Brussels, 23 June 2017

COST 014/17

DECISION

Subject: **Memorandum of Understanding for the implementation of the COST Action "Stem cells of marine/aquatic invertebrates: from basic research to innovative applications" (MARISTEM (Marine invertebrate stem cells)) CA16203**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Stem cells of marine/aquatic invertebrates: from basic research to innovative applications approved by the Committee of Senior Officials through written procedure on 23 June 2017.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA16203
STEM CELLS OF MARINE/AQUATIC INVERTEBRATES: FROM BASIC RESEARCH TO INNOVATIVE APPLICATIONS (MARISTEM (Marine invertebrate stem cells))

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);
b. "COST Action Proposal Submission, Evaluation, Selection and Approval" (COST 133/14);
c. "COST Action Management, Monitoring and Final Assessment" (COST 134/14);
d. "COST International Cooperation and Specific Organisations Participation" (COST 135/14).

The main aim and objective of the Action is to foster the study of marine/aquatic invertebrate stem cells (MISCs) for innovative ideas relevant to various biomedical disciplines. The Action aims at consolidating and strengthening the fragmented European MISC community, and integrating the MISC field with biomedical disciplines. 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 56 million in 2016.

The MoU will enter into force once at least five (5) 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.

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.

TECHNICAL ANNEX

OVERVIEW

Summary
The 'stem cells' discipline represents one of the most dynamic areas in biology and biomedicine. While adult marine/aquatic invertebrate stem cell (MISC) biology is of prime research and medical interest, studies on stem cells from organisms different from the classical models (e.g., human, mouse, zebrafish) have not been pursued vigorously.

Marine invertebrates as a whole portray the largest biodiversity and the widest phylogenetic radiation on Earth, from morphologically simple organisms (e.g., sponges, cnidarians), to the more complex molluscs, crustaceans, echinoderms and protochordates. Likewise, they illustrate a kaleidoscope of MISC-types that participate in the production of enormous novel bioactive-molecules, many of which are of significant potential interest for human health (antitumor, antimicrobial). MISC further participate in aging and regeneration phenomena, including whole-body regeneration, the knowledge of which can be clinical relevant.

Up to now, the European MISC-community is highly fragmented and very scarce ties were established with biomedical industries to harness MISC for human welfare.

Thus, this COST Action aims at:

- consolidating the fragmented European community working on MISC;
- promoting and coordinating European research on MISC-biology;
- stimulating young researchers to approach research on MISC-biology;
- developing, validating, training and networking of novel MISC tools and methodologies;
- establishing the MISC discipline in the front interest of biomedical disciplines;
- establishing collaborations with industries to exploit MISC as sources of bioactive molecules.

Areas of Expertise Relevant for the Action	**Keywords**
● Biological sciences: Stem cell biology ● Biological sciences: Apoptosis ● Biological sciences: Systems evolution, biological adaptation, phylogenetics, systematics ● Biological sciences: Cell signalling and cellular interactions ● Biological sciences: Environmental and marine biology	● aquatic/marine invertebrates ● adult stem cell ● regeneration ● cell cultures ● bioactive molecules

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

Research Coordination
● Consolidation of the European community of scientists involved in marine/aquatic invertebrate stem cell research.
● Coordination of marine/aquatic invertebrate stem cell research, sharing of methodologies/databases used in marine/aquatic invertebrate stem cell research in various European countries and updating of scientific and technical guidelines for standardisation of methods, techniques and protocols, in order to maximise the extent and the quality of the results.

● Establishing collaborations with industry for technology transfer and the exploitation of marine/aquatic stem cells in the fields of biomedicine and biotechnology.
● Coordinate collaborative and scientific ties, at international level, with scientists working on marine/aquatic invertebrate stem cells.

Capacity Building
● Strengthening the European Community on marine/aquatic invertebrate stem cells through data sharing, setting up new collaborations among participants.
● Promoting interactions of Action members in order to establish a defined identity and profile in the European field of marine/aquatic invertebrate stem cells and establish ties with European networks and scientific societies/institutions in related fields.
● Stimulating contacts and the development of a joint research agenda in order to strengthen future research on MISC.

1) S&T EXCELLENCE

A) CHALLENGE

I) DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Main aim of this COST Action is to foster, at the European level, the knowledge of the biology of marine/aquatic invertebrates stem cells (MISC) in order to build innovative ideas relevant to various biomedical disciplines. In line with the Ostend declaration and the Marine Board, specific aims of this Action are: (**a**) to overcome the scientific boundaries and obstacles facing the European MISC community; (**b**) to consolidate the fragmented community; (**c**) to integrate the MISC field with biomedical disciplines (such as aging, cancer, immunology, regeneration, stem cells biology, etc.); (**d**) to strengthen the European research community on MISC. This will be done by promoting: **i**) joined research on stem cell biology; **ii**) sharing of innovative ideas and discussion on compared models; **iii**) coordination of research in MISC biology; **iv**) establishing ties with biomedical and biotech industries to harness MISC for animal and human welfare as well as sources of molecules of interest for biotechnological purposes; v) training the next generation of biologists in MISC research.

II) RELEVANCE AND TIMELINESS

Marine/aquatic invertebrates represent a large biodiversity assemblage of pluricellular organisms and the widest phylogenetic radiation on Earth, with more than 2,000,000 species formally described (95% of the overall animal biodiversity). They have been employed as laboratory models for more than 150 years and contributed to elucidate various biological problems.

If, up to few years ago, the use of invertebrates as model organisms was limited by the paucity of –omics data, the situation has rapidly changed and is still changing. Today, the genomes and various transcriptomes of many marine/aquatic invertebrate species (Radiata, Ecdysozoa, Lophotrochozoa and Deuterostomata), as well as many recombinant proteins of invertebrate origin, are available. New technologies such as RADsec, RNAsec, next-generation sequencing methods, shotgun sequencing, ChIP-sequencing), epigenome characterisation, mass spectrometry and protein profiling, reverse-phased protein microarrays, combined with novel bioinformatic tools, have revolutionised the available tool-box of research methodologies. These can be applied to further develop the use of marine/aquatic invertebrates as reliable model organisms in biological research, with focus on their stem cells.

