Project context and main objectives

Cardiovascular diseases are the leading cause of death in the western world. In the second half of the 20th century researchers have recognised the major risk factors that contribute to heart attacks, heart failure, stroke and other cardiovascular diseases. Among these risk factors are hypertension (high blood pressure), diabetes, smoking, hypercholesterolaemia (high cholesterol levels) and others. In clinical practice these factors can be used to estimate a patient's risk for experiencing cardiovascular events. However, precise assessment of current cardiovascular health and precise prediction of cardiovascular risk are not possible. The aim of EU-MASCARA is to find biomarkers of cardiovascular risk that could help to better assess cardiovascular risk than the currently used risk factors. EU-MASCARA focuses on a range of complex and novel markers that have been shown to provide a comprehensive view of a patient's cardiovascular status. By detecting very early stages of cardiovascular diseases that are not yet causing symptoms these markers could therefore help to assess a patient's risk for developing symptomatic disease. Many of the complex biomarkers in EU-MASCARA derive from recently developed approaches that characterise a large number of molecules in blood and urine samples. Among these are metabolomics (the study of small molecules that derive from the biochemical processes in the body) and proteomics (the study of all proteins in a given sample). EU-MASCARA also looks at other markers such as genetic markers and factors related to cardiac structure and function and inflammation.

In the second period the consortium aims were:

1. To complete the patient recruitment and standardise phenotypes across different study cohorts with the outlook of finalising the data analysis platform that will bring together different complex biomarker sets from partner laboratories for integrative analysis. The cohorts with prospective follow-up had to be identified and the Consortium had to decide on appropriate cohorts for comprehensive biomarker analysis.
2. To continue the biomarker analyses in the areas of genetic variants and microRNAs, proteomics, metabolomics, inflammation and microalbuminuria and markers of cardiac stress and collagen turnover. These analyses had to mirror the overall shift from cross-sectional to longitudinal analysis in this period of the project.
3. The data integration and a more detailed health economic analysis. Data integration, although it features throughout the project, has seen significant progress due to the large amount of multifaceted biomarkers available in this period . Health economic analyses also started already in Period 1 but had to continue now based on the biomarkers selected for Consortium wide analysis
4. The dissemination and exploitation of the project results. The initial plans devised in the 1st period had to be fine-tuned and balanced between needs of academic partners

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and SME partners who have different priorities with regard to scientific output and economic gain.

Work performed since the beginning of the project and the main results achieved so far

Period 2 was characterised by the transition from cross-sectional to longitudinal analysis of data. Pilot data from Period 1 were now available and all laboratories were able to handle the assigned sample volume and deliver high quality data. A selection of biomarkers involving laboratories in all scientific partners was made for comprehensive analysis across the consortium.
The consortium decided to perform the comprehensive analysis not on samples pulled together from different studies. The integration of different phenotypic data would have been impossible and would have reduced the power to detect meaningful biomarker profiles. Instead, the consortium followed the path that was successfully established in Period 1, i.e. to focus consortium wide activities on core cohorts and supplement these data with further analyses in other clinical cohorts. The core cohorts that were chosen for Period 2 were "FLEMENGHO" and the "Generation Scotland: Scottish Family Health Study".
By the end of Period 2 the biomarker analysis of the "FLEMENGHO" cohort was nearly complete. Samples have been analysed for genetic, proteomic, metabolomic and cardiac markers. Samples for assessment of inflammatory markers were in the process of being shipped to partner RTS by the end of this Period. The urinary proteomic data have already been analysed and defined new signatures of diastolic dysfunction that are predictive of the development of heart failure. Similarly, the cardiac stress and genetic data have been analysed and published. Metabolomic data are available and following on from the 2014 consortium meeting an analysis strategy and pipeline was established with the aim to complete this work in Period 3.
The "Generation Scotland: Scottish Family Health Study" cohort has provided an initial set of 150 samples to all partners to establish the sample access policies and to perform analysis of biomarkers that could explain the cardiovascular risk associated with a history of pre-eclampsia. Samples on the main phenotype of Period 2 (incident heart failure) have now been identified and will be distributed to consortium partners in Period 3.
A new feature of the work programme is the establishment of bilateral and trilateral collaborations between consortium partners. In the 2013 meeting in Leuven, the consortium realised that specific questions result from the overall work that can be better addressed in focussed collaborations between expert partners rather than making consortium wide attempts that would slow down the progress. These collaborations have been extremely successful and resulted in large number of published scientific output that will inform the final Period of the project.
Another new feature of Period 2 was the integrative data analysis. Whilst methods have already been developed in Period 1 it was not until Period 2 that sufficient amounts of

