MEMORANDUM OF UNDERSTANDING

Subject: Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1202: European Network on Microvesicles and Exosomes in Health and Disease (ME-HAD)

Delegations will find attached the Memorandum of Understanding for COST Action as approved by the COST Committee of Senior Officials (CSO) at its 185th meeting on 6 June 2012.
MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action BM1202
EUROPEAN NETWORK ON MICROVESICLES AND EXOSOMES IN HEALTH AND DISEASE (ME-HAD)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 “Rules and Procedures for Implementing COST Actions”, or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The aim of this Action is to foster a multidisciplinary approach to research on ME, accelerating progress on the levels of basic science and technology, physiology, pathology and translational research, with the ultimate goal of exploiting ME for clinical application, achievable only through co-ordinated efforts and valorisation thus recruiting interest and funding from industry.

3. 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 40 million in 2012 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

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A. ABSTRACT AND KEYWORDS

Microvesicles and Exosomes (ME) have attracted much recent interest because of their potential functions, use as disease biomarkers and possible therapeutic exploitation. Due to their enormous relevance, through nationally-funded projects throughout Europe, this relatively new field of research is quickly expanding. There are, however, many associated issues that need to be addressed to ensure optimal research performance and collation and cross-interpretation of data. These issues range from the need to derive a consensus on fundamental guidelines/nomenclature and optimal techniques for vesicle isolation and analysis, to more advanced issues such as collating emerging data and expertise towards better understanding, and thus exploiting, the physiological and pathological roles of ME. There is also urgency to identify gaps in ME knowledge and how best collaborative research can address these questions. This COST Action will, therefore, create a network of European experts, fostering a multidisciplinary approach to enhance both basic understanding and translational potential of ME. To achieve this, this Action will bring together a critical, yet currently fragmented, mass of academic, clinical and industry expertise from COST and non-COST countries in a field where Europe plays a leadership role, while developing a forum for free-exchange of concepts and training of young European researchers.

**Keywords**: Exosomes; microvesicles; cell-to-cell communication; translational research; intersectoral training

B. BACKGROUND

B.1 General background

The research topic of this COST Action is microvesicles and exosomes (ME). Cell-derived vesicles or extracellular vesicles is often used as a general term for small vesicles, expelled by most cells and ranging from 30-1000nm in diameter. Exosomes (a nano-sized sub-family of vesicles), first described in 1983, have gained substantial interest since recently shown to play a role in intercellular communication. These discoveries marked the beginning of many distinct research efforts in this field. As ME have implications in both health and disease, diagnostics and therapy, attention of basic scientists, clinicians and industry has been attracted.
Increasing interest in ME is evident and many European research groups, based in academia, clinics and industry have initiated research programs –funded though national initiatives– for the study of microvesicles and/or exosomes. Evidence for the increased interest include the fact that the 1st international conference on this topic (in 2005) attracted 25 attendees, while the 2011 workshop in Paris had 250 attendees; with many hopefuls turned away due to space limitations. As a further indication of the growing interest in this area, by 2003 PubMed listed 20 publications on ME, with >200 publications in the past year alone; frequently led by Europe. More than 700 researchers signed up to the recently-established online non-funded International Society, led by Europe, and dedicated to extracellular vesicles. The Society and this Action are highly complementary.

Despite increasing ME research, there is no consensus or guidelines yet as to how these entities should be defined, purified, analysed etc. Ultimately, without a platform to bring together relevant stakeholders to reach a consensus, each group- while, undoubtedly, performing important locally-funded research- typically must define and then apply their own criteria in this regard. Furthermore, no organised co-ordination, exchange of ideas, techniques, materials and people exists between these numerous European research groups and there is no forum for fundamental co-operation aimed at bringing together emerging data or for collectively identifying where gaps in our knowledge exist and how best we can work together to answer these questions.