There are three major routes of involvement where MISC are highly relevant and timely:

a) The understanding of fundamental biological processes. MISC are key players in the biology of aquatic invertebrates, with a central role in many biological processes. While much is known on adult stem cells and their properties in vertebrates (primarily mammals) and some model terrestrial invertebrates (i.e., *Drosophila*), very little has been learnt on the nature and properties of MISC. Aquatic invertebrates exhibit multiple cell types with stem cell attributes. Studies revealed that, in contrast to the prevalence of diverse oligopotent and unipotent stem cells in vertebrates, marine invertebrates appear to display the communal spread of multipotency and pluripotency, with adult stem cells that give rise to cell lineages characteristic of more than a single germ layer, sometimes with somatic and germ line potentials. In addition,

unlike vertebrates, in aquatic/marine invertebrates, stem cells are disseminated and widespread inside the animal body, i.e., not associated with a regulatory microenvironment (niche). It is also of notice that transdifferentiation (today, a topic of great interest when trying to understand how to “reprogram” a cell) is ubiquitous in invertebrates. These observations delineate common and unique properties of MISC, possibly tailored to suit the varied life history traits, ecology and developmental modes characteristic of aquatic invertebrates.

b) Alternatives to the use of vertebrates. The recent directive of the EU (2010/63/EU) on the protection of animals used for scientific purposes is highly restrictive for the use of the complex, classical vertebrate models in biological research. Following the general scientific trend and to bypass the restrictions imposed by the directive on behalf of the protection of animals, researchers turned to use vertebrate cell lines and primary cultures to get answers to a wide array of biological questions. This greatly limits the comprehension of biological phenomena at the organismal level as well as from evolutionary perspectives. Invertebrates (cephalopods excluded) are not included in the EU directive and offer the possibility of *in vivo* analyses. In many cases, they not only successfully replace mammals or other vertebrates as laboratory animals in biological research but also provide added values for their simpler body organization or less genetic complexity and for presenting phenomena that do not occur in vertebrates (like whole body regeneration, concealed vs. programmed aging, etc.).

c) The importance for the biomedical and biotech industries. Marine biotechnology is an emerging discipline aiming at use of marine bioresources for biotechnological applications (Blue Technology). However, industries traditionally lack familiarity with the marine environment. The latter, with its vast genetic richness, is a potential source of new products of social value, as marine organisms produce molecules (enzymes, biopolymers, bioactive compounds, secondary metabolites) that can find applications in various fields, such as nutraceutics, cosmetics, antibiotics, disease-fighting drugs, antifouling products, biomaterials and more. In addition, marine invertebrates portray a kaleidoscope of MISC types that participate in the production of this enormous list of novel bioactive molecules, (antitumour, antimicrobial, and anti-inflammatory) with significant potential interest for human health. MISC also participate in unique aging and regeneration phenomena, including whole-body regeneration, lack of senescence/aging, and the presence of unique stemness systems, without formation of tumours, the knowledge of which can be of great help in medicine. The increasing availability of genomes from marine animals will allow the rapid detection of the gene networks involved in the biological phenomena listed above as well as biosynthetic pathways of new useful bioactive molecules and metabolites by the functional analysis of cloned gene libraries. In addition, marine organisms and their cells are of great use to study the impacts of environmental stressors, global warming and ocean acidification on biota.

The European MISC community, however, confronts scientific boundaries and obstacles:

a) Ties with biomedical and biotech industries. Current European marine biotech initiatives are mainly focussed on identifying novel molecules and biosynthetic pathways, but there is a critical lack of cellular models able to express biomolecules and test their effect on environmentally significant organisms. At present, most of the research on MISC is primarily fundamental, leading to papers published in scientific journals; but precisely this may stand in the way of commercialisation and economic logics. The future of marine biotechnology (including MISC research) for industrial and medical applications is enormous. The global market for marine biotechnology is estimated at $ 4.1 billion in 2015 and has the potential to reach $ 4.8 billion by 2020 and $ 6.4 billion by 2025 (http://www.smithersrapra.com/market-reports). The marine environment accounts for the great majority of the ecosystems and living species of the Earth and marine biotechnology follows the diverse opportunities emerged, in a wide variety of biotech areas and medical fields from basic research to industrial applications. In the European Community, the interest in marine biotechnology from the scientific community, and to some extent from industry, has grown rapidly in the past decade owing to the recognition of the sheer scale of opportunity presented by the largely unexplored and immense biodiversity of European seas and oceans and the increasing availability of molecular tools to explore this biodiversity. However, cellular tools are critically lacking to validate these molecular studies. Such important barriers and challenges need to be tackled at various levels

for Europe to remain a key player in marine biotechnology research. Part of the problem is the lower spending of gross domestic product on research and development in the marine arena and another obstacle is that the EU takes too long to transform research and innovation results into marketable products.

b) Social implications. Boosting marine innovation through biotechnology-related activities is specifically mentioned in Horizon 2020: under the priority 'Better Society'. This is widely supported by the notion that the successful development of the marine biotechnology sector in Europe would be performed under industry-academic collaborative environment. But recent development in green biotech, nanotechnology and synthetic biology have shown how critical the social acceptability of new technological knowledge has become. Social acceptance of the MISC technology, and marine biotechnology as a whole, faces an important challenge in convincing a large array of stakeholders that, on one hand, it does not build on irresponsible knowledge, and, on the other hand, it can fulfil the numerous promises it has announced. This COST Action will help identifying the main bottlenecks that could impede the development of the MISC discipline and, therefore, provide guidance in dealing with the corresponding policy issues, with input onto the recent European move towards Responsible Research and Innovation initiative.

c) Need for coordinated European research on marine/aquatic stem cells. The community engaged in MISC in COST countries is highly fragmented, each laboratory working on its cell of choice, using different methodologies. The preliminary census throughout COST countries led to identifying >200 MISC scientists in different institutions, located in coastal and in inland laboratories, employed by government, universities and research institutions. Additionally, numerous scientists are employed by biotechnology entities. In other marine sciences fields (e.g., oceanography), the need for sharing costly research instruments (e.g., vessels) has led to higher research integration. This is not the case in MISC research in COST countries and therefore, far less scientific innovations have been produced, negatively affecting this discipline.

