Brussels, 13 April 2018

COST 040/18

**DECISION**

Subject: **Memorandum of Understanding for the implementation of the COST Action "Catalysing transcriptomics research in cardiovascular disease" (CardioRNA) CA17129**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Catalysing transcriptomics research in cardiovascular disease approved by the Committee of Senior Officials through written procedure on 13 April 2018.

**MEMORANDUM OF UNDERSTANDING**

For the implementation of a COST Action designated as

**COST Action CA17129**
**CATALYSING TRANSCRIPTOMICS RESEARCH IN CARDIOVASCULAR DISEASE (CardioRNA)**

The COST Member Countries and/or the COST Cooperating State, accepting the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action (the Action), referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any new document amending or replacing them:

a. "Rules for Participation in and Implementation of COST Activities" (COST 132/14 REV2);
b. "COST Action Proposal Submission, Evaluation, Selection and Approval" (COST 133/14 REV);
c. "COST Action Management, Monitoring and Final Assessment" (COST 134/14 REV2);
d. "COST International Cooperation and Specific Organisations Participation" (COST 135/14 REV).

The main aim and objective of the Action is to accelerate the understanding of transcriptomics in cardiovascular disease and further the translation of experimental data into practical applications for diagnostics and therapies. This will be achieved through the specific objectives detailed in the Technical Annex.

The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 52 million in 2017.

The MoU will enter into force once at least seven (7) COST Member Countries and/or COST Cooperating State have accepted it, and the corresponding Management Committee Members have been appointed, as described in the CSO Decision COST 134/14 REV2.

The COST Action will start from the date of the first Management Committee meeting and shall be implemented for a period of four (4) years, unless an extension is approved by the CSO following the procedure described in the CSO Decision COST 134/14 REV2.

## OVERVIEW

**Summary**

This Action aims to create an interdisciplinary network to accelerate the understanding of transcriptomics in cardiovascular disease (CVD) and further the translation of experimental data into usable applications to improve personalized medicine in this field. CVD remains the leading cause of death worldwide and, despite continuous advances, better diagnostic and prognostic tools, as well as therapy, are needed. The human transcriptome, which is the set of all RNA produced in a cell, is much more complex than previously thought and the lack of dialogue between researchers and industrials and consensus on guidelines to generate data make it harder to compare and reproduce results. Currently, there is no network to address the complexity of transcriptomics in CVD, offering an advantage to this Action. It aims to provide opportunities for collaboration between stakeholders from complementary backgrounds, allowing the functions of different RNAs and their interactions to be more rapidly deciphered in the cardiovascular context for translation into the clinic. This Action will generate grant proposals to advance understanding of the transcriptome's role in CVD and to translate findings into clinical applications, thus fostering personalized medicine and meeting a current public health challenge. CardioRNA will refine guidelines for transcriptomics investigations in CVD to increase reproducibility of results, facilitating clinical product development. It will disseminate knowledge and allow capacity-building through different types of meetings, prioritizing students and early career investigators. Thus, this Action will advance studies on cardiovascular transcriptomics, generate innovative projects and consolidate the leadership of European research groups in the field.

| **Areas of Expertise Relevant for the Action** | **Keywords** |
|---|---|
| ● Biological sciences: Transcriptomics | ● cardiovascular disease |
| ● Biological sciences: Epigenetics and gene regulation | ● transcriptomics |
| ● Medical biotechnology: Transcriptomics for medical biotechnology | ● best practices and guidelines |
| | ● translational research |
| ● Clinical medicine: Cardiovascular diseases | ● personalized medicine |

**Specific Objectives**

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

● Further the understanding of the transcriptome's role in cardiovascular disease by establishing a multidisciplinary network.
● Foster collaborative initiatives in cardiovascular disease transcriptomics.
● Develop improved guidelines for best practices and experimental standards that offer the greatest potential for cardiovascular transcriptomics studies.
● Stimulate development and optimization of RNA-based products for prognostic, diagnostic and therapy for cardiovascular disease management.

Capacity Building

● Facilitate collaborations in the subject matter by acting as a stakeholder platform.
● Facilitate the design of studies on the translation of research into products for cardiovascular disease.
● Ensure knowledge, skills and expertise exchange through training schools and STSM, particularly to young researchers and students.

# 1. S&T EXCELLENCE

## 1.1. CHALLENGE

### 1.1.1. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Cardiovascular diseases (CVD) remain the main cause of death worldwide despite technological and medical advances and is responsible for debilitating a significant number of patients annually. To discover novel diagnostic and prognostic tools and develop new treatments, understanding the molecular mechanisms in a comprehensive view is essential. Such knowledge can forward personalized medicine, where interventions are tailored to the individual patient, potentially improving the lives of millions of people and reducing healthcare costs.

One way to promote this area is by studying the transcriptome, which is the set of all different types of RNA produced in a cell and that may be involved in the pathogenesis of several diseases, including cardiovascular ones. However, the human transcriptome is much more complex and challenging to study than previously thought, and new types of RNA are frequently revealed. Currently, there are relatively isolated research groups working on the same subject and sometimes using different standards to generate data, making it harder to compare and reproduce results. This is particularly true when aiming to develop products for personalized medicine because they must deliver accurate information and high reproducibility.

This COST Action will provide a platform to coordinate the efforts of different groups and offer opportunities for collaboration between clinicians and scientists from interdisciplinary backgrounds to more rapidly decipher the role of different forms of RNA in CVD and allow to transfer such knowledge into practical applications for diagnostics and therapies. CardioRNA members aim to refine and implement guidelines for transcriptomic studies in CVD, tackling the whole investigation pipeline in order to improve comparison between studies from different laboratories and reproducibility of results, allowing faster development of new RNA-based tools for personalized medicine. This Action also aims to enable enhancement and **continuity of the network** efforts in improving the understanding of the transcriptome in the cardiovascular context and advancement of personalized medicine. Finally, it has the goal to provide a platform to train the next generation of researchers and students in the field.

Thus, **this Action aims to help alleviate the global burden of CVD by addressing the following challenges**: increase the knowledge of the role played by the complex transcriptome in CVD by coordinating efforts from different groups; refine common standards and best practices that will offer the greatest potential for transcriptome studies in CVD as well as increase reproducibility between studies and advance product development; and assure continuity of studies focusing on translational research and public-private partnerships to enhance personalized medicine.