The Participants, who developed this Action, believe that it is imperative that the European research community works together on these issues in a manner that is inclusively of all interested stakeholders and so includes not only academics, clinicians and industry from COST countries, but also from non-COST countries. Overall, the main motivation for this COST Action entitled Microvesicles and Exosomes in Health and Disease (ME-HAD) is to link up experienced and early-stage researchers, to generalise previous work and address the challenges outlined above at a pan-European level. So having considered all possible options including the European Science Foundation (ESF), European Space Agency (ESA), EUREKA or Framework Programme 7 (FP7), the Participants believe that an interdisciplinary Action, ensuring that Europe continues to lead this field, is the most relevant framework to success.
B.2 Current state of knowledge

Although it has long since been realised that eukaryotic cells release complex structures, termed membrane vesicles, into their extracellular environment, only in the past decade has it been realised that these entities are not merely junk or debris indicative of cell death, but that they may be micro- or nano-maps of their cell of origin and may be of both physiological and pathological relevance.

Microvesicles and exosomes (ME) are often defined and sub-grouped first-and-foremost on size and proposed origin (exosomes ~30nm-100nm, endosomal origin; microvesicles >100-1000nm, from the cell membrane). However, there is no reason to believe that microvesicles of 30-100nm cannot also exist and the relevance of size per se is unknown. Furthermore, once the vesicles have been released, their origin cannot be identified as unique markers for the different vesicle types have not yet been identified. Vesicles for analysis are typically isolated from medium conditioned by cultured cells, serum, saliva and other body fluids, based on size; through a range of methods (See D.2 for more details), based on the Researcher’s choice but not standardised between laboratories. Similarly, basic quantification and characterisation of the resulting ME populations are varied and non-standardised (again, see D.2). Collaborative efforts to review this area and reach a consensus on guidelines will, undoubtedly, lead to more robust output from European research and help fast-track exploitation of its clinical relevance through academic, industry, clinical collaborations.

Considering origin and biogenesis of ME, exosomes are believed to be generated in endosomal compartments termed Multivesicular Bodies (MVBs) that pack and store molecules in membrane-bound structures. These MVBs may then fuse with the cell membrane, releasing their contents of small vesicles extracellularly. Since 1987, these small vesicles are termed exosomes. In contrast, so called microvesicles are typically described as derived from the cell membrane. Frequently the term extracellular vesicles or cell-derived vesicles are used to include both microvesicles and exosomes; although some authors sub-divide their description as exosomes and “other vesicles”. However, the origin of isolated vesicles cannot be studied retrospectively, as markers specific for different vesicle types are unknown. So, in this regard, there is no consensus on the relative importance of separate mechanisms of biogenesis. Adding to the complexity with regards to terminology, ME from different origins are sometimes randomly assigned other titles (e.g. those isolated from blood are sometimes termed “microparticles”; from seminal fluid, “prostasomes”; from cancer/tumour cells, “oncosomes”, etc.).
Notwithstanding the necessity for networking and a consensus on terminology and methodology, it is important to highlight the importance that has been attributed to MEs to date. Major milestones include the observation, in 1996, that these vesicles play a role in intercellular communication. In brief, EBV-transformed B-lymphocytes were found to secrete exosomes that bore MHC II bound to antigenic peptides, essential for the adaptive immune response. In 1998, dendritic cells were also reported to secrete exosomes that stimulate T cells, thus initiating adaptive immune response and ultimately anti-tumour activity in mice. Arising from this, a limited number of exosomes-based cancer vaccines are being explored in clinical trials.

Global analysis of ME contents, possible so far, indicates that they contain both molecules common to ME from different origins and others specific to a given cell of origin. ME from normal cells shows that typically the proteins included are from the cell membrane, endocytic pathway, and cytoplasm, but rarely from the ER, mitochondria, nucleus and Golgi apparatus. However, MEs from abnormal (e.g. cancerous) may also originate from other organelles e.g. “leaky” mitochondria. In addition to proteins, ME also contain mRNAs and non-coding RNAs. In fact, further support for a role of exosomes in cellular communication was the observation, in 2007, that mRNAs secreted within exosomes from a “donor” cells can be translated by “recipient/target” cells, at least \textit{in vitro}. Relevance of exosomes \textit{in vivo} has also recently (2011) been shown, where exosomes administered into mice prepared niches for attaching tumours cells/metastasis. Another seminal example (2011) involved pay-loading dendritic cell exosomes with Beta-secretase 1 (BAEC1)-specific siRNA, administering i.v. and then observing knock-down, in the brain, of BAEC1 i.e. an important protein in Alzheimer’s. Additionally, exosomes have been successfully used as delivery systems for chemical drugs (e.g. curcumin) that show more efficacies when delivered in exosomes than in their soluble form.