d) Training of next generation researchers. Unlike the ongoing Blue Biotech European projects, focussed on molecules and genes of biological interest, this COST Action is mainly biodiversity oriented, with particular attention to stem cells and their potential for applied research. In general, there is a deficiency of European students and young scientists with a thorough knowledge of the marine/aquatic biodiversity and receiving practical training in cutting-edge techniques and cellular approaches underpinning ongoing developments in marine biotechnology, including MISC. There are also needs to support high quality new students and young researchers with a broad education in marine science, medicine and biotechnology that are ready to become leaders in this rapidly expanding field. Additionally, there is a shortage of university programs with focus on the sea in integrated courses, research and practical experience within the university, and through partnerships with the local biotech industry. It is therefore a prime mission to prepare a new generation of talented students for careers in the MISC discipline (research and industry). This field will not be developed without having constant flow of young scientists irrigating the various aspects of research in MISC. Up to now, small number of graduate students are engaged with MISC, primarily due to the lack of established and standardised methodologies. Addressing of specific needs in education and training in the field of marine biotechnology (including MISC), will bring together crosscutting disciplines such as marine biology, cell biology, bio-informatics, systems biology, synthetic biology, etc.

B) SPECIFIC OBJECTIVES

I) RESEARCH COORDINATION OBJECTIVES

The following objectives aim to overcome the fragmentation within the European MISC community, strengthen the collaboration among the European research community on MISC, foster the MISC discipline at the academic level and link the MISC research with biomedical disciplines (such as aging, cancer, immunology, regeneration, stem cells biology, etc.).

• Consolidation of the European community of scientists involved in MISC research. This will be done through the: **i**) organisation of annual meetings for data sharing; **ii**) promotion of Short-Term Scientific Missions for technical training; **iii**) creation of an Action website and a newsletter acting as a discussion Forum for MISC central to the MISC community; **iv**) setting up new collaborations among participants; **v**) organisation of workshops on MISC and specific methodologies related to MISC, in particular addressing the bottlenecks in MISC identification and *in vitro* culture; **vi**) publication of an updated review on MISC in a qualified scientific journal; **vii**) organisation of participants in Working Groups to address the scientific tasks described below.
• Coordination of MISC research, sharing of methodologies/databases in order to maximise the extent and the quality of the results.
• Establishing collaborations with industry for technology transfer and the exploitation of MISC in the fields of biomedicine and biotechnology.
• Coordinate collaborative and scientific ties, at international level, with scientists working on MISC, primarily from the USA and Japan.

II) CAPACITY-BUILDING OBJECTIVES

• Strengthening the European Community on MISC through setting up new collaborations among participants and promoting Short-Term Scientific Missions for technical training.
• Promoting interactions of Action members in order to establish a defined identity and profile in the European field of MISC and establish ties with European networks and scientific societies/institutions in related fields.
• Stimulating contacts and the development of a joint research agenda in order to strengthen future research on MISC.

C) PROGRESS BEYOND THE STATE-OF-THE-ART AND INNOVATION POTENTIAL

I) DESCRIPTION OF THE STATE-OF-THE-ART

Stem cells in multicellular organisms, possess the unique ability to remain in undifferentiated state and, upon demand, originate cells that differentiate. This has been well established in vertebrates and terrestrial invertebrates, where much of the research activities have been linked to applied aspects of stem cell biology. However, the excessive focus on the applicative outcomes of stem cell biology poses the risk of missing the wide range of stem cell properties that are represented in various multicellular taxa, primarily in taxa belonging to the aquatic invertebrates. In addition, working on vertebrate systems is extremely expensive and raise several ethical concerns.
Not only MISC share whole ranges of novel biological properties but, unlike vertebrates, are also key participants in the aging and regeneration phenomena. The comparison of well known phenomena in model systems with novel phenomena, associated with the biology of MISC, can help to better understand processes and phenomena in mammalian stem cells biology, including natural chimerism and cancer, ageing and senescence, immunity and autoimmune responses, and more, all difficult to explain or understand in the human context.
A careful literature search attests that studies on stem cells from organisms, not defined as the classical model organisms (such as human, mouse, zebrafish, etc.), have not been pursued vigorously. This is because these cells are generally few in number, sometimes not specifically characterised, are studied in biological systems not amenable for *in vitro* work and, probably, are less plastic.
Recent studies have shown that the best groups of organisms for stem cell research are the marine/aquatic invertebrates. These organisms possess numerous types and lineages of stem cells that can offer, once studied in the lab, important clues in the understanding of stem cell biology. Stem cells are present in marine/aquatic organisms, in either (morphologically) simple organisms, such as cnidarians, sponges and flatworms, or those of more complex taxa, such as crustaceans, echinoderms and urochordates. Unique, for many of the above list of marine invertebrates, is the understanding that some adult MISC are pluripotent, capable of

developing both the germ line and somatic tissues, and involved in asexual reproduction and regeneration. Marine organisms challenge the prevailing dogmas on stem cell structures, niches and cell lineages biology. They also challenge the existing concepts on the genetic and epigenetic control of stem cell differentiation. Due to their simpler morphological and tissue organisation and the accessibility of some of them to genetic manipulation, marine/aquatic invertebrates are reliable and efficient model systems to investigate the molecular basis of stemness and stem cell regulation. This can be done by analysing the molecular interactions between stem cells and their niches under controlled in vivo conditions.

II) PROGRESS BEYOND THE STATE-OF-THE-ART

Many of the features revealed in MISC are not recorded in stem cells from vertebrates, including the almost absence of association with regulatory microenvironments (niches), their widespread distribution (and high percentage) in all marine animal, their totipotency, the feeble distinction between somatic and germ stem cells lineages, among other features. However, while the literature on stem cell from vertebrates is rich and expanding at an exponential rate, investigations on MISC are very limited and scarce, despite the results pointing that MISC are very important in various biological processes. Such processes involve understanding of mechanisms promoting cell growth and differentiation, regeneration and budding typical of marine invertebrates, tissue homeostasis of marine organisms (including those that may live for decades), aging and senescence.

The concepts of stem cell-based organogenesis, aging, cancer and regeneration are interrelated and shared through evolution. Therefore, stem cells are not only entities of biological organisation, accountable for the formation and regeneration of specific tissue and organ systems, but also units in the complex evolutionary selection process. Thus, a clear understanding of the relationships between stem cells and the above-reported processes and the possibility of their conservation will push the MISC community in Europe greatly ahead of the current state-of-the-art.

