuttle walk-test were done. Echocardiographic recordings were sent to a core echocardiographic laboratory (Nancy) for analysis. Blood and urine samples were taken for both local, including standard haematology and biochemistry profiles, and core-laboratory tests, including markers of collagen metabolism. Blood samples for core-laboratory assessments were taken after a period of supine rest after a 12-lead electrocardiogram had been recorded.

Randomisation and Blinding

Patients were randomised in a 1:1 ratio to Spironolactone or control using random permuted blocks stratified by site. Randomisation lists for each site were created by the Study Coordinating Center

(Leuven) using statistical software (SAS 9.3) and aweb-based management system. All persons evaluating key tests, the clinical endpoints committee, and those conducting laboratory biomarkers tests were kept blind to the treatment allocation.

Randomised Interventions

Spironolactone was initiated at 25 mg/day with the intention to titrate to 50mg/day but with the possibility of down-titration to 25mg every other day. The dose of spironolactone could be decreased, stopped or reinitiated according to a pre-specified algorithm driven by changes in serum potassium and renal function. The control group received no additional treatments.

Follow-up

After randomisation, patients had follow-up visits after one week and after one, two, three, six and nine months to assess serum potassium, renal function and blood pressure. At one month and the end of study visit, patients had the same assessments done as at baseline. Originally, the end of study visit was planned to occur at nine months. Due to slower than planned recruitment, it was decided to extend the period of enrolment but due to the fixed duration of grant funding, this meant that the end of study visit occurred between 3 and 9 months for some patients. In order to try and increase the rate of recruitment some assessments were made optional including assessments of resting pulse-wave velocity and cardiorespiratory monitoring during the shuttle walk-test and, at the one month visit, only safety assessments were mandatory.

Primary Aim and Endpoint

The primary aim was to investigate whether spironolactone alters extra-cellular matrix remodelling, assessed by measuring a by-product of the synthesis of Type-III collagen, Procollagen Type III N-Terminal Peptide (PIIINP), in patients at increased risk of developing heart failure and whether this effect is greater in those with increased plasma concentrations of Galectin-3.

The primary outcome measure was change in serum concentrations of PIIINP from baseline to nine months, measured using a radio-immunoassay in a core laboratory. The primary endpoint was the interaction between such changes and plasma concentrations of Galectin-3 measured at baseline.

Secondary Aims and Endpoints

Secondary aims were to investigate whether spironolactone induced changes between baseline and one month and baseline and end of trial in:-

1. Serum or plasma concentrations of other biomarkers of extracellular matrix turnover including carboxy-terminal propeptide procollagen type I (PICP), a marker of increased synthesis and carboxy-terminal type-I telopeptide (CITP) and Galectin-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 assessed by pulse-wave analysis (BPLab* - Germany) before and after nitrates (patients may opt out of nitrates)
4. Exercise capacity using a shuttle walk test if data on this test are available whilst wearing either
5. The clinical composite of incident heart failure or atrial fibrillation, non-fatal myocardial infarction, stroke or CV death.
6. The incidence greater than 20% decline in eGFR
7. Incident hyperkalaemia (serum potassium >5.5mmol/L) or hypokalaemia (serum potassium <3.5mmol/L)

8. The incidence of gynaecomastia and/or breast pain
9. Hypotension, falls and fractures

Statistical Analysis

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 proposed sample-size provided sufficient statistical power for exploratory analyses of the main secondary endpoints listed above. For example, the trial had 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). Further gain in statistical power was anticipated by treating Galectin-3 as a continuous variable and using methods for repeated measurements.

Statistical analyses were carried out using Stata ® version 15.1. The primary efficacy analyses was done using the intention to treat principle on the full analysis set, comprising all randomized patients who received at least one dose of trial medication. The safety set comprised all patients that received at least one dose of the trial medication and had at least one post-baseline safety assessment and was analysed according to the treatment received. The per-protocol set comprised all randomised patients without any major protocol violation, defined prior to un-blinding.