Overall the importance of studying and understanding ME is evident by their implication in cell communication and mis-communication; in antigen-presentation; tumour immune suppression; tumour-stromal interactions; metastasis; angiogenesis; drug-resistance; stem cell differentiation; neurodegeneration; reproduction; and other processes and that the contents of vesicles in body fluids provide valuable information on the state of the tissue of origin. Yet we don’t yet know the breadth and scope of their relevance in either physiological or pathological circumstances.
We do know, however, that -depending on their source and target cells- these vesicles incorporate and transfer selected mRNA, non-coding RNAs, and proteins. These, thus, have potential as biomarkers for disease which can be harvested from body fluids overcoming the requirement for biopsies (ie non- or minimally-invasive) as well as "self" delivery systems which could be harnessed for therapeutic purposes.

This Action is innovative as it brings together experience and young researchers from academic, clinical and industry settings all who share a common goal of progressing the field of European ME research in a logical and co-ordinated manner. This new integrative interdisciplinary approach for the critical concerted assessment of terminology/nomenclature, methodology, experimental design and data interpretation will advance from current state-of-play regarding the basic understanding to the diagnostic and therapeutic potential of ME. This action will also improve creativity and entrepreneurial mindset among ME researchers. The collaboration with industrial partners will allow us to explore the exploitability of European-based research with respect to biomarker and drug generation.

B.3 Reasons for the Action

The main reason for this Action is to enable high-quality collaboration and valorisation between experienced and early-stage academic, clinical and industry-based European researchers involved in ME research, maintaining Europe’s dominant position within this rapidly developing field.

Despite the scientific, medical and thus potential economical relevance of understanding and exploiting ME, a number of unresolved scientific questions remain in urgent need to be addressed in order to accelerate the successful translation of basic research findings into medical application. Addressing these scientific issues, while this field is still at a relatively early stage, is important to ensure comparability of current and emerging research output and to advance our understanding of the relevance of these vesicles. Future progress in this field critically depends on resolving these objectives now. To achieve this, we must create a network of researchers from European and non-European countries to facilitate fruitful networking and collaboration, hopefully leading to improved understanding of the role of ME in both physiology and pathology.
Advancing upon this, there is also an urgency—and importantly, the goodwill—to bring together emerging data and expertise from across Europe to aid us advance our understanding of both the physiological and pathophysiological functions of ME; to determine how best ME may be exploited for therapeutic reasons; to draft principles for large-scale production of ME for therapeutic uses; and, crucially, to identify where gaps in our collectively knowledge of ME exist and thus how best we can work together to answer these questions.

Currently research activities in this space are scattered throughout many laboratories with no framework for coordination and knowledge exchange. This inclusively interdisciplinary COST Action is considered the optimal way to synergise the Participants’ existing research activities in this field and to immediately improve exchange of knowledge and people within its community, acceleration the solution to these scientific problems. This Action will advance basic scientific and early clinical progress and form a strong foundation for future scientific and clinical application.

### B.4 Complementarity with other research programmes

With regards to complementarily with ongoing European research, no Framework Call has been issued on this topic and no consortium has been funded at an EU level. However, there exists a strong support of associated research activities by Framework Programme and other European fora *i.e.* consortia focussing on conditions where ME are likely to contribute including immunology, cancer, angiogenesis, tissue regeneration.