This COST Action aiming at strengthening the European research community on MISC and promoting joined research among Action members on comparative stem cell biology, developing innovative ideas and technology, is the most appropriate tool for significant European progression, breaking through the current state-of-the-art. A rapidly growing number of investigations of MISC, as well as a more integrated European scientific community engaged with MISC research, will offer exciting new avenues for advancing knowledge of MISC biology as well as providing novel systems for medical and economical applications.

III) INNOVATION IN TACKLING THE CHALLENGE

Interdisciplinary approaches are crucial to tackle the challenging MISC topics and they are central to the proposed holistic, integrated approach to managing the MISC community. Pluripotent MISC provide powerful and physiologically relevant systems to characterise the regulatory mechanisms that control cellular differentiation at all organisational levels of biology, also reflecting on mammalian systems. The following three key strategies to target current and future scientific and economic awareness have been identified:

a) need for standardised and optimised protocols. Innovative technologies and strategies that are being and will be employed to decipher the mechanisms that control the directed differentiation of pluripotent stem cells, are the base rationale for the scientific approach in tackling the challenge. There is the need to set up and share good protocols for rearing marine invertebrates and for MISC isolation, culture and phenotyping. This should be done on a wide spectrum of marine invertebrate species and requires the integration of different laboratories, in European countries, and small biotech companies in each specific European country. The COST Action is probably the best and most innovative approach to tackle all the challenges that were listed above. Innovation will be achieved by addressing scientific problems with clear objectives, as those indicated in section 3 of this proposal, and through the frequent interactions among the Action members. This will also enhance competitiveness of the research.

b) need for consolidation of the fragmented scientific arena. The scientific community in the European countries, currently engaged with MISC biology, is fragmented and composed of too few numbers of established scientists. The recruitment of a new generation of young scientists interested in MISC, the consolidation of the fragmented MISC community in Europe, the frequent meeting among members, through participation in workshops, meetings and Short-Term Scientific Missions to other laboratories, will favour the generation of new ideas and joint research projects in the field of MISC.
c) need for contacts with industries. The MISC field needs to develop connections with relevant biotech industries and it faces with the challenge in convincing the multiple types of stakeholders (i.e., those reported in para 2.B.I) that, through its know-how and expertise, it can achieve what is promised. Contacts with industries interested in exploiting MISC for their regenerative potential and/or the production of bioactive molecules or metabolites useful in animal/human welfare and/or biotechnological applications will be established. Marine/aquatic organisms have revealed and still can reveal unexpected gene regulatory pathways of interest in regenerative biology; in addition, they are known sources of valuable biologically active substances for the pharmaceutical, biotech and food industries. Many marine secondary metabolites are known for their highly effective antioxidant, antibacterial, antifungal, antifouling and antitumor activities. Ideally, the possibility to produce such bioactive compounds using MISC-derived immortalised cell-lines, would be of great importance in developing societal improvements and advances. An academic-industry collaborative approach can add new value to applied MISC research.

D) ADDED VALUE OF NETWORKING

I) IN RELATION TO THE CHALLENGE

Stem cell science is an emerging global industry in which European countries fiercely compete for economic advantage in an arena where, currently, USA and Japan dominate. In contrast, most European groups (academics, as well as companies and SMEs) are dispersing their scientific efforts using different biological models and methodologies, where no stream of knowledge and intellectual property are available. There is thus a pressing need for expertise, approaches and tools. The only way forward is by consolidating a network of MISC groups sharing expertise. It is not possible to be competitive in the international arena if research groups/projects are not promoted through collaborative, intra-EU actions. Networking is one of the most efficient approach for consolidation of the European MISC community and raising European competitivity and leadership. Such networking should focus on the biological diversity of MISC and their structures, totipotency properties, development and other understudied, unique or culturally valuable (e.g., aging, cancer), biological/applied aspects. **Thus, the Action will develop European leadership and expertise in MISC discipline**.
The fragmented research community currently limits European competitiveness also in technology transfer to potential end-users. For example, there are, currently, only few SMEs in COST countries that consider MISC as part of their R&D plan. This Action will not only offer a unique nucleus of commercial importance, but also the pooling of individually acquired knowledge within a single community, offering the participants better access to international grant agencies or to products under development in each country/group. **Thus, this COST Action will have a competitive ability to develop innovative, effective, and flexible adaptation strategies that will address multiple national, regional, and global priorities in key economic and social sectors.**
Networking will help to discuss novelties and research results, attract students to the MISC discipline, provide opportunities to mix newcomers with important players in the MISC arena and stem cells biology discipline and learn about the potential offered by the markets. In addition, amalgamating the European community working on MISC (both academy and industries) may pose significant challenges to the future of European stem cell science.
Particular attention will be paid to the training of early-stage researchers by organising training schools, by favouring their mobility through Short-Term Scientific Missions and their participation to scientific meetings and workshops. The coming generations of young

researchers (trained in the MISC discipline) will be the future leaders in marine science. Networking will also give additional strength to outreaching activities, in particular those promoting the societal awareness of the importance of sea and its biodiversity.

II) IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Despite the great efforts, variety of funding schemes and actions within Horizon 2020, no former or existing scientific networks/projects in Europe dealt/are dealing with MISC and, up to-date, no individual entity (either private or national institute) in the European MISC discipline has gathered a critical mass of researchers and knowledge to become a globally leading contributor. There is an urgent need to increase the cohesion between scientific institutions and industry in this field via the creation of a COST Network. Indeed, because of the scientific community fragmentation, it is not known whether methodologies developed for other animal systems are being utilised in developing MISC or have failed; likewise, failed experiments/approaches are not being presented nor published in peer-reviewed journals. This makes exchange of information utterly crucial in the research community, sharing promising methods to avoid replication of failed or redundant procedures. Therefore, it is expected that de-fragmentation of the MISC scientific community can revolutionise this field, resulting in novel generic technologies/products.

This COST Action will be tightly connected with various European associations that deal with cell cultures. One such important organisation is the European Collection of Authenticated Cell Cultures (ECACC), a supplier of authenticated and quality controlled cell lines. The same implies for the Horizon 2020 research and innovation programme called EuroStemCell that tries, among its major goals, 'to help European citizens make sense of stem cells, by providing independent, expert-reviewed information and road-tested educational resources on stem cells and their impact on society'. The MISC participants will also team with the ECVAM (https://eurl-ecvam.jrc.ec.europa.eu), which is the EU reference Centre for the development and validation of alternative testing methods to replace, reduce or refine the use of laboratory animals in biomedical sciences with an emphasis on toxicology assessment.