### 1.1.2. RELEVANCE AND TIMELINESS

Non-communicable diseases are the major global disease burden, with CVD as the leading cause of death, accounting for 17.5 million deaths per year, a number that is estimated to increase.[1] The **socio-economical burden of CVD due to premature deaths and reduced quality of life is substantial**. It

has been estimated to cost €210 billion annually to the European Union alone.[2] Considerable parts of the world are at significant risk for CVD, which, as a chronic disease, may be caused by cumulative biological, behavioural and social risks. Nonetheless, the main risk factors contributing to CVD tend to be consistent worldwide: hypertension, diabetes, poor diet and obesity, physical inactivity and smoking.[3]

The modern lifestyle that promotes sedentary behaviour and ingestion of high-calorie foods has resulted in an obesity epidemic around the globe and is related to the increasing incidence of chronic diseases, particularly cardiovascular ones.[4] Such aspects of modern lifestyle result in abnormal gene expression, contributing to the development of pathologies that significantly increase the risk for CVD development, such as obesity, atherosclerosis, type 2 diabetes, and hypertension. **The high and increasing prevalence of these chronic conditions has contributed to the pandemic of CVD**.[5]

Environmental factors, such as the above-mentioned, can affect gene expression, which is reflected by the transcriptome. For many years, emphasis was given to the study of protein-coding genes giving rise to messenger RNA (mRNA). More recently, the emergence of non-coding RNA genes (ncRNA) has allowed the scientific community to appreciate the full complexity of the transcriptome. The discovery of various new classes of RNAs and their different functions has had profound implications for molecular biology and medical research (Figure 1). The importance of ncRNAs in physiological and pathological processes, such as CVD, has been frequently reinforced, and they have major regulatory roles in gene expression.[6, 7] Thus, understanding the interactions between the different types of RNA, both coding and non-coding, and how they affect gene expression in CVD, is necessary to forward CVD management.

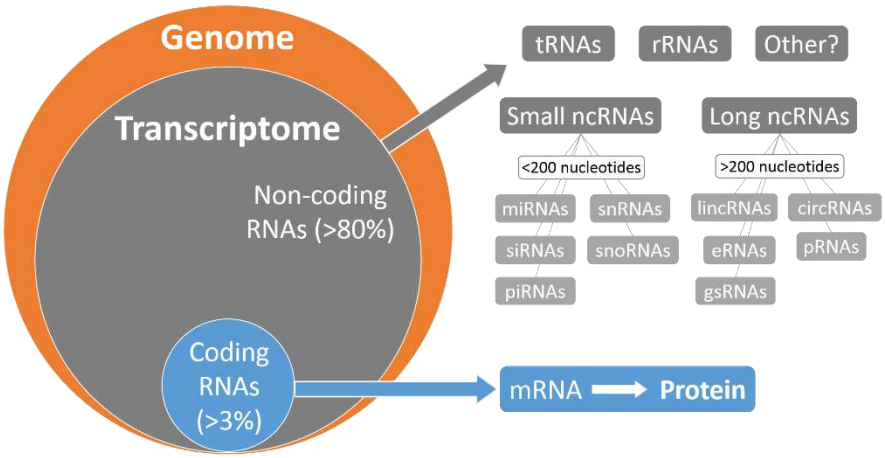


*Figure 1. Coding and non-coding RNAs in the human genome.*

In addition, knowledge of the transcriptome regulation and recent technological developments to manipulate gene expression (RNA interference, antagomiRs, gapmers, short hairpin RNA viral-mediated delivery of ncRNAs, e.g.) allow investigation of the role and value of RNAs to personalize healthcare of patients affected by CVD. Thus, the transcriptome and **particularly ncRNAs may have a more direct application in the clinical practice**. Importantly, despite most ncRNAs are found inside cells, they have been consistently identified in body fluids, including the blood.[8] The types and amounts of different ncRNAs vary and have distinct and specific profiles in different pathophysiological states,[9, 10] leading to the possibility of using these molecules as non-invasive markers of disease.[11, 12] Several studies have suggested that specific ncRNA profiles may be used as diagnostic and prognostic tools for different pathologies, including cardiovascular ones.[13]

Early and accurate diagnosis and prognosis for CVD patients can optimize clinical decision making to better adjust interventions for individual patients. Currently, **optimal treatment selection and dosage are restricted due to limited awareness of molecular and environmental information of patients**.[14] A shift in medicine to focus on individuals rather than populations promises a more proactive and predictive approach.[15] This is the concept of personalized medicine, which is committed to inspect, diagnose and monitor risk to allow that patients receive treatments tailored to their molecular and individual configuration;[14] or, if not to the individual, treatments tailored to a subgroup of individuals that share similar traits and molecular outline.[16]

However, personalized medicine presently represents a more theoretical concept than a practical one, with the one-size-fits-all approach being still broadly used to manage CVD. To put the promise of personalized medicine in practice, tools that stratify patients and improve healthcare need to be

accessible in the clinic, making translational research essential, meaning that basic research findings are converted into usable applications for clinicians and patients. The basis of personalized medicine includes targeted therapies and biomarkers, which are molecular indicators of diagnostic and prognostic of disease and treatment efficacy. The **transcriptome provides an important source of therapeutic agents and biomarkers** as continually demonstrated by different research.[17-19] Although new technologies to study the transcriptome enable information of its role in CVD mechanisms, much remains to be elucidated as new levels of complexity are unveiled. The slow speed at which advances are occurring in translational research suggests there **are bottlenecks to be resolved.[15] Thus, there is a crucial and urgent need for a collective effort in order to advance in the field by deciphering the role of the transcriptome in CVD and create new possibilities for the development of innovative products for CVD management.**

## 1.2. OBJECTIVES

### 1.2.1. RESEARCH COORDINATION OBJECTIVES

This COST Action **aims to accelerate the understanding of transcriptomics in CVD so personalized medicine advances faster in this field**, addressing a current public health challenge. It proposes to constitute a network to offer opportunities for **collaboration between clinicians, academic researchers from interdisciplinary backgrounds and industry** to achieve breakthroughs and allow the transfer of basic science into usable applications (Figure 2). CardioRNA aims to **refine guidelines** from the design to the analysis of transcriptomics data to enable better comparison between studies from different laboratories and reproducibility of results, facilitating the development of products.