In reality, however, ME studies reported to date are likely to mark the beginning of enormous research efforts in this field and an interdisciplinary COST Action is the optimal way to merge current -and advance future- activities. Regarding future worldwide activities, undoubtedly efforts in this field will result in diagnostic, pharmaceutical and clinical applications. Advancing on Europe’s lead, ME-HAD will accelerate scientific progress within Europe, representing a collective advantage compared to non-European scientific and commercial competitors.
C. OBJECTIVES AND BENEFITS

C.1 Aim

The aim of this Action is to foster a multidisciplinary approach to research on ME, accelerating progress on the levels of basic science and technology, physiology, pathology and translational research, with the ultimate goal of exploiting ME for clinical application, achievable only through co-ordinated efforts and valorisation thus recruiting interest and funding from industry. For successful scientific progress and, ultimately, clinical application of ME, researchers in this field - from academia, clinical settings and industry - will now work together on the following 4 challenges, to amalgamate past findings and observations in this field and progress to working together to logically synergise future studies on ME in Europe:

- Basic science & associated nomenclature: to critically evaluate both currently used terminology and methodological/technological approaches for ME isolation and analysis by developing a forum for free exchange of ideas and data, leading to a template for best practice available through ME-HAD website and the recently-established Society, ISEV;
- Physiological relevance of ME: to integrate and critically evaluate former data on the physiological role(s) of ME and, subsequently, advance upon current research to further determine the role of these vesicles under normal, healthy circumstances;
- Pathophysiological relevance of ME: to integrate and critically-evaluate available data on pathophysiological roles of ME; again, extending on existing research to further elucidation of their role in diseases such as cancer, neurodegenerative, allergic & autoimmune diseases;
- Diagnostic & therapeutic potential of ME: to advance our collaborative research on the relevance of ME as minimally-invasive biomarkers; progress research on ME as therapeutic delivery vectors (e.g. vaccines); and, where possible, designing inhibitors of ME release or function as potential therapies for conditions where such an approach would be relevant.
C.2 Objectives

Secondary objectives are to:

- Reach out and include scientists, clinicians, industry-based researchers from all European countries with an interest in ME, consolidating and shaping Europe’s ME research;
- Promote strong links between participants through the website (also facebook, twitter, YouTube lectures), meetings, workshops, summer schools, Short-Term Scientific Missions (STSM); particularly to broaden the training and mobility of early-stage researchers;
- Train 100 or more young investigators in ME through Summer School participation and/or STSMs;
- Facilitate larger, more ambitious scale collaborations, by encouraging >1 joint European grant/year;
- Increase general public awareness and promote ME research via at least one open forum per year and/or press releases.

In quantitative terms, the achievements of the objectives outlined here will be evaluated using the following parameters:

- Number of active Partners from COST countries (currently 10 countries), Partners from non-COST countries (currently 4 participants), and number of companies involved (currently 4 companies);
- Number of training activities and number of young researchers trained in ME-associated state-of-the-art technologies;
- Number of STSMs supported by the Action;
- Number of appointments of researchers who moved from one to another research laboratory within the Action;
- Number of appointments of researchers who moved from a research laboratory outside of the Action to a research laboratory within the Action;
- Number of collaborative applications submitted for funding, with a special focus on contracts signed with European industry;
- Number and quality (impact factors, citations) of collaborative peer-reviewed publications;
- Number of invited presentations of collaborative research projects;
- Number of academic promotions/other career advancement steps, particularly of young researchers;
- Number of pre-clinical and clinical studies, by the COST Action members, focused on ME;
- Number of comprehensive reports and communications to the general press.