This COST Action will interact with the Blue Growth initiatives included in other European science consortia/networks/platforms (Assemble, Euromarine, Eurostemcells, EMBRC, Corbel, ERA-MBT, EuroSyStem, Neurostemcell, Neurostemcellrepair), and will contact participants to current and former EU projects on marine and stem cell science, such as MarBEF, Euroceans, MarineGenomics, OptiStem, ESTOOLS.

2) IMPACT

A) EXPECTED IMPACT

I) SHORT-TERM AND LONG-TERM SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS

The aim of this Action is to mobilise the European scientific expertise in MISC research and associated technological resources so as to provide professionals with relevant state-of-the-art information, methods and tools for the amelioration and treatment of diseases (gene regulatory networks, antimicrobials, antimitotic compounds), and for pharmaceutical and biotechnological purposes (enzymes, polymers, secondary metabolites). The heavy investment in developing and sharing technologies, from genomic/proteomic tools to high throughput analysis of chemical compounds, necessitates support from a solid network of scientists with complementary expertise. The MISC Network will provide such a wide range of expertise. The Network will use resources to determine how and when to involve end users in knowledge dissemination plans. Knowledge translation (synthesis, dissemination, exchange, and ethically sound application of knowledge) will be used to improve MISC activities between researchers and knowledge users. Interactions may vary in intensity, complexity, and level of engagement depending on the research, findings, and needs of particular knowledge users.

Scientific-technological short term impacts

• creation of an Europe-wide research network to answer to the key questions on MISC;
• promotion of the interactions among scientific institutions interested in MISC;
• release of updated standardised scientific protocols/technical guidelines for rearing of marine/aquatic invertebrates and MISC isolation and culture;
• creation of registry/repository for sharing data on MISC research;
• creation of a website and a newsletter as a forum to link MISC community;
• common publications in peer-reviewed and open access scientific journals describing optimised protocols for MISC isolation and rearing.

Socio-economic short term impacts:

• increase of public awareness on importance and potential of MISC.
• starting collaboration with industries for the exploitation of MISC.
• training initiatives for students/young researchers.
• encouraging the creation of new networks for fund-raising opportunities

Scientific-technological long-term impacts:

• new insights on the biology of MISC and on mechanisms that control their in vitro growth. Results will represent a commonly distributed know-how.
• contribution to a wide range of biomedical discipline (e.g., regenerative medicine, aging and cancer). The study of MISC will increase commonly shared and evolutionary perspectives in these disciplines, towards a more comprehensive understanding.
• positive impact on regeneration biology as marine/aquatic invertebrates have uniquely regeneration potential and can contribute to the comprehension of the constraints preventing large scale regeneration in vertebrates. MISC can also be used to assay the impact of different chemicals in their ability to regenerate tissues.
• new strategies for sustainable exploitation of marine bioproducts, and for development of alternative ecotoxicological tests for biomedical and biotechnological industries. The management of intellectual and industrial property rights arising from this Action will ensure that potential benefits of MISC project results are shared fairly and reasonably among the institutions of the COST Action participants.
• better understanding the impact of environmental stressors (temperature, acidification, etc.) in regeneration processes and in resilience of challenged aquatic ecosystems.
• MISC can differentiate in a variety of cell lines, including hemocytes and, among them, immunocytes. Therefore, the study of MISC differentiation to immunocytes can provide a better elucidation of the behaviour of the immune system in reared, edible marine invertebrates and help in control diseases and viral infections in aquaculture plans of marine edible organisms.

Socio-economic long term impacts:

• change of public perception concerning marine invertebrates towards a full awareness of the socio-economic importance of marine biodiversity, promoting the sensible use of marine resources and the protection of the marine environment for future generations. As a matter of fact, degraded marine ecosystems provide fewer goods and services than healthy habitats via decreased abundance of living species. As reported, marine invertebrates, can be the source of cellular systems reliable for sustainable biotechnological production of new bioactive molecules useful for human and animal health and other applications (e.g., antifouling, enzymes for biocatalysis, biopolymers, products of interest to pharmaceutics, nutraceutics and cosmetics) of interest to biomedical and biotech industries.
• change of the social acceptance for the MISC importance in day to day life.
• awareness of MISC potential by stakeholders, contacted through specific meetings with SMEs representatives. Even if still at early stages, MISC research is opening up competitive niches of potentially lucrative avenues for the development of protocols and technologies. Similarly, the MISC market is backed by biomedical research and bioprocessing.
• the availability of MISC will also increase potential monetary benefits to society by adding novel tools for scientific disciplines, including mammalian stem cells biology. This reflects the objectives of the European Strategy for Marine & Maritime Research and the last European Science Foundation positional paper on marine biotechnology. MISC also represent one of the targeted topics in the EU consortia ASSEMBLE and EMBRC, aiming to promote marine laboratory infrastructures.

• Young European researchers with careers in the MISC discipline will become the new generation of MISC researchers in Europe.

B) MEASURES TO MAXIMISE IMPACT

I) PLAN FOR INVOLVING THE MOST RELEVANT STAKEHOLDERS

At the start of the Action, a dissemination plan will evaluate maximizing impacts using a **who** (relevant endusers)-**how** (dissemination plan)-**when** approach.
The most relevant stakeholders are the following:
• **SMEs**, in particular: **i**) the antifouling paint sector that can take advantage from new natural antifouling products preventing the growth of the bacterial film that triggers the adhesion of encrusting organisms, without any concern for the environment and alternative to those currently in use, that have profound effects on the biocoenoses once in the environment; **ii**) the fine chemical sector, for a wide range of materials; **iii**) the nutraceutic and cosmetic sector, interested in new useful bioactive molecules; **iv**) the pharmaceutic and medical device sector, where new antimicrobials are required to face the increasing number of bacterial strains resistant to penicillin-based antibiotics; **v**) the human health sector, as new antimitotic compounds can be of great help in the treatment of some kinds of cancer;
• the medical community interested in alternative molecular mechanisms of aging, differentiation, tumour formation and regeneration operating in marine/aquatic invertebrates;
• the broader scientific community studying stem cells, their role and differentiation pathways, that can gain additional knowledge from the behaviour of MISC;
• the general public that can have benefits from the results of MISC research;
• the European networks interested in stem cells and MISC, such as: **i**) EuroStemCell; **ii**) the Horizon 2020 research and innovation program aiming to help European citizens making sense of stem cells; **iii**) the ECVAM, European Commission reference Centre for the development and validation of alternative testing methods to replace, reduce or refine the use of laboratory animals in biomedical sciences; **iv**) the ECACC, European Collection of Authenticated Cell Cultures, a supplier of authenticated and quality controlled cell lines.