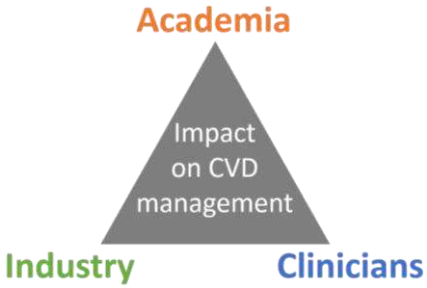


*Figure 2. Different backgrounds of the network members and their interactions to impact CVD management, thus healthcare.*

The Action will lead to creating new possibilities of basic research and of product development for personalized medicine. CardioRNA members believe that by coordinating research activities through an **interdisciplinary network, more meaningful results can be achieved**. Furthermore, the platform that will be provided by the Action will allow easier public-private partnerships to support the translation of the new knowledge to the clinic.

Researchers from pertinent disciplines will connect through CardioRNA to organize activities in order to achieve the research coordination objectives. The table below describes the research coordination SMART objectives: Specific, Measurable, Achievable, Relevant and Timely.

| Specific | Measurable | Achievable | Relevant | Timely |
|---|---|---|---|---|
| Further the understanding of the transcriptome's role in CVD by establishing a network | Number of institutions and investigators joining the network | Active and increasing collaborative research activity in the field as seen in the biomedical literature | Increased knowledge on the subject has noteworthy implications for the healthcare of patients with CVD | Expansion of the network will be continuous (month 1-48) |
| Foster collaborative initiatives in CVD transcriptomics | Number of Action outputs such as projects submitted for funding and scientific publications | Collaborations will allow optimisation and standardization of protocols, thus helping to deal with the complexity of studies | Join expertise to aid European research groups improve and consolidate their research capacity and leadership in this field | Project application to different funding bodies per year (month 12-48) |
| Develop improved guidelines for best | Production of specific | Different guidelines and techniques are | Increasing standardization will | Publish documents on 1) best |

| | | | | |
|---|---|---|---|---|
| practices and experimental standards that offer the greatest potential for cardiovascular transcriptomics studies | documents (peer-reviewed publications and others) | successfully used in this field of research | facilitate comparison and reproducibility of results, allowing faster interpretation of results and development of new tools to the clinic | practices on collection and processing of biological material (month 24); 2) experimental standards for RNA analysis in CVD (month 40) |
| Stimulate development and optimization of RNA-based products for prognostic, diagnostic and therapy for CVD management | Number of projects that target translational research and personalized medicine | Partnerships from the network will facilitate study designs on the translation of research knowledge into medicinal products | Regards healthcare improvement of patients with CVD | Projects on clinical products development (month 48) |

### 1.2.2. CAPACITY-BUILDING OBJECTIVES

CardioRNA will convene researchers from COST countries and International Partner Countries (IPC) to **increase scientific dialogue and provide training** in the role of transcriptomics in CVD through different types of meetings, involving students and early career investigators (ECI). It will support them to attend meetings, training schools and short-term scientific meetings (STSM) within the Action. The following table describes the capacity-building SMART objectives.

| Specific | Measurable | Achievable | Relevant | Timely |
|---|---|---|---|---|
| Facilitate collaborations in the subject matter by acting as a stakeholder platform | Number of strengthened and new research collaborations, publications and student exchanges in the field | Experts from different fields are able to exchange knowledge and share expertise | Concerns advancement of a field of knowledge that impacts healthcare | Yearly meetings of the whole network (month 1-46) |
| Facilitate the design of research studies on the translation of research into products for CVD | Number of partnerships between researchers, clinicians and industry and joint publications/research studies between such stakeholders | The network will be comprised of experts with complementary backgrounds and sharing common interests | Tackles a public health challenge and development of translational research | Continuous aid to form partnerships and design research studies (month 1-48) |
| Ensure knowledge, skills and expertise exchange through training schools and STSM, particularly to young researchers and students | Number of training schools and STSMs | COST provides support for the meetings to occur | Knowledge, skills, and expertise transfer is essential to advance in the field and train the next generation of scientists | At least yearly training schools and/or STSMs (month 6-46) |

## 1.3. PROGRESS BEYOND THE STATE-OF-THE-ART AND INNOVATION POTENTIAL

### 1.3.1. DESCRIPTION OF THE STATE-OF-THE-ART

The transcriptome represents all genes expressed in a tissue in a given biological state. In contrast to DNA sequence that is constant in an individual, there is great variation in gene expression in different tissues and in different pathophysiological circumstances.[20] **Specific signatures of the transcriptome have been associated with CVD phenotypes and prognosis**, while investigation of RNA frequently

points to the structural and functional versatility of these molecules.[19] Although only 1.5% of the human genome represent protein-coding genes, most of the genome is transcribed, leading to the production of different types of RNA.[21] A more recent assessment of transcripts has revealed a variety of RNA types with different sizes and shapes that don't encode proteins but have important regulatory functions, revealing further complexity to gene expression.[22] Among these ncRNAs, microRNAs are probably the most studied class. It is estimated that they regulate over 60% of mRNAs in humans[23] by inhibiting their translation into proteins. Furthermore, a single microRNA can target several mRNAs and each mRNA can be targeted by multiple microRNAs.[24] They have also received much attention because, unlike most RNAs, they are stable in the blood and their **diversity and abundance may reflect distinct health states**. Such characteristics make these small molecules very good biomarker candidates for clinically relevant parameters. Besides microRNAs, there are other types of ncRNA, including circular RNAs (also stable in the blood) and the heterogeneous class of long non-coding RNAs (lncRNAs), playing diverse functions in the cell, possibly as cell-to-cell communicators and with further potential as biomarkers for CVD.[18, 25]

Most of the available information about the transcriptome in CVD patients comes from studies in non-cardiac tissues and cells, such as blood and blood cells, mainly due to their accessibility.[26] Nonetheless, particularly ncRNAs have been associated with nearly all cardiovascular processes, from normal heart development to stress response in adults, regulating heart hypertrophy, contractility, fibrosis, apoptosis and gene expression. They also modulate gene expression and cell fate in vascular endothelial and mural (smooth muscle cells and pericytes) cells, playing a role in vascular biology.[27, 28] Still, there is much to explore and learn about the biological mechanisms of ncRNAs contributing to homeostasis and CVD, a task that may be complicated partly due to their diverse mechanisms of action.[25] This hampers the exploitation of the full potential of RNA-based therapeutics. **Since ncRNAs are important regulators of pathophysiological processes in the cardiovascular system and are present in the blood in protected forms that prevent their degradation, they have potential to be used as diagnostic and prognostic markers and as therapeutic targets**.[18, 25]