The characteristics of patients who were screened, but who were not included in the trial (either refused or ineligible) were summarised along with reasons for refusal or ineligibility. Baseline characteristics by randomised group were summarised for categorical variables using frequencies and percentages, and for continuous variables using mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate.

The analysis of the primary end-point PIIINP (change from baseline to the final visit) used analysis of covariance (ANCOVA). A linear regression model was fitted, including a binary variable to indicate the treatment group (placebo/spironolactone), a binary variable to indicate whether Galectin-3 was above or below median and baseline PIIINP. An interaction term was included to evaluate the additional impact of spironolactone in patients with Galectin-3 above median. Residual analysis was used to examine the fit of the model to the assumptions of linear regression and data were transformed to meet the assumptions of linear regression.
Changes in PIIINP were also analysed using Galectin-3 as tertiles or as a continuous variable.

Secondary endpoints were analysed using ANCOVA for continuous endpoints or multi-variable logistic regression or multi-variable Cox regression in the case of dichotomous and time to event endpoints respectively. When data were missing, appropriate multiple imputation methods were used depending on the scale and pattern of the missing data. No adjustments were made to allow for Type-1 error for multiple comparisons in view of the exploratory nature of this mechanistic trial.

Results

Between January 2016 and June 2018, 877 patients were screened for and consented to participate in the HOMAGE trial of whom 561 were eligible for participation and 527 were randomised (**Figure 1** – Consort Diagram). The main reason for exclusion was a plasma NT-proBNP or BNP below the required range; 5% were found to have atrial fibrillation and 4% had a left ventricular ejection fraction <45%. The characteristics of screened and randomised patients were similar apart from the plasma concentration of natriuretic peptides and a greater use of aspirin amongst those randomised (71%) compared to those who were not (60%). Most patients had coronary artery disease, about 40% had a history of myocardial infarction and about 70% had undergone a coronary revascularisation procedure. Almost 80% had a history of hypertension and more than half had a systolic blood pressure >140mmHg despite most patients being on at least one anti-hypertensive agent. Almost 40% had diabetes mellitus and more than 20% had micro-albuminuria.