II) DISSEMINATION AND/OR EXPLOITATION PLAN

Scientific community
Publication of results as joint research articles and presentations at important international conferences in the field, together with organisation of courses and seminars will help us to improve the visibility and impact of this Network on an international scale. The detailed list of these dissemination activities includes:
• writing collaborative review articles on MISC research in peer-reviewed, high impact (possibly open access) scientific journals;
• editing a scientific book focused on MISC and/or regenerative biology;
• exploiting courses/workshops/meetings to disseminate the main outcomes of the Action among scientists;
• promoting workshops/teaching activities on MISC in the European universities through the initiative of the participants of this COST Action;
• promoting interuniversity agreements aimed to an International PhD program on MISC;
• addressing students, in mentoring activity, to research on MISC for their degree thesis;
• creating new networks, within the MISC community, research proposals/projects resulting from the networking activities that will occur under this Action;
• participating to international conferences on stem cells. This will provide good opportunities to share the results obtained within this COST Action with a wider scientist network.
SMEs
• promoting transfer of knowledge, expertise and technical skills from this COST Action to the stakeholders as possible end-users through specific meetings/workshops;
• organising specific workshops/meeting with industries to help the interaction with the biotech world. The contact with some SMEs interested in the above-reported field has already started and they will be invited to specific workshops, as indicate at section 3.A.II. Furthermore, the

European Networks cited above, will be contacted by the Management Committee of this COST Action and representatives will be invited to the meetings/workshops.

General public

It is clear that a pressing problem the European countries are facing in recent times is the communication between scientists and the general public. The striking features of simple aquatic organisms to which the general public is exposed during leisure activities at the seaside are good ambassadors to communicate about the potential of European research to improve daily life. These planned activities are of special concern and have been considered:

• activating and maintaining active, even after the closure of this COST Action, an open website, as a preferential platform to share protocols, methods, etc. and to offer accessible knowledge to the general public;

• working together with Innovation and Press offices of participants' Institutions and coordinating activities with other European bodies for efficient outreaching activities to introduce the lay public to the research performed by the network.

C) POTENTIAL FOR INNOVATION VERSUS RISK LEVEL

I) POTENTIAL FOR SCIENTIFIC, TECHNOLOGICAL AND/OR SOCIOECONOMIC INNOVATION BREAKTHROUGHS

Stem cell biology in vertebrates has a great deal to offer to society and industry. The ability to culture vertebrate cells in the lab has supported tremendous breakthroughs in science over the years, from the very foundations towards cell therapy and tumour stem cell biology. It is evident, in the mammalian stem cell biology discipline that stem cells have been invaluable for treating a number of intractable diseases and that boundaries are pushed and frequent discoveries are made. It is above dispute that innovative technologies in the mammalian stem cells arena continue to proliferate, striving to advance the research. There are two main critical issues in the clinical/commercial translation of stem cells intellectual properties and products: (**1**) entrepreneurial exploitation of breakthrough ideas and innovations, and (**2**) regulatory market approval. Thus, the commercial development of stem cells products and innovations reflects potential high risks due to technology risks, changing policies, changing markets, and management changes, in the highly dynamic field, but on the other hand- the benefits incurred from a successful approach are tremendous. What is surprising about the recent stem cell breakthrough in the mammalian systems, is that researchers find new discoveries that could not be found if the research was not focussed on the stem cells biology. The same implies for the MISC discipline that answers the strategic breakthrough needs of wide applied biotechnology and healthcare issues and provides additional innovative facets that are not found in the mammalian stem cells discipline:

a) Comparative aspects. Comparative approaches on MISC essentially consist of examining whole genome structures, gene arrangement and rearrangement, stem cells lineages, stem cells properties (such as stemness capabilities, structures, etc.) with the aim of delineating the evolution of gene families and cell lineages, the cellular and molecular basis of adaptation (including the identification of cells potentially involved in niche adaptation) as well as the evolutionary relationships at various taxonomic levels. The high *in vivo* plasticity of MISC shapes, structures, cell replacements, proliferation processes and cell lineages, in different invertebrate taxa, make a comparative study approach highly valuable. In the mammalian stem cells arena, only few comparative studies are available and this confines many prediction on stem cells origins, activities and fates.

b) Environmental approaches. They deal with the understanding of the functional significance of cellular variation in MISC - the basic unit of selection - in natural biological entities. This includes the use of various genotyping approaches to delineate the structure of inter- and intraspecific biodiversity of MISC, as well as the metagenomics approach to MISC, which treats entire organisms (sometimes even populations) as carrying a single living entity. This was never done in the vertebrates in spite of various theoretical approaches.

c) Evolutionary perspectives. MISC may also provide some understanding of evolutionary relationships between different phyla and within-phylum groups. This is particularly valid for

organisms that possess MISC types with major evolutionary importance (such as colonial urochordates), either with respect to phyletic novelty or to structural cell lineages that can only be investigated using an evolutionary approach. There is still only a poor understanding of stem cells origins and their importance in governing the dynamics of stem cell populations over evolutionary time.
d) Changing of current dogma(s) such as disposable soma, irreversibility of aging, germ/somatic cell barriers, as demonstrated by the capacity of whole body regeneration from small fragments or the ability to rejuvenate, easily found in marine invertebrates.

3) IMPLEMENTATION

A) DESCRIPTION OF THE WORK PLAN

I) DESCRIPTION OF WORKING GROUPS

Five Working Groups (WG) have been identified, each WG is involved in the analysis and development of different specific topics.