High-throughput sequencing technologies combined with bioinformatics and computational biology have recently helped expand the field of transcriptomics.[29] Three main techniques are diffusely employed to assess RNA expression: RNA sequencing (RNA-seq), microarrays and quantitative real-time polymerase chain reaction (qPCR). The first is used to obtain a complete profile of RNA in a sample, while the others are used to detect previously characterized RNAs.[30] All techniques have been widely used to enlighten our understanding about the transcriptome. **However, the field is still in its infancy and faces many challenges**. First is the complexity of the whole transcriptome due to its various players, their interactions and different mechanisms of action. These relationships and functions are still to be determined. Another issue is the lack of consensus on all steps of the process on how to best perform and interpret experiments of CVD transcriptomic studies. Comparison of results and replication of experiments are imperative to advance in gene expression studies. For example, the quality of RNA is crucial to obtain reliable results,[26] especially because RNA profiles can be easily disturbed by sample collection and processing.[31] For microRNA studies, in particular, different sample types (e.g., serum, plasma, blood collection in heparin) and measurement platforms can affect the microRNA quantification results.[32] Moreover, there is an urgent need to develop internal and better external reference materials for such studies.

Thus, transcriptomics holds potential to explain fundamental biological phenomena in the developing field of ncRNAs within a CVD context and to advance medicine through the development of new diagnostic and therapeutic tools. However**, in order for transcriptomics to fulfil the promise of improving healthcare, technical advances and a better understanding of the biological functions of ncRNA must be accomplished**. This Action aims to both refine and standardise RNA analyses and reveal ncRNA molecules that might be useful to detect disease, predict its progression and treat it.

### 1.3.2. PROGRESS BEYOND THE STATE-OF-THE-ART

The proposers of CardioRNA believe the critical challenges regarding transcriptomics studies in CVD can be significantly mitigated by coordinating efforts from different groups through this Action, leading to progress in the quality and speed of findings in the field and, hence in healthcare. The topics to be tackled in this Action all contribute to such advancement, and **networking will allow researchers to identify and come up with innovative ways to answer difficult biological questions** regarding the role of RNA in CVD.

Organization of events and meetings during this Action will pave the way for discussions and knowledge exchange about RNA interactions that may prevent or lead to CVD, identify gaps in the current transcriptomics in CVD research and define future directions of study, all adding to the current state-of-the-art. The Action will support continuity of studies and of projects focusing on basic and translational research. By targeting best practices on every step along a transcriptomics CVD study pipeline, the Action aims to increase comparison and reproducibility of results between different laboratories that will interest investigators beyond CardioRNA members. Among the current partners are experts on Clinical Biobanking and Biospecimen Science who can provide a valuable contribution to best practices for CVD sample collection and processing and aid in the production of guidelines to build reference materials for CVD, for instance. Furthermore, the elaboration of an inventory of available patient cohorts classified by main CVD will substantially help researchers find adequate cohorts for their studies and may facilitate collaborations.

The immediate benefits of this Action will be the advancement of scientific collaboration across Europe and the unification of transcriptomics research in CVD through the network, **involving clinical, academic and industrial partners.** This will allow the CardioRNA participants to **increase their competitiveness for European funding and establish leadership in the research field**.

### 1.3.3. INNOVATION IN TACKLING THE CHALLENGE

To our knowledge, there has not been any other COST Action on CVD. Within such a broad field, CardioRNA offers to join researchers interested in one complex molecular area, the transcriptome, to understand its role in CVD. The Action is directed at **overcoming the relative isolation of investigators of various backgrounds, clinicians and industrial** working in the field due to a lack of coordination, tools, and resources to provide the adequate means for collaboration. **By combining different expertise and skills**, a strong added value is included in the Action, which will be important to **promote discoveries and innovative research studies**, including ones for development of products for personalized medicine. In bringing a large group of experts together, CardioRNA will **endorse a solid agreement on best practices in transcriptomics studies in CVD**, for which raising awareness about its importance beyond the Action's participants will be an important goal. In the long term, new technology and intellectual property may result from collaborative research started during the Action.

## 1.4. ADDED VALUE OF NETWORKING

### 1.4.1. IN RELATION TO THE CHALLENGE

The potential applications of transcriptomics to aid manage CVD are clear; however, knowledge of its role in this context is still coming to light, especially as technologies progress and novel players and interactions emerge, together with new possibilities. Existing collaborations between groups can be significantly enhanced by a broader coordinated structure. This COST Action is well-suited for **strengthening ongoing collaborations and establishing new ones** by the creation of a large, interdisciplinary and international network to jointly orchestrate and **strategically plan integrated activities that will result in meaningful advancements in the field and, eventually, in healthcare**.

In order to maximize the potential of European research in the important and still developing field of transcriptomics and speed up breakthroughs, **coordination of efforts is paramount to make a greater impact**.

Networking through this Action will allow not only initiation of important discussions about the transcriptome's role in CVD, but also to **search, identify and define new directions of study and plan future projects benefiting from the expertise of members from different disciplines**. In addition, networking is key to deal with the current lack of consensus for best practices in transcriptomics studies for CVD, which hinders particularly the development of RNA-based products. Appropriate procedures and standards throughout the entire process to generate data from RNA are key to determine the quality of information and its later applications. Thus, an organized network with a dedicated working group (WG) focusing on best practices will **facilitate the establishment, acceptance, and dissemination of standard practices due to a large number of participants and their widespread locations, extending to their own networks worldwide**.

#### 1.4.2. IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

The challenge described here requires a **systemic approach to transcriptomics in CVD**, relevant to different areas, such as research, medicine, industry, and public health. This COST Action is **unique in its holistic and systems approach, bringing together the currently scattered knowledge in order to advance our understanding of CVD and decrease its global burden**. Currently, there is no network with an international and multidisciplinary approach in transcriptomics of CVD. In many circumstances and especially for certain types of ncRNAs (e.g., lncRNAs), knowledge is fragmentary despite their diagnostic and therapeutic potential. Current Ongoing European and international initiatives on CVD are currently limited to only one type of RNA at a time (mostly small RNAs) or to only one CVD. Such specialization causes missed opportunities to understand the role of RNAs in this field since different CVD are regulated by common ncRNAs. CardioRNA has the **added value of gathering a multidisciplinary holistic and international initiative to integrate and translate the knowledge in the area**. CardioRNA counts with the participation of active members of the European Society of Cardiology (ESC) Working Groups, which will facilitate the promotion of research collaborations and networking opportunities and the dissemination of this Action's outcomes at the European level. **CardioRNA will embrace the CVD national level initiatives currently in place, encouraging and facilitating connections** with the international network of players built through this Action in a constructive manner. Collaborating with specialized networks and consortia will provide greater impact of this Action and advancements in the field.