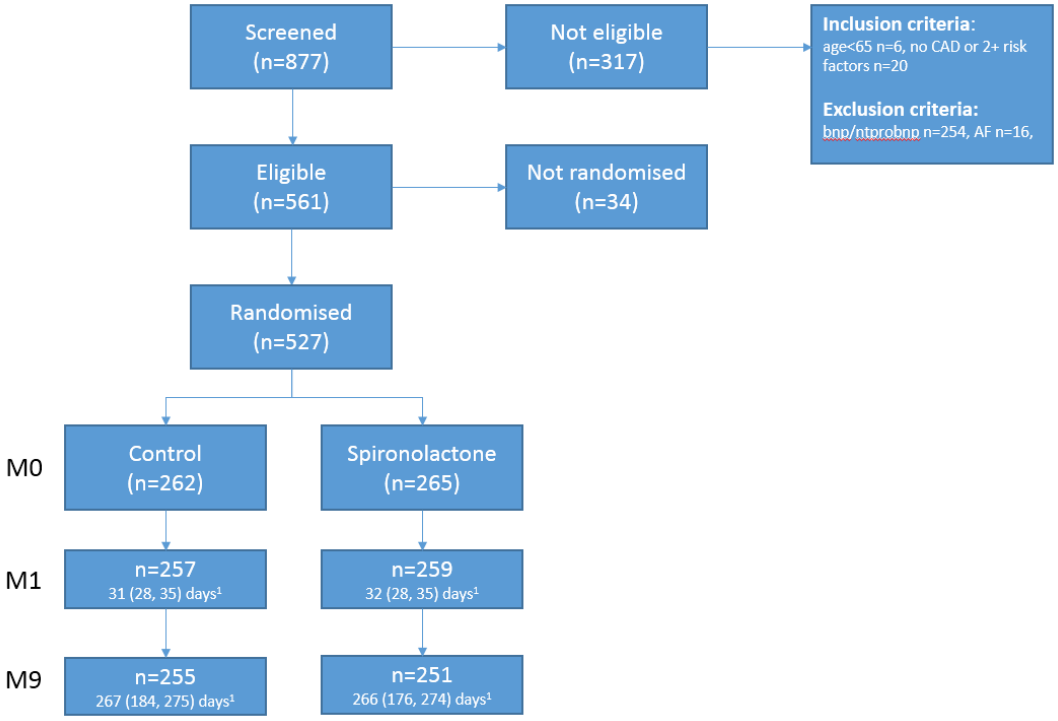


Figure 1: Consort-diagram. [1] Median (IQR) days between randomisation and visit

The median age of those randomised was 73 years and 26% were women. There were no significant differences in patient-characteristics or results of investigations for patients assigned to spironolactone or control. Median eGFR was greater than 70mL/minute/1.73m^2 and median serum potassium was 4.3mmol/L. Plasma concentrations of NT-proBNP were modestly elevated. Half of patients had a systolic blood pressure of >140mmHg despite anti-hypertensive therapy.

Patients reported, on average, moderately severe breathlessness on moderate exertion but not orthopnoea or tiredness and reported a generally good quality of life. The shuttle walk-test distance was mildly reduced compared to healthy people of similar age and sex. The rise in heart rate was modest, probably reflecting treatment with beta-blockers. There was a substantial increase in systolic blood pressure during exercise. On echocardiography, median left ventricular ejection fraction and left ventricular end-diastolic volume were normal but median left atrial volume was mildly increased. Collagen fragments and biomarkers measured, to date, were also similar between groups.

Most patients were followed for 9 months, with a minority for only 3 months (**Figure 2**)

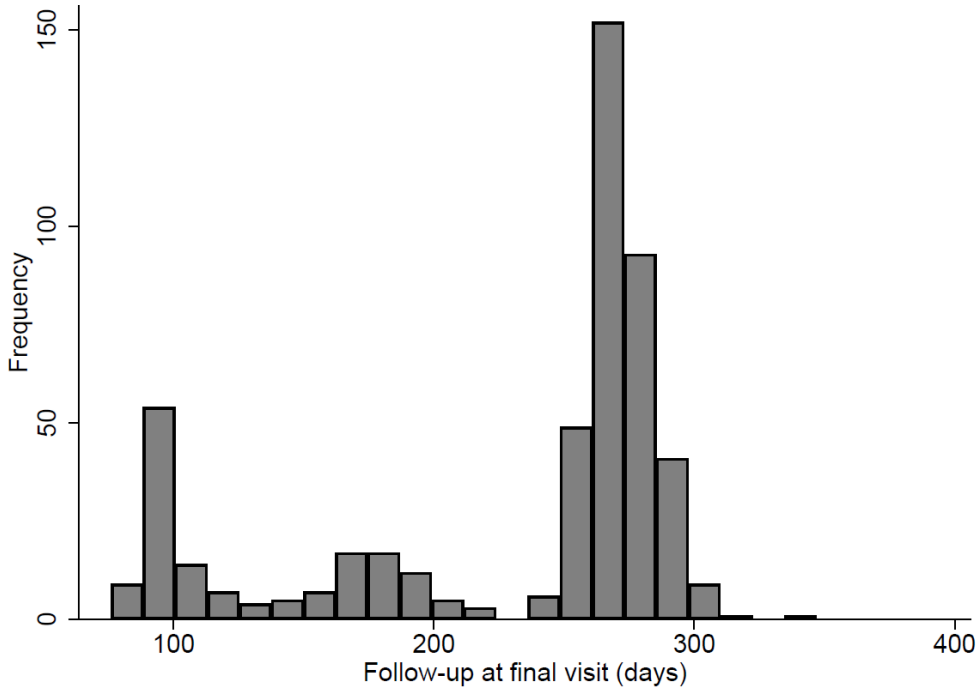


Figure 2: Distribution of Final Follow-up Visits

Comment

The HOMAGE randomised trial has successfully enrolled a substantial cohort of patients with cardiac dysfunction at high-risk of developing heart failure; the intended target population. A large proportion of patients completed all follow-up visits, 506 (96%) had an end of study visit and almost 500 have an available blood sample for analysis of the primary endpoint. It is our intention to report the outcomes of the trial at the European Society of Cardiology meeting in Paris in August 2019. The results are under embargo until that time.