WG 1 - Developing protocols for raising marine/aquatic invertebrate stem cells under *in vitro* conditions
WG1 coordinates the activities of a series of tasks devoted to the development of common protocols, problem solutions and tools (e.g., the development of resource services) in order to foster integration of research institutions. This will guide the development of shared services and solutions not only within the research institutions but also into the working environments of stakeholders and users to lay a solid foundation for long-term cooperation.
Task 1.1 - new marine invertebrate models and access to marine resources
Task 1.2 - the problem of endosymbionts in establishment of pure or mixed cell cultures of MISC
Task 1.3 - methods for stem cell enrichment in culture
Task 1.4 - immortalisation of marine/aquatic invertebrate stem cells
Task 1.5 - cryopreservation of marine/aquatic invertebrate stem cells
Deliverables:
1.1 Report listing the reference laboratories, institutions, marine stations for the supplying of marine/aquatic invertebrates;
1.2 Publication reporting on common protocols for MISC identification, isolation, enrichment, immortalisation, rearing, storage;
1.3 Guidelines to solve the problem of endosymbiont contamination that, up to now, made fruitless the efforts of in vitro rearing of MISC.

WG 2- “omics” to characterize the MISC phenotypes
Technical and scientific capabilities to support the cooperation are coordinated by WG2: molecular/biochemical profiling of novel model organisms needs data services for annotation, analysis and archiving.
Task 2.1 - comparative functional genomics and transcriptomics of marine/aquatic invertebrate tissues or derived MISC
Task 2.2 - comparative proteomics of marine/aquatic invertebrate stem cells
Task 2.3 - differentiation molecular pathways of MISC
Task 2.4 - development of strategies for “manipulating” stem cells (knockdown, CRISPR, transgenesis, etc.)
Deliverables:
2.1 Report on biochemical and biomolecular stem cell markers for aquatic invertebrates
2.2 Creation of a shared, trans-European open access database with molecular data of the organisms of interest, continuously implemented by the COST Action participants
2.3 Report on biochemical and biomolecular stem cell markers for aquatic invertebrate organisms;

2.4 Report detailing the strategies for “manipulating” MISC (knockdown, CRISPR, transgenesis, etc.);
2.5 Comparative evaluation report on proteomics of MISC.

WG 3- Blue biology: MISC as model systems for the study of (see tasks)
Task 3.1 - evolutionary aspects of stem cell differentiation and development
Task 3.2 - cancer, aging and senescence phenomena in marine/aquatic invertebrates
Task 3.3 – regeneration in marine/aquatic invertebrates
Deliverables:
3.1 Publication on genes, signal transduction pathways, proteins involved in development, senescence, regeneration, prevention/induction of cancer of MISC;
3.2 Publication on conserved detoxification pathways that allows MISC to survive in organisms exposed to altered environmental conditions;
3.3 Review paper on the main evolutionary steps/passages in the evolution of development, senescence, regeneration.

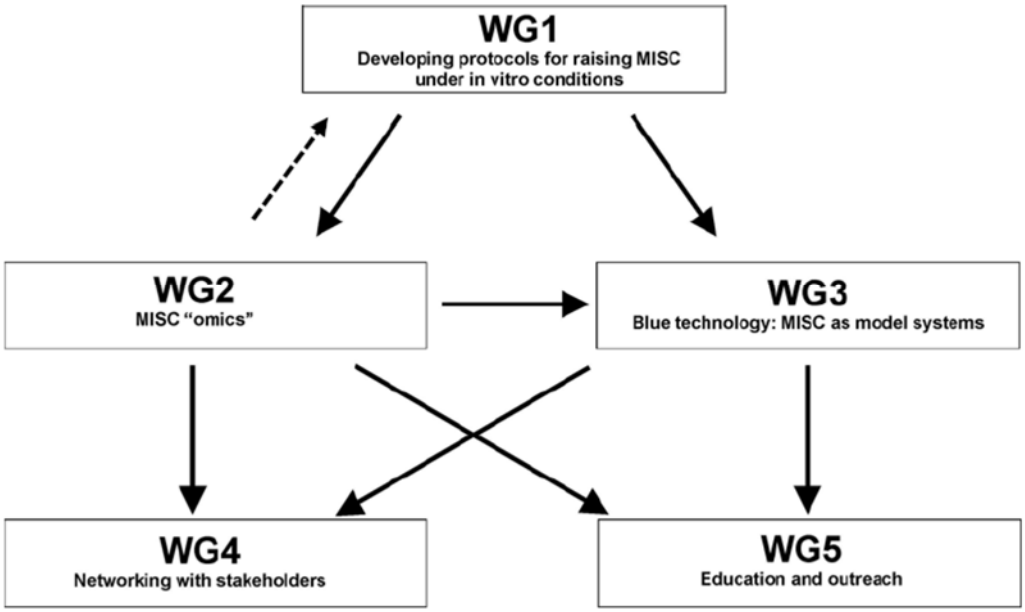
WG 4- Networking with stakeholders
The evaluation of the potential of MISC to provide useful biomolecules is the focus of WG4
Task 4.1- bioactive molecules from marine/aquatic invertebrates. The technology developed in culture may be instrumental in solving some practical tasks in marine biotechnology, e.g., the generation of cell cultures producing complex bioactive compounds.
Task 4.2 Identifying stakeholders in COST countries. Stakeholders will be contacted from the beginning of the COST Action and invited to specific workshops in which the potential applications of MISC will be discussed.
Task 4.3 Workshop with stakeholders.
Deliverables:
4.1 Special issue on identified bioactive molecules (antimicrobials, anticancer, opsonins, enzymes) in marine/aquatic invertebrates of potential use in human health, pharmaceutics, nutraceutics, cosmetics, antifouling paint formulation.
4.2 Report on identified stockholders interested in MISC in COST countries.