# 2. IMPACT

## 2.1. EXPECTED IMPACT

#### 2.1.1. SHORT-TERM AND LONG-TERM SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS

CardioRNA proposers expect widespread coordination of various parties interested in the context of transcriptomics in CVD. It anticipates exchange among the members and knowledge transfer to students and young researchers. Consequently, this Action will **provide the basis for significant advancements for CVD transcriptomics and translational research as well as personalized medicine, impacting society in addition to the scientific community**.

In the **short-term**, this Action will have the following impacts: 1) research collaboration and joint grant applications for future national and European funding; 2) increase competitiveness of the network participants for European funding and the impact of resulting research; 3) encourage translational

research due to participation of industrial partners in the network; 4) stimulate innovative perspectives to study the transcriptome due to CardioRNA's multidisciplinary team; 5) provide opportunities and scientific training for ECI and students through workshops, training schools and STSMs.

The **long-term** impacts of the Action include: 1) expand fundamental knowledge about the role of the transcriptome in CVD to improve its prognostics, diagnostics, and treatment; 2) increase the quality of data generated and reproducibility of studies of the CVD transcriptome, thus raising the quality and impact of publications; 3) establish a leadership of both the network members and the European research axis in the proposed research field; 4) foster personalized medicine and consequently healthcare, through outcomes of public-private collaborations.

## 2.2. MEASURES TO MAXIMISE IMPACT

### 2.2.1. PLAN FOR INVOLVING THE MOST RELEVANT STAKEHOLDERS

CardioRNA already includes three types of stakeholders: **researchers with 11 different specialities** (Molecular Biology, Biotechnology, Cardiology, Computer Science, Information Systems, Clinical Research, Epidemiology, Epigenetics, Clinical Biobanking, Biospecimen Science, Molecular Genetics, Pharmacology, and drug discovery), **clinicians** (cardiologists, intensive care specialists) **and industry in biotechnology**. The following stakeholders will be encouraged and invited to join the Action: additional industry partners, European and National Cardiology Societies and scientists with a background in population science and health economics, systems biology and bioinformatics. As educators, the Cardiology Societies are particularly relevant to help disseminate and promote CardioRNA findings and proposed best practices and guidelines delivered by WG2. Networking efforts from participating members will be important to achieve higher dissemination of the initiative and its outcomes to attract new relevant stakeholders. A key feature of this Action to **appeal to new stakeholders will be its long-term prospects to ensure the network lasts beyond the four years of COST Action**. In that sense, CardioRNA will be introduced to develop long-term relationships and help understand participants' expectations by creating an amicable and supportive environment.

### 2.2.2. DISSEMINATION AND/OR EXPLOITATION PLAN

The dissemination and exploitation plan will be coordinated by WG4 and strategies may be updated based on participants' feedback. The **main aims of CardioRNA dissemination and exploitation plan** are: to inform about the challenges and promote the solutions proposed by this Action; to engage stakeholders and funding bodies; to promote knowledge transfer and interaction with industry partners; and to maximize the impact of the Action's outcomes. To achieve this, the main **dissemination** methods include: 1) an open-access website with information and news about CardioRNA's developments and activities and information materials generated (e.g., reports, best practices guidelines, and patient cohort inventory). In addition to being a rapid communication tool, the website will facilitate access and sharing of information and organization of initiatives within the network; 2) publish biannual electronic newsletters to communicate major CardioRNA news, events, and publications; 3) conferences, workshops, and training schools organized by this Action will be open to outside participants, broadening its reach; 4) present this Action at international conferences, seminars, and workshops; 5) publications in peer-reviewed journals, including position papers, will be the main form of dissemination of the Action's results for the scientific community.

**Exploitation** will include: 1) preparation of research projects for collaborative and interdisciplinary grant applications, providing higher competitiveness; 2) benefit from the industry members' expertise on intellectual property to guide projects preparation and their execution; 3) collaborations to write manuscripts will allow for publications in higher impact peer-reviewed journals, also increasing visibility

and reach; 4) promotion of dialogue with industry members to identify areas for potential innovation; 5) capacitation of the future generation of scientists and researchers.

## 2.3. POTENTIAL FOR INNOVATION VERSUS RISK LEVEL

### 2.3.1. POTENTIAL FOR SCIENTIFIC, TECHNOLOGICAL AND/OR SOCIOECONOMIC INNOVATION BREAKTHROUGHS

The main potential innovations from this Action will be the result of increasing collaboration through a coordinated initiative to optimize returns on scientific investment, filling the gap of unmet knowledge and clinical needs in CVD personalized medicine. Thus, the Action will have **two main potential impacts: a scientific and technological and a socioeconomic one**. From the scientific and technological point of view, the combination of expertise from the participating research groups at the front line of transcriptomics in CVD will lead to significant breakthroughs in the knowledge level in this field and potentially to novel intellectual property for clinical applications. This, in turn, will positively impact healthcare. However, to be successful in understanding transcriptomics in CVD and fulfilling its innovation potential as biomarkers and therapeutic targets, **standardization of best practices is urgent**. This topic will be addressed by WG2, which will **devise strategies to minimize low reproducibility of experiments, diversity of sample types and their collection and processing**. Harmonization of procedures is a pre-requisite to reproducibility and further industrial developments of translational research findings.[12] Many new therapeutic or biomarker candidates fail to be reproduced/validated due to unstandardized methodologies for blood collection, marker assessment, or even inadequate statistical analyses. **CardioRNA will provide new lines of conduct to reduce the rate of failure to validate new findings from research laboratories**, increasing the chances of bringing to commercial application novel RNA targets. To minimize the risk associated with harmonization of procedures, the consortium already gathers experts in Biobanking, Biospecimen Science, and Bioinformatics, fields that cover topics necessary for procedures' standardization. Discussions and exchange of experiences within these experts will reduce the risk of failure to provide new standardization guidelines to an acceptable level. Such endeavour can only be achieved through the cooperation and coordination of research groups active in the field, which this Action will promote.