1.4 Potential impact and the main dissemination activities and exploitation of results (10 pages max)

A-MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS

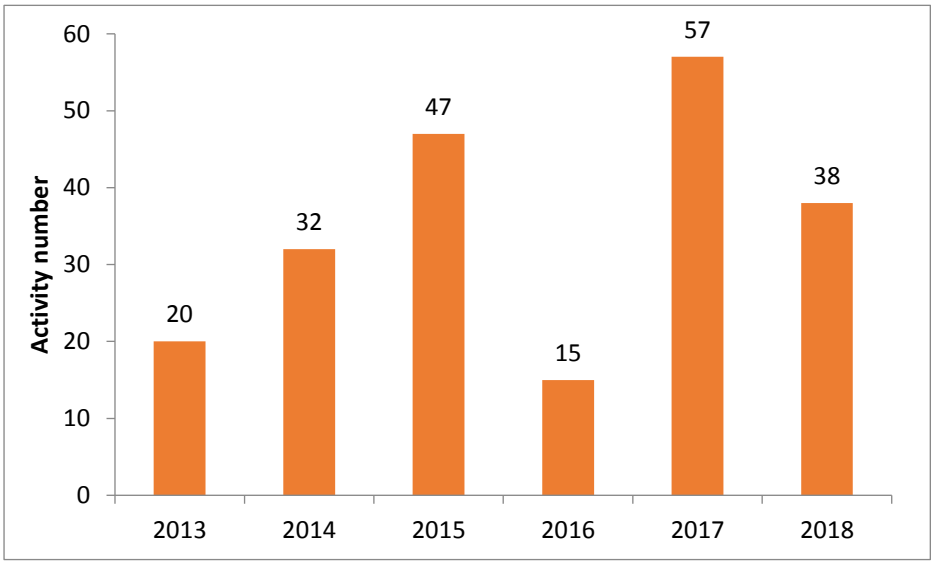
The main goal of the HOMAGE project was to provide a novel biomarker that will greatly improve the early diagnosis of heart failure, identify patients at risk of developing heart failure and characterise patient subsets with a greater likelihood of response to preventive therapies. The first step in this process was the development of the common database consisting of existing cohorts to validate the most promising biomarkers for the predication of incident heart failure in elderly patients at risk of or with actual left ventricular dysfunction. This allowed to determine a biomarker footprint for new-onset heart failure. The second step was to try to prevent the onset of heart failure in an at-risk population. The study is still under analysis.

The HOMAGE coordinator and partners have attended and/or were invited to more than 150 meetings over the 6 year consortium duration and could disseminate significantly the strategy and the main results of the consortium reached so far.