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data were available to perform cross-platform analysis of multifaceted biomarkers. The exemplar cohort for this task has been InGenious HyperCare where details on left ventricular mass and a comprehensive polyomic dataset were available from the work in Period 1. Strategies to combine such datasets were developed and delivered and are now ready to be applied to datasets generated in Periods 2 and 3. Similarly, there has been a much stronger focus on health economic analyses that became more concrete with the now available data from Periods 1 and 2.
The consortium had two meetings, one in Leuven (2013) and one in Milan (2014) that reshaped some of the work plans in order to take advantage of the multitude of data generated in Period 1. Publications that derived from the project are growing in number and impact and feature the collaboration between the project partners and the consortium as a whole. The partners presented data at numerous national and international conferences and are now ready to organise a symposium at the upcoming European Meeting on Hypertension and Cardiovascular Protection in Milan 2015.
With regards to exploitation, the consortium made attempts to address the specific needs of academic and SME partners. For the former, the scientific output in the form of papers and conference contributions was the top priority. For the latter the commercial exploitation and generation of IP was an equally important task. Procedures were put in place to address specifically the needs of SME partners in the final Period of the project.

Description of the expected final results and their potential impacts and use

The main aim of EU-MASCARA is to validate biomarker candidates that improve cardiovascular risk prediction. Within the project there is a focus on novel biomarkers, particularly those derived from "omics" strategies, which have already been shown to be associated with cardiovascular diseases in robust pilot studies but not yet translated into larger prospective cohorts.
The programme of work is roughly divided into two phases: a first cross-sectional phase where relevant biomarkers are tested in association studies and a second phase where the most robust markers are taken into prospective cohorts. Despite the already existing evidence on individual markers this two-pronged approach is necessary as biomarkers that derive from a multitude of different approaches have never been analysed together in a comprehensive fashion. The two phases also facilitate the development and establishment of a consortium-wide infrastructure focussing on (1) standardised clinical phenotyping and data handling; (2) technological aspects of biomarker analysis; and (3) integrative approach to data analysis. Work in Period 2 has, however, shown that the analysis of complex data has to be much more dynamic than originally anticipated. Regular feedback and interrogation of data from cross-sectional and longitudinal studies helped to focus on meaningful biomarkers that could in the future help to understand the pathophysiology of cardiovascular disease, predict individual risk and help to target existing to and develop novel therapies for those at highest risk.

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With these overall objectives in mind the Consortium will focus on a number of very specific tasks in the final Period.

1. Any outstanding data analysis within individual cohorts will be finalised. Efforts have been made to ship biosamples to EU-MASCARA laboratories and completion of the wet lab work is expected early in Period 3. This will be achieved by Consortium-wide collaboration as well as by bilateral and trilateral collaborations between Consortium partners. From these studies we expect a number of well-defined results that will inform further analyses and future strategies.
2. Cross-sectional and longitudinal data obtained from the core cohorts "FLEMENGHO" and "Generation Scotland: Scottish Family Health Study" will be integrated within and across cohorts to identify molecular signatures associated with cardiovascular risk and disease. This work will be led by the coordinating partner but will span across all participating sites who will provide expert input for interpretation of these data.
3. Health economic analyses will be conducted to inform policy makers and stakeholders of the economic and societal value of biomarker assessment in populations at different a priori cardiovascular risk.
4. Results of the project will be presented prominently at a symposium during a major cardiovascular congress in Europe and will continue to be published in relevant cardiovascular journals.
5. The specific exploitation needs of academic and SME partners will be further explored and addressed to in order to maximise intellectual, societal and economic gain as a result of this project.

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