CardioRNA will also boost the success chances of personalized medicine endeavours, which require know-how from industry. The network's **industry partners are small and medium enterprises (SME) leaders in technology and experienced in developing biomarkers into diagnostic tools, discovering and designing drugs, and introducing technologies and devices into laboratories worldwide**. Thus, this Action can also contribute to socioeconomic innovation by accelerating development of products for the clinic, allowing doctors to better tailor treatment to the needs of each patient. For example, in some cases, a standard treatment is administered to every patient because there are no tools to stratify specific groups at risk, leading to increased patient burden due to unnecessary treatment and excessive healthcare costs. Additionally, RNA-based therapy potentially represents a powerful tool for personalized medicine due to their specific expression patterns associated with distinct pathologies. But for being in its early days, it faces challenges related to scientific and business aspects. **CardioRNA is planned to deal with these risks by building an interdisciplinary network to be able to have a systems approach** to the complexity of the transcriptome and CVD and the population variability. Hence, **we expect to give the CVD community successful case studies that can inspire and orient future efforts for therapy development**.

# 3. IMPLEMENTATION

## 3.1. DESCRIPTION OF THE WORK PLAN

### 3.1.1. DESCRIPTION OF WORKING GROUPS

CardioRNA will be organized into four interrelated working groups (WG) to optimize the accomplishment of the main proposed goals. Three WGs will be dedicated to develop science-related activities and meet the research coordination goals. One WG will communicate within and outside the network and support the other WGs to develop their networking activities. The latter will also be mainly responsible for disseminating the Action's activities and enhancing its impact, particularly in the European community. All WGs will be responsible for achieving capacity-building objectives, particularly contributing to training activities.

The whole network will meet once a year to monitor progress. Fast modes of communication will be used between the members, such as email, teleconferences, and Skype calls, in-between on-site face-to-face meetings. The CardioRNA website will provide an important way to communicate and exchange information, particularly regarding events and trainings. All WGs will provide reports evaluating the development of activities and results achieved.

**WG1: Regulatory function of the transcriptome**

The human transcriptome is composed of coding and non-coding transcripts. Among the non-coding transcripts, one distinguishes short ncRNAs and lncRNAs. The latter group is the largest and most diverse class of transcripts and exerts regulatory functions that ultimately control cell identity and behaviour. In particular, this regulatory layer integrates developmental and environmental cues to shape the various cellular responses implicated in development and disease. Although microRNAs have been extensively studied in the context of cardiovascular disease, lncRNAs need to be systematically investigated. A better understanding of their importance as regulators of the gene programs controlling the response to stress should allow identification of new targets for improving diagnosis and treatment. WG1 will focus therefore on identifying novel ncRNAs and characterizing their regulatory functions in cardiovascular pathophysiology, including their interactions with protein-coding transcripts. One aspect that will be addressed is the difference of ncRNA expression according to gender. This WG will provide training in the various approaches and techniques that are used to probe and manipulate the transcriptome in order to study the role of ncRNAs in the cardiovascular system. To reach these goals, WG1 will be composed of clinical and basic scientists with different expertise ranging from epigenetics to systems biology.

WG1 Objectives

- Analyse transcriptomics data to gain insights into the molecular mechanisms implicating RNAs behind CVD;
- Discuss the specific roles of regulatory RNAs in cardiovascular homeostasis and disease;
- Examine the importance of RNA partners (DNA, RNA, proteins) in RNA functions;
- Identify research gaps in transcriptome analysis in CVD, and define future directions of study;
- Organize meetings, STSMs and/or training schools to address these different topics.

WG1 Tasks: 1) Review the current knowledge in RNA biology in CVD and identify research gaps; 2) promote ongoing research to determine the role of different types of RNA in CVD; 3) identify important research topics to be explored in the future; 4) organize WG1 meetings, STSMs and/or training schools; 5) provide progress reports for WG4.

WG1 Milestones: 1) Initial meeting to define strategies for WG1; 2) focused meetings with network members; 3) WG1 meetings, STSMs and/or training schools.

WG1 Deliverables: 1) Scientific publications and review papers stating WG1 main conclusions; 2) list of relevant research topics to be studied; 3) WG1 meetings, STSMs and/or training schools.

**WG2: Best practices and experimental standards**

Comparison of protocols and data from different laboratories is necessary to facilitate the comprehension of results and advance knowledge in CVD transcriptomics. It also directly impacts the development of tools that can be used for diagnosis and monitoring of CVD. In order to compare results, generated data must meet common standards, which should be from the collection of samples to the generation and analysis of data, so that variations are minimized and results are accurate and reproducible. Sample collection, storage, and quality testing are critical, particularly when dealing with RNA due to its sensitive nature. Although guidelines exist for separate steps of sample collection, data generation, and analysis, a complete guideline comprising all steps that are specific for CVD is still

lacking. WG2 will focus on the best practices for cardiovascular transcriptomic studies from the crucial issue of sample collection and processing, which directly impacts final results, to the main techniques used to study the transcriptome (qPCR, microarray and RNA sequencing) and the statistical analysis. Protocols, technologies, and procedures will be assessed and standard strategies will be delineated for better quality control and study comparison, tackling all the steps along the process. This Action aims to bring together in WG2 experts from relevant disciplines to discuss and come up with guidelines to generate reference materials for CVD and also for the production of internal quality control materials that can be used in CVD transcriptome analyses. Participating members with unique knowledge will share their expertise through meetings, STSMs, and training schools.

WG2 Objectives

- Propose guidelines for collection and processing of biological material for cardiovascular transcriptomics analysis, including quality testing and storage, and define strategies for their implementation among different laboratories;
- Review standard protocols and suggest refinements for the generation of data on RNA from specific types of samples;
- Choose standard protocols for each analysis methodology (qPCR, microarray, and RNA-seq), including sample type and handling that offer the greatest potential for CVD transcriptomics studies;
- Generate guidelines to produce reference materials for CVD and for the production of normalizers and internal quality control materials for CVD RNA analyses;
- Organize WG2 meetings, provide training for ECI and students through STSMs and/or training schools.