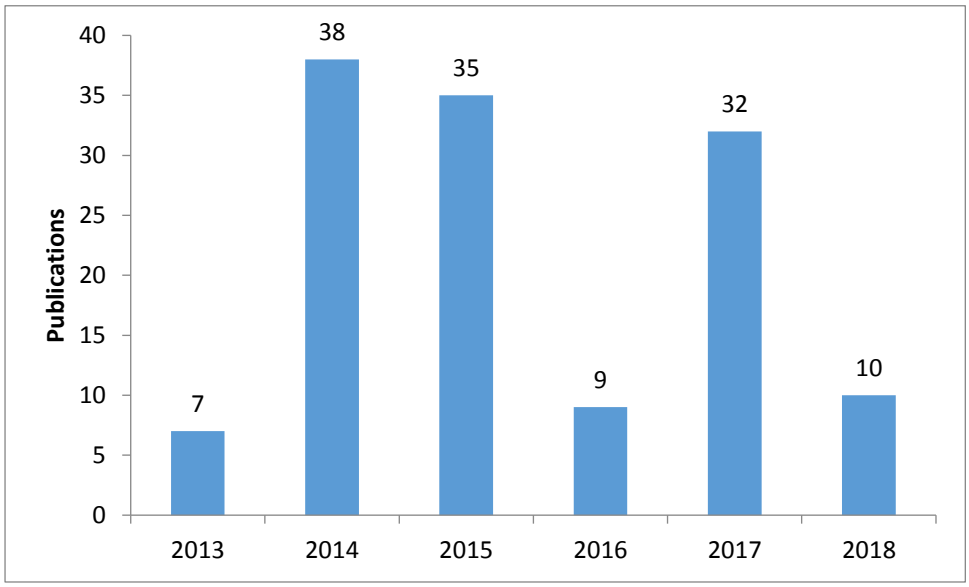
HOMAGE co-organized with INSERM annual meetings in Cannes France, on April each year, during all the duration of the consortium. These meetings, likely to continue annually, entitled “Transatlantic Heart Failure Biomarker Working Group” assemble transatlantic international experts in the field. The objective is to share HOMAGE and other results. Year on year HOMAGE has initiated and/or influenced a number of new collaborations, with potential long-lasting outcomes. This year the programme will be expanded as to have one full day meeting with the HOMAGE partners, back to back to the main “Transatlantic Heart Failure Biomarker Working Group”

HOMAGE successfully organized a 2-days public workshop on January 10 and 11th, 2019 in Barcelona, Spain. The workshop featured the main actors in heart failure biomarkers from all over the world to present their latest results and discuss the main findings of the HOMAGE project. Representative of regulatory agencies and pharmaceutical groups were invited and presents to talk about the future of the HOMAGE discoveries.

Partners in HOMAGE took opportunities to present and communicate around HOMAGE. They went to International and European meetings and presented HOMAGE related results and activities in more than 172 occasions (109 European, 62 worldwide) with 20 press coverage, 129 oral presentations and 23 posters (graph below).



The work in HOMAGE led to more than 132 publications in 47 journals with a high representation in hypertension related journals. Despite these high numbers, the impact of HOMAGE is yet to come. The major results have not been exploited yet and will led to important publication in 2019-2020. HOMAGE trial baseline caracteritics will be presented for the first time at the ESC (European Society of Cardiology) meeting in August 2019 and first results will be submitted for presentation at the AHA (American heart Association) annual meeting 2019.



Hypertension (24)	BMC medicine (2)	American journal of cardiology	European journal of preventive cardiology
Plos one (11)	BMC medical genetics (2)	American journal of epidemiology	FASEB
Journal of Hypertension (8)	Artery Research (2)	American journal of medical sciences	Free radical biology and medicine
European journal of heart failure (7)	JACC heart failure (2)	Biochimica et biophysica acta-molecular basis of disease	JASN
Journal of the AHA (6)	Kidney international reports (2)	BMC genetics	Journal of cardiovascular translational research
Nephrology dialysis transplantation (5)	Cardiovascular research (2)	BMJ open	Kidney international
American journal of hypertension (5)	Circulation heart failure (2)	Canadian journal of cardiology	Medicine science
Blood pressure (5)	Hypertension research (2)	Cardiovascular Diabetology	Metabolism clinical and experimental
European Heart Journal (4)	Scientific reports (2)	Circulation	Netherlands heart journal
JACC (4)		Clinical and experimental hypertension	Plos medicine
Journal of human hypertension (3)		Clinical Kidney journal	Proteomics-clinical applications
International journal of cardiology (3)		Clinical science	QJM-monthly journal of the association of physicians
International journal of nephrology and renovascular disease (2)		Environmental research	

B-POTENTIAL IMPACTS

Heart failure is a life-threatening syndrome with substantial morbidity and mortality and is a burden to patients, their careers, and health systems. Estimates of heart failure incidence and prevalence are difficult to generate. Accurate epidemiological estimates of heart failure, however, are crucial to

ensure resources are appropriately and adequately allocated to treat patients with existing disease, and to inform prevention methods among those at risk. It is problematic that the benefits and risks of treatment are uncertain for an already large and growing number of patients.