**C.3 How networking within the Action will yield the objectives?**

Networking within the Action will yield the objectives by:

- Drafting guidelines on best practice for ME analysis and principles for their future large-scale production for therapeutic purposes;
- Facilitating exchange of expertise among participating countries, by contributing to a powerful combination of expertise materialised by efficient exchange of state-of-the-art scientific and technological knowledge between Participants;
- Collating/critically-evaluating data from complementary studies, increasing understanding of ME in physiological and pathophysiological circumstances; this clearer understanding potentially contributing to new biomarkers, targets, vectors for therapeutic research;
- Training and exchange of young researchers between partner laboratories within the network;
- Co-ordinating the initiation and completion of collaborative projects, with a particular emphasis on those which are multi-disciplinary (Academia - Clinics - Industry);
- Improving our European and, indeed, international visibility in order to attract researchers and investors and industry (*i.e.* brains and money [back] to Europe);
- Identifying gaps in our ME knowledge, improving creativity, and drafting a roadmap to further collaborative studies aimed at addressing these gaps not only during but also beyond the 4 years of this COST Action, by leveraging off the success of ME-HAD.
C.4 Potential impact of the Action

As detailed in Section B, despite the scientific, medical and thus potential economical relevance of understanding and exploiting ME, a number of unresolved scientific questions remain in urgent need to be addressed in order to correctly interpret and consolidate output from European research in this field. This Action will set standards and guidelines in European ME research, as well as promoting free exchange of ideas and training opportunities for young researchers. It will enhance Europe’s leadership in this relatively new, but quickly evolving, research area. The collaborative efforts between experts in different areas of ME research is expected to provide a strong platform for ultimately translating scientific progress into clinical applications. By promoting co-operation across disciplines, sharing information on so-called “negative experiments” in addition to successes, the Action will reduce redundancy and support the efficient use of resources, thus being of immediate benefit to European scientific progress. In fact, potential future application scenarios beyond this Action include: minimally-invasive biomarkers for diseases; new targets against which to develop therapeutics; better “self” delivery systems pay-loaded and targeted to cells of choice. Overall, by accelerating basic science, cooperation with diagnostic and pharmaceutical companies, and translation to bedside, this Action could contribute substantial benefit to science, economy, public health and Society by helping to open up new markets for medical application (i.e. seeding novel European biomedical industry).

C.5 Target groups/end users

The Target Groups will be European academic, industry-based and clinical researchers. Early-stage researchers will particularly benefit from being actively involved in leading and steering a relatively new, but very relevant field of research that is currently led by Europe. End-users also set to benefit would be spin-off companies and Small and Medium Enterprises (SMEs) aiming at exploiting ME for diagnostic and/or therapeutic applications. As clinical application is the ultimate aim, we envisage the ultimate “end-users”, beyond the 4 years of this Action, being clinicians caring for patients. All these groups were represented in the preparation of this Action.
D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The Objectives listed in Section C form the template around which this COST Action’s scientific programme is structured. Four main research challenges have been defined which represent the major focal points that must be collectively addressed for further efficient scientific progress and success in the field of ME research in Europe. Each area identified below broadly represents the responsibility of participants to set the standards and to facilitate integration within the Action: (i). Basic science and associated nomenclature; (ii). Physiological relevance of ME; (iii). Pathophysiological relevance of ME; (iv). Diagnostic & therapeutic potential of ME.

While the co-operation and integration of efforts invested in these 4 challenges is required to research the scientific goals, this Action has been structured in such a way as to remain open during the implementation stage to every European research group interested in ME, whether from academia, clinics or industry. This open structure will allow timely fostering of novel activities not foreseen during the preparation of this Action. Furthermore, while competition is widely considered to be an important driving force for scientific progress, ME-HAD will concentrate on coordination, cooperation and exchange among European participants in order to improve their opportunities in the world-wide competition in this field. Participants of this COST Action agree that reciprocal knowledge transfer within European academic, clinical and industrial research represents the best means to address these 4 challenges.

D.2 Scientific work plan methods and means

While this COST Action will not support specific research projects per se, it will substantially accelerate scientific progress by supporting co-ordination, co-operation and exchange among its participants who are already locally funded to perform research related to ME.