WG2 Tasks: 1) Devise best practices guidelines towards sample collection, handling, storage and quality testing for cardiovascular transcriptomics analysis; 2) review current pipelines for RNA analysis using qPCR, microarray and RNA-seq to draft a document suggesting improved pipelines for each in the context of CVD; 3) identify lacking experimental details in CVD transcriptomics publications and suggest criteria of critical information that should be published to enable reproducibility of results; 4) find the best normalization targets and their reference ranges for normalization in CVD transcriptomics; 5) write a technical report with guidelines to generate reference materials for CVD transcriptomics studies; 6) organize WG2 meetings, STSMs and/or training schools; 7) provide progress reports for WG4.

WG2 Milestones: 1) Initial meeting to define strategies for the design of best practices and experimental standards and their implementation; 2) WG2 meetings to review and discuss guidelines; 3) STSMs and/or training schools on best practices and experimental standards in CVD transcriptome.

WG2 Deliverables: 1) Consensus document/publication with best practices for sample collection, handling, and storage for CVD transcriptomics research; 2) consensus document/publication with experimental standards for all steps of RNA analysis using qPCR, microarray, and RNA-seq for CVD transcriptomics research; 3) refinement of guidelines for publishing minimum information regarding CVD transcriptomics; 4) a peer-reviewed publication with the guidelines developed by WG2 and the relative advantages of adopting them, so that the CVD community at large will benefit; 5) a peer-reviewed publication / technical report on the production of reference materials for CVD and for the production of internal quality control materials applied for CVD; 6) WG2 Meetings, STSMs, training schools.

**WG3: Development of cohort inventory**

The main goal of WG3 is to create an inventory of cohorts from healthy individuals and CVD patients in order to provide awareness of the available cohorts that can be used in research, thus, facilitating collaborations. Such an inventory, with key characteristics of the cohort (paying particular attention to gender) and information on the principal investigator, will be made available to the public through this COST Action's website and possibly through websites from other relevant organizations and institutions (e.g., Biobanking and BioMolecular Resources Research Infrastructure Directory, International Society for Biological and Environmental Repositories). Although potential ethical and Material Transfer Agreement issues regarding shipment of clinical samples between countries may arise, this is not within the domain of this Action.

WG3 Objectives

- Create an inventory of available cohorts classified by CVD;

- Generate a document detailing the cohorts' main characteristics and principal investigators contact information.

WG3 Tasks: 1) Gather information on current cohorts available for CVD studies; 2) create an inventory of the available cohorts suited for CVD transcriptomics studies; 3) make the inventory public through this Action's website and other websites and media; 4) additional dissemination strategies, e.g. main publication to advertise the inventory; 5) organize WG3 meetings; 6) provide progress reports for WG4.

WG3 Milestones: 1) WG3 meetings; 2) list of information about cohorts for CVD studies; 3) publication in various media of the generated inventory.

WG3 Deliverables: 1) Document with an inventory of available patient cohorts classified by main CVD and with the principal investigators' contact information; 2) publication of the inventory on the Action's website and other media; 3) advertisement of the availability of the cohort inventory in major cardiology journals.

**WG4: Dissemination**

WG4 will be responsible for promoting communication, knowledge exchange and bring new members to the network. This WG will organize yearly CardioRNA network meetings and provide support for the different WGs to organize their meetings, workshops, STSMs and training schools. It will also be responsible for providing updates on the activities of the network and disseminating the Action's progress and initiatives to the public.

WG4 Objectives

- Establish effective communication systems within the network to strengthen knowledge exchange;
- Expand the Action's network by recruiting new members and private partners;
- Develop and implement a dissemination strategy to increase the visibility of this Action;
- Provide continuous updates about the information and activities of the network;
- Organize yearly network meetings;
- Support WGs to organize topic-specific meetings, workshops, training schools and STSMs;
- Raise awareness to the importance of experimental standards in CVD transcriptomics studies.

WG4 Tasks: 1) Set-up the Action's website that will support the network activities and will be used for outreach purposes; 2) organize yearly network meetings and support other WGs to organize their events; 3) create a member database with information about each member of the Action; 4) invite new members and private partners; 5) disseminate the efforts of the network.

WG4 Milestones: 1) First Management Committee (MC) Meeting; 2) Action's website running; 3) whole network meetings.

WG4 Deliverables: 1) Action's website; 2) database with main information about each member of the network; 3) meetings and materials for the meetings; 4) outreach texts published on the Action's website.

### 3.1.2. GANTT DIAGRAM

| Activity | Year 1 | | Year 2 | | Year 3 | | Year 4 | |
|---|---|---|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |
| Kick-off meeting | | | | | | | | |
| Website launch | | | | | | | | |
| Website update | | | | | | | | |
| Management Committee meetings | | | | | | | | |
| CardioRNA network meetings | | | | | | | | |
| Individual WG topic-specific meetings | | | | | | | | |
| Workshops | | | | | | | | |
| STSMs | | | | | | | | |
| Training schools | | | | | | | | |
| Final Action meeting | | | | | | | | |

### 3.1.3. PERT CHART (OPTIONAL)

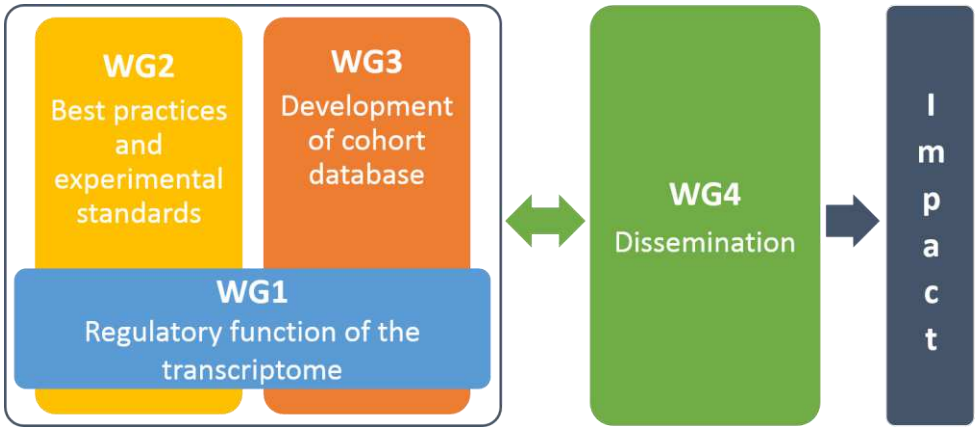


*Figure 3. Overall interrelations between the five CardioRNA WGs created to achieve the goals described.*