Successful treatment of patients with cardiovascular disease (CVD) has markedly improved through the deployment of new therapies typically evaluated in large randomized controlled trials to provide evidence-based guidance. While this approach has reduced morbidity and mortality, the total burden of CVD maintains an upward trajectory as the population ages and developing countries adopt a Western lifestyle. Further, patients who now frequently survive an acute cardiac event often subsequently require long-term treatment for chronic conditions (e.g. chronic heart failure [HF]).

Chronic heart failure specialists today are currently in transition from standard practice to personalized medicine, but this transition has been slow and inefficient reflecting insufficient knowledge and lack of confidence in modern advanced testing as well as medical inertia. This inertia is in part due to entrenched viewpoints regarding application of HF therapies deeply engrained in clinical practice guidelines.5

Development and implementation of biomarker-based personalized medicine in HF offers potential to significantly reduce clinical and financial burdens associated with HF

The impacts of HOMAGE are articulated around 3 main axes:

(1) Uncovering and understand mechanisms of heart failure progression to identify new therapeutic targets. From a clinical trial perspective, biomarkers are now a pivotal component to HF clinical trials, and use of biomarkers in trial design triples clinical trial success rate. According to a recent report by Amplion, BIO and BioMed Tracker that examined 10 000 clinical trials, the likelihood of approval of a new drug increases from 1 in 10 to 1 in 4 when trials incorporate biomarkers as an entry criterion and/or surrogate end-point (http://www.amplion.com/blog/amplion-bio-and-biomedtracker-release-new-report-on-ground-breaking-role-biomarkers-have-in-increasing-likelihood-of-approval-loa). In the study, researchers focused on biomarkers that were used to identify patients who were most likely to respond to a developmental drug, which in turn increased the chance that a therapeutic benefit will be demonstrated. Additionally, the biomarker may contribute to a better understanding of pharmacological aspects of candidate drugs, as well to improved characterization of subtypes of disease to aid selection of specific therapeutic interventions.

Increased focus on biomarker-based precision medicine is causing a paradigm shift in drug development in which companies that originally focused on developing blockbuster drugs are now embracing programs with narrower indications, shorter development timelines, and reduced costs. The cost of current phase III trials, averaging 50 000 USD per patient (http://www.pharmalive.com/clinical-trial-costs-are-rising-rapidly) is becoming unsustainable. The pressure is less excessive in HF than in other CV diseases, such as anti-thrombotic therapy in acute coronary syndromes, where the size of trials is even higher, mainly because of the lower event rates as compared with HF. Still, mechanistically-bioprofiling patients, so as to enroll those more likely to be responders is now being considered seriously in pharmaceutical research. Beyond circulating peptide biomarkers and DNA-based biomarkers, RNA-based biomarkers hold promise to better understand the pathophysiology of HF syndrome and in tailored personalized strategies. Indeed, various RNA species (either microRNAs [miRNAs] or long noncoding RNAs [lncRNAs]) can be found as cell-free RNA in the circulation. In particular, the levels of a number of circulating miRNAs have been reported to change in response to HF and are proposed to be putative biomarkers of HF; much research is yet needed to validate their real clinical value on

top of current prediction strategies. In contrast to miRNAs, the identification of lncRNAs as biomarkers of HF is still at its infancy.
Ultimately, patients will benefit because they will be less likely to be prescribed drugs that are not going to provide them any benefit, and therefore expose them only to potential adverse effects.