WG 5- Education and outreach
This WG will coordinate the training of young researchers and dissemination of results, in order to strengthen the human capital, within Europe with expertise on MISC, establish contacts between researchers, and shared best practices in the field of MISC.
Task 5.1 - training of the next-generation researchers
Task 5.2 – training schools
Task 5.3 - dissemination (in & out) of research results
Task 5.4 - website
Task 5.5 - society and science
Deliverables:
5.1 Overview publication on MARISTEM Action on MISC, as 'Research Ideas & Outcomes".
5.2 Creation of an Open website, as a preferential platform to share protocols, methods, etc. and to offer accessible knowledge to the general public.
5.3 Scientific book focused on MISC and/or regenerative biology

II) GANTT DIAGRAM

	Year 1		Year 2		Year 3		Year 4	
General action management								
First General Action meeting	+							
Management Committee (MC) meetings		+		+		+		+
Core group (CG) meetings	+	+	+	+	+	+	+	+
Launch of Action website	+							
Final meeting/conference								+
WG1 Developing protocols for raising marine/aquatic invertebrate stem cells under in vitro conditions								
Task 1.1 New marine invertebrate models and access to marine resources	+	+	+	+	+	+	+	+
Task 1.2 The problem of endosymbionts in establishment of pure or mixed cell cultures of MISC		+	+	+	+			
Task 1.3 Methods for stem cell enrichment in culture		+	+	+	+			
Task 1.4 Immortalisation of MISC			+	+	+	+	+	+
Task 1.5 Cryopreservation of MISC			+	+	+	+	+	+
WG2 "omics" to characterize the MISC phenotypes								
Task 2.1 Comparative functional genomics and transcriptomics of marine/aquatic invertebrate tissues or derived MISC	+	+	+	+	+	+	+	+
Task 2.2 Comparative proteomics of MISC	+	+	+	+	+	+	+	+
Task 2.3 Differentiation molecular pathways of MISC		+	+	+	+	+	+	+
Task 2.4 Development of strategies for "manipulating" stem cells (knockdown, CRISPR, transgenesis, etc.)				+	+	+	+	+
WG3 Blue biology: MISC as model systems for the study of (see tasks)								
Task 3.1 Evolutionary aspects of stem cell differentiation and development	+	+	+	+				
Task 3.2 Cancer, aging and senescence phenomena in marine/aquatic invertebrates					+	+	+	+
Task 3.3 Regeneration in marine/aquatic invertebrates			+	+	+	+	+	+
WG4 Networking with stakeholders								
Task 4.1 Bioactive molecules from marine/aquatic invertebrates		+	+	+	+	+		
Task 4.2 Identifying stakeholders in COST countries		+	+	+	+	+	+	+
Task 4.3 Workshops with stakeholders			+		+		+	
WG5 Education and outreach								
Task 5.1 Training of the next-generation researchers		+	+	+	+	+	+	+
Task 5.2 Training schools			+		+		+	
Task 5.3 Dissemination (in & out) of research results			+	+	+	+	+	+
Task 5.4 Website	+	+	+	+				
Task 5.5 Society and science		+		+		+		+

III) PERT CHART (OPTIONAL)



IV) RISK AND CONTINGENCY PLANS

The main risks of the Action are related to scientific tasks of WG1, with a current lack of success in propagating cultures of MISC. The current stall in cultivating MISC is directly related to the major obstacles listed above, including the fragmentation of the scientific community working on MISC and the lack of networking, the deficiency of European students and early career scientists trained in the MISC discipline, the lack of connections with industries and more advanced models of mammalian stem cells (technology transfer to potential end-users), the general lack of knowledge of MISC biology and on the intraspecific and interspecific communication of MISC cellular components for their survival. This is why this Action targets the above weakening points. The positive aspects of the Network, in terms of sharing protocols, advancing the use of marine systems, comparing the stem cell biology or the regeneration processes in non-conventional metazoan models, and studying the environmental effects of toxic components, far outweigh the main risks of the Action. The need of networking and coordination in this field is so evident that, even in the presence of negative results in establishing immortalised MISC, the Action will undoubtedly generate new tools for research and exert a positive influence on Action Members, next-generation researchers and European researchers in the field of invertebrate stem cells.

B) MANAGEMENT STRUCTURES AND PROCEDURES

The management and organisation of this Action will be carried out according to "Rules and Procedures for Implementing COST Actions". The Action will be managed by the Management Committee (MC); MC Members will be nominated by the COST National Coordinators of the Participating COST Countries. The MC will elect during the first MC meeting the Chair, the Vice-Chair, the WG Leaders, the STSM Coordinator/ Committee. The MC will make decisions on the Action's activities, evaluate the progress of the scientific tasks from WG reports, evaluate the progress of deliverables, prepare all required scientific reporting and promote the contacts with other relevant EU networks. The Core Group (CG) will be constituted and will consist of the Action Chair, Vice Chair and WG Leaders, STSM Coordinator and other key position deemed necessary by the MC. During the first MC meeting, the MC will decide on the mandate of the CG.

C) NETWORK AS A WHOLE

Cellular (stem cells), genomic, proteomic and bioinformatic technologies are advancing rapidly and it is often difficult for those involved in fundamental biological research aspects to keep up to date and have access to the latest tools. These tools are often developed by laboratories that are restricted in their access to suitable and tractable models with which to fully exploit the full potential of their powerful tools and their application to important healthcare problems. This Action is unique in combining a network of European laboratories that are at the forefront of using organismal, cellular and genomic technologies with biologists studying the fundamental aspects (such as stem cell biology, aging, regeneration and tumour formation), end users that are associated with industries (five different sectors were outlined in section 2.B.I) and human wellbeing in the European, also tackling with the pressing environmental challenges our seas and oceans face right now.

This COST Action aims at a novel research collaboration within a scientific community that, up to now, is highly fragmented. Within this Action, for the first times, participants will join their forces to common objectives for the consolidation of research on MISC at the European level in order to strengthen this emerging field in academies (e.g., by promoting the institution of university courses devoted to MISC) and create synergy with R&D Institutions. Up to now, research on MISC in Europe has been very limited, with scattered expertise, and hampered by low funds and scarce attention by the scientific community. Putting together participants from different countries on common objectives related to MISC, has the potential of producing a strong scientific impact in the field. In this context, this Action will be a comprehensive, integrated, multidisciplinary genomic and proteomic approach to understand the basic biology of stem cells and regeneration. One of the main aims will be, in the long term, to improve and enhance treatment of disease by utilising homologous gene networks and gene products to mobilise natural adult stem cell populations and to create pluripotent cells. Others aims are to provide the scientific community with new biomolecules for applicative research.

The network will be the connecting avenue for all the people and institutions carrying out work on aquatic organisms in Europe, with a critical mass of more than hundred researchers, using the following model organisms: Porifera, Cnidaria, Platyhelmintes, Mollusca, Echinodermata, Crustacea, Tunicata.