### 3.1.4. RISK AND CONTINGENCY PLANS

As in most large-scale projects, a number of potential risks can be identified. One that has already been addressed is the inability of participants to commit to their tasks. Indeed, in order to significantly minimize this challenge, many participants have been involved in the preparation of the Action to increase their commitment to implementing their duties. Additional main potential risks for successfully completing this Action and its contingency plans are presented in the table below, sorted by **likelihood** (low: 1; medium: 2; high: 3).

| | Risk | Contingency plan |
|---|---|---|
| 1 | Few interdisciplinary interactions among members. *Potential impact*: low | Promotion of interdisciplinary discussions and scientific exchanges through seminars and roundtable discussions, e.g. *Responsible*: WG leaders |
| 2 | Network members not working together effectively. *Potential impact*: medium | Several members of CardioRNA have collaborated with each other through publications or projects, displaying their openness to work together. In order to create a team cohesion, clearly defined roles for the members will be specified, so everyone knows what he or she is responsible for. Furthermore, every member will be encouraged to ask questions and listen to one another. *Responsible*: WG leaders |
| 2 | Excessive costs for annual whole network meetings. *Potential impact*: medium | Organize concomitant topic-specific WG meetings and CardioRNA network meetings and encourage multiple roles for attendees. *Responsible*: MC |
| 2 | Loss of motivation from participants throughout the Action. *Potential impact*: high | Actively involve members in developing the tasks and organizing meetings, workshops and training schools; acknowledge people who made key contributions to achieving the Action's goals; assign tasks to newcomers as a condition to participate in the Action; rotate WG leaders, foster co-authorship on scientific publications and involve participants in dissemination activities. *Responsible*: WG leaders |
| 2 | Lack of adherence to experimental standards. *Potential impact*: medium | Main biomedical-cardiology-genomics journals will be approached and recommendations to include as a submission requirement a checklist associated with the guidelines will be performed. *Responsible*: WGs 2 and 4 |
| 2 | Cohort organization (WG3 topic). *Potential impact*: low | Only well-defined and extensively characterized and validated patient cohorts from consortium experts will be considered. *Responsible*: WGs 2 and 3 |
| 3 | Disagreements between participants on standardization, reproducibility and best practices (WG2 topic). *Potential impact*: high | Understand the dynamics of each conflict and elect a moderator to manage to address them, including to suggest that participants develop sets of criteria for rating solutions. *Responsible*: MC, WG2 |

| | | |
|---|---|---|
| 3 | Inter-individual variability and disease complexity<br>*Potential impact*: medium | One Action's goal is to address this risk by creating a holistic network with a systems-based approach by experts in the field.<br>*Responsible*: WGs 1, 2 and 3 |
| 3 | Delays in the implementation of tasks due to deficient coordination.<br>*Potential impact*: medium | WG leaders will be responsible to coordinate activities, monitor progress of the work and adjust as necessary. They should assign more than one participant to each task and also provide progress reports to WG4 to receive support. *Responsible*: WG leaders |
| 3 | Design issues on translational research projects to develop diagnostics or prognostics products.<br>*Potential impact*: high | Involvement of experienced SMEs has already taken place and further engagement of industry partners will be encouraged.<br>*Responsible*: WG4 |

## 3.2. MANAGEMENT STRUCTURES AND PROCEDURES

The Action management structure will match both the organization of the CardioRNA network and the COST rules. It will be mainly comprised of the MC appointed by the COST National Coordinators to implement, manage and supervise the Action's activities, an Action Chair and Vice Chair to ensure the Action meets its objectives in time and within budget, and Working Group leaders. The Grant Holder will have support from internal departments to assist with the Action's financial management, project management, dissemination and website building, and maintenance.

The MC, Action Chair, Vice Chair, and WG leaders will exchange ideas to agree on a detailed work plan for the Action. Throughout the Action, they will discuss its progress and make improvements as necessary and identify any new risks and contingency plans to allow its success. In addition to face-to-face meetings, the MC will often communicate by teleconferences to ensure the appropriate development of the Action and its scientific quality. WG4 will work more closely with the other WG leaders to support organization of meetings, workshops and training schools and report to the MC. All WGs will organize their topic-specific meetings during the yearly CardioRNA network meeting.

Procedures will follow the ethical regulations on patient data protection, biological material handling, and transfer, and the stakeholders will be informed about the ethical issues associated with the science discussed in the Action.

## 3.3. NETWORK AS A WHOLE

The Action will be represented by scientists with interdisciplinary backgrounds and SMEs distributed throughout Europe and beyond, **allowing the Action's initiatives to expand within the participants existing collaboration networks, dispersing to still other countries**. Furthermore, research groups from **Inclusiveness Target Countries** (ITC) are part of the proposers with major leadership roles from this Action's conception. Both the main subject of CardioRNA and the lack of coordination of efforts to deal with it are widespread, making cooperation between this Action and IPCs mutually beneficial. Additionally, this Action will promote spreading of excellence and innovation-driven entrepreneurship in health to these countries.

Overall, the participants will comprise a group of experienced scientists with the backgrounds necessary for this Action: Molecular Biology, Biotechnology, Cardiology, Translational research, Computer Science, Bioinformatics, Clinical Research, Epigenetics, Biobanking, Biospecimen Science, Molecular Genetics, and Pharmacology. This variety of disciplines will be essential to address the challenges of the Action, ensuring exchange of **complementary knowledge and expertise necessary to deal with the complexity of the transcriptome in CVD and of translational research**. Particularly, experts in biobanking and biospecimen will significantly contribute to addressing the challenge of common standards and best practices and the participation of experts in translational and transcriptome research in cardiology and SMEs is especially relevant to elaborate projects directed towards the clinic for

personalized medicine. WG4 will work to maintain engagement of the participants and to approach new partners from different disciplines as needed.

This Action envisions to closely involve ECIs to inform and train them in a collaborative environment. They will be involved in the Action development, playing roles in WGs, workshops and training schools. Such participation will enhance their qualifications and strengthen and **allow continuity of cooperation in the European research axis**. Furthermore, **female scientists, both early-career and established, have mostly contributed to the preparation of the Action** and to delineate its objectives and structure. Thus, it is expected and encouraged that they will play major roles in the Action's management, including in WG leadership.

Interactions induced by this Action will **consolidate existing collaborations and provide the opportunity to establish new interdisciplinary ones**. Knowledge exchange during meetings, workshops, training schools and STSMs will facilitate the expansion of research cooperation within the network.

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