(2) Moving towards personalized and precision medicine by identifying specific mechanistic bioprofiles. Chronic heart failure specialists today are currently in transition from standard practice to personalized medicine, but this transition has been slow and inefficient reflecting insufficient knowledge and lack of confidence in modern advanced testing as well as medical inertia. This inertia is in part due to entrenched viewpoints regarding application of HF therapies deeply engrained in clinical practice guidelines.
Chronic heart failure doctors currently rely heavily on clinical acumen and imaging techniques (from echocardiography to the more recent MRI and PET), and only rarely request a cardiac biopsy to establish the diagnosis. Companion biomarkers, obtained from simple venipuncture, may emerge in this new era as the liquid biopsy of the heart. The experience gained in HF trials incorporating measurement of circulating biomarkers suggests such markers (e.g. natriuretic peptides) can aid in diagnosis, provide prognostic information useful for monitoring risk, and in certain contexts might be used for titration of therapy. Biomarkers may also aid the identification of apparently healthy people who are at excessive risk for developing cardiac disorders. However, major gaps in care exist between randomized clinical trials and real-world practice.
As proof of concept, Lupón *et al*. recently developed a novel HF risk calculator that included multi-markers reflective of different pathophysiological pathways in heart failure (amino-terminal pro-B type natriuretic peptide [NT-proBNP], soluble [s]ST2, and highly sensitive troponin T [hs-TnT]); the Barcelona Bio-HF calculator (BCN Bio-HF calculator; www.bcnbiohfcalculator.org) is a web-based calculator that refines risk-stratification and allows for quick and easy interactive calculations of prognosis and life expectancy at the individual patient level.
In a similar vein, a clinical and biomarker score was recently developed to predict relevant reverse remodeling (R2), the ST2-R2 score, which contains five clinical variables (i.e. non-ischemic etiology, left bundle branch block, HF duration, baseline LV ejection fraction, and beta blocker treatment) and a biomarker closely associated with LV remodeling (ST2). This score was recently shown to predict relevant R2 and was internally and externally validated. The ST2-R2 score provided proof-of-concept that R2 is a predictable phenomenon, and the addition of the interleukin receptor family member sST2 to several clinical parameters significantly improved accuracy of prediction. Additionally, patients with higher ST2-R2 scores had approximately 80% lower risk of death relative to the patients with the lowest scores. These data may be important in the management of HF patients and may influence treatment decision-making, i.e. in patients with a very high probability of significant R2 the clinician might opt for medical treatment first before planning on device implantation, while patients with low probability of R2 might be treated more aggressively.
A number of limitations of multi-marker panels should be acknowledged. These include potential multiplexing and analytical challenges in assaying multiple markers at once as well as the challenges of interpretation for the clinician due to different cut-offs for each of the separate markers. Nevertheless, it can be anticipated that scoring calculators and algorithms will increasingly use circulating biomarkers in combination with clinical variables to allow appropriate surveillance and fully informed counselling of HF patients, their families and other stakeholders in the process of patient care.

(3) Identifying prevention stratagem to prevent the development of heart failure. Novel biomarkers may also identify specific pathways involved in risk, where drugs interrupting such mediator bio-targets have not yet been explored in appropriately designed trials. In a state-of-the art paper, Braunwald postulated seven major classes of biomarkers contributing to the biomarker profile in HF. These included myocardial stretch, myocyte injury, matrix remodelling, inflammation, renal dysfunction, neurohormonal activation, and oxidative stress. A single biomarker is unlikely to reflect all the facets of the HF syndrome, and a multi-marker strategy may better characterize the complexity of HF. For example, though galectin-3 (a mediator of inflammation-induced tissue fibrosis) is a relatively weak predictor of risk in HF, specific inhibitors of galectin-3 might be of specific benefit in those with elevated concentrations. Similarly, insulin like growth factor binding protein 7 (IGFBP7) was recently linked to the presence of diastolic abnormalities in patients with both HFrEF and HFpEF; IGFBP7 is also prognostic in these patients. Given the pivotal importance of diastolic abnormalities in both HFrEF and HFpEF, it is tempting to speculate whether targeted interference of IGFBP7 might improve such abnormities with consequent improved prognosis. The promise of precision medicine is underpinned by large-scale biologic databases, omics, diverse cellular assays, and digital health technology. On this background circulating biomarkers, some of them already available and FDA/CE approved, may be at the forefront.

At the core of the concept of precision medicine is the idea one may identify a biological signal towards which therapies might be applied to specifically counteract pathophysiology. An example of this would be the use of tyrosine kinase inhibitor treatment for vulnerable tumours. This approach fundamentally differs from the generic use of biomarkers to decide on a broad range of non-specific HF therapies that do not clearly interact with the biology of such biomarkers.

In order to be used as a bio-target, a circulating biomarker must represent a mediator of disease, or be tightly linked to a mediator. For example, NT-proBNP is a marker of disease, but its closest mediator is BNP; infusion of BNP has been explored as a method of bio-targeting the risk in HF, though the benefits of this approach have been mixed, and at no time was this therapeutic approach enhanced with biomarker measurement to assist in decision-making. Enhancement of various beneficial mediators through neprilysin inhibition is another example of bio-targeting mediators of HF risk; however, once again the opportunity for more directed application of such therapies has not been explored.

To resume, HOMAGE consortium was and is able to significantly move forward the field of biomarkers in heart failure. Mechanisms leading to heart failure were uncovered, specific prevention strategies were and will be identified. The work performed in HOMAGE will yield results for years to come, will be taken in consideration for the 2020 ESC recommendation and will likely have large repercussions on heart failure prevention.

1.5 Partners involved and coordinator's contact details

Part. #	Participant organisation name	Organisation short name	Country	Principal Investigator
1A	Institut National de la Santé et de la Recherche Médicale	Inserm	FR	Faiez Zannad
1B				Alexandre Mebazza
1C				Philippe Rouet
1D				Florence Pinet
2	Inserm Transfert SA	IT	FR	C. Dascher-Nadel
3	Fondation Transplantation	EDDH	FR	Stephanie Grojean
4	ACS Biomarker BV	ACS	NL	Heico Breek
5	*Randox Clinics Limited (until 31/03/2016)*	*RTS*	*UK*	*Jim Curry*
6	*Medizinische Universitat Graz (Until 30/11/2014)*	*MUG*	*AT*	*Burkert Pieske*
7	*The University of Manchester (Until 31/04/2016)*	*UNIMAN*	*UK*	*Mamas Mamas*
8	Fundacion Para La Investigacion Medica Aplicada	FIMA	SP	Javier Diez
9	University College Dublin, National University of Ireland, Dublin	UCD	IL	Kenneth Mac Donald
10	University of Hull	HULL	UK	Andrew Clark
11	Universiteit Maastricht	UM	NL	Stephane Heymans
12	Istituto di Ricerche Farmacologiche Mario Negri	IRFMN	IT	Roberto Latini
13	Medizinische Hochschule Hannover	MHH	DE	Thomas Thum
14	Katholieke Universiteit Leuven	KU Leuven	BE	Jan Staessen
15	London School of Hygiene and Tropical Medicine	LSHTM	UK	Tim Collier
16	*Emory University Non Profit Corp (Until 30/09/2014)*	*EMO*	*USA*	*Javed Butler*
17	University of Glasgow	UG	UK	Christian Delles
18	Mosaiques Diagnostics GmbH	MOS	DE	Harald Mischak
19	The Research Foundation of State University of New York *(Until 31/01/2018)*	Stony Brook	USA	Javed Butler
20	University of Keele	KU	UK	Mamas Mamas
21	TATAA Biocenter Ab	TATAA	SW	Jens Björkman
22	Charité - Universitaetsmedizin Berlin	CHARITE	DE	Burkert Pieske

Coordinators' contact details

Prof. Faiez Zannad
Centre d'Investigation Clinique Inserm, CHU, Université de Lorraine.
Institut Lorrain du Coeur et des Vaisseaux
4, rue du Morvan
54500 VANDOEUVRE-LES-NANCY, France
f.zannad@chru-nancy.fr

1.6 Project logo and public website

HOMAGE www.homage-hf.eu