ME-HAD’s work-plan is to achieve its objectives through 10 networked tasks (T1-T10) implemented through 4 highly-interdependent Working Groups (WGs).
WG1 (Tasks/T 1-3): Guidelines for nomenclature and analysis

The various defining terms and methods for ME isolation, verification and analysis will be considered by all interested members of this Action to reach a consensus on the most appropriate terminology and methods moving forward. As detailed more below, this challenge is of fundamental importance and due consideration will be given to this when interpreting data related to Challenges 2-4 below. Furthermore, if and when new techniques for ME isolation and analysis become available, knowledge of these will be exchanged at the earliest stage possible among members of the COST Action.

As mentioned in B.2., microvesicles and exosomes for analysis are typically isolated from medium conditioned by cultured cells, serum, plasma, saliva and other body fluids, based on size; through a range of methods, selected on the given Researcher’s preference but not standardised between European laboratories. These include series of filtration and/or ultracentrifugation techniques to separate nano-sized and larger vesicles, sometimes followed by flotation into sucrose-gradients to further purify sub-populations of similarly-sized small vesicles based on differential density and separate out non-membranous macromolecular complexes. What has yet to be determined is whether size is really a key differentiating characteristics between ME populations. What is also not currently understood is whether ME increase their size under certain pathological conditions that may confer stress on cells (link to WG3). Additionally, besides the commonly used filtration and centrifugation methods, flow cytometry (FC), chromatography, immunoadsorption/bead capture, microfluidics, and other commercially-available polymer-based extraction such as ExoQuick™ are reported. Furthermore, basic quantification and characterisation of the resulting ME populations typically includes some combination of the following analysis i.e. protein content/surface proteins by Western blotting, transmission and/or scanning electron microscopy, FC, Dynamic Light Scatter (DLS), Nanoparticle Tracking Analysis based on Brownian motion (NTA), atomic force microscopy, and Mass Spectrometry (MS). A comparative evaluation of experience and success with all methods assessed and results generated will be of substantial help to both experienced and new researchers in this field in selecting the optimal combination(s) of methods for subsequent collaborative studies.
This will be achieved through:

Task-1. Evaluating currently used terminology for ME

- Collecting current knowledge on terminology used in describing and defining microvesicles and/or exosomes and subsequently integrating, critically evaluating this data;
- Summarising and distribution of this data within ME-HAD to derive harmonisation and a consensus on what European researchers believe to be the most relevant terminology;
- Dissemination of this information to all international researchers active in ME research.

Task-2. Collating and evaluating methods used for ME isolation and analysis

similarly to Task-1, but with a different specific focus.

- (i) Collating of current knowledge and expertise on methods and technologies for isolating microvesicles and/or exosomes (with emphasis not only on successful outcome, but also considering “negative” data to ensure we collectively learn from this);
- (ii) Collating of information on methods and technologies for analysing ME; and integrating, critically-evaluating data from (i) & (ii);
- Summarising and distribution this data within ME-HAD, in order to derived harmonisation and a consensus on ME-related methodology;
- Dissemination of this information to international researchers active in ME research; again, setting recommendations and inviting input for overall harmonisation in this field, lead by Europe.

From Task-1 & Task-2, develop and publish of a Compendium “Handbook of Terminology & Techniques in Microvesicles & Exosomes Research” [note: Meth. Mol. Biol., Springer, have expressed an interest in partnering on this].
Task-3. Establishing principles for ME large-scale production

- Advancing from the outcome of Tasks 1&2, ME-HAD will establish principles for ME large-scale production, identified as necessary for future clinical applications.
- Overall, WG1 will provide international guidelines established through a European network; proposing specific methods for ME analysis, based on shared expertise; and deriving potential means of their large-scale production for future therapeutic purposes.

WG2 (Tasks 4-5): Physiological importance of ME

As is the situation described for WG1, very good research has been done throughout pockets on Europe indication microvesicles and/or exosomes have a role to play in normal, healthy conditions, including communication necessary for immune-protection, embryonic- and other stem cell-differentiation, platelet function, embryonic cell-derived, etc. So, in the context of above, data from COST Action members’ prior research considered suitable for integrating and/or directly comparing will be co-analysed in order to establish the breadth of our collective understanding of the physiological role(s) of ME. This will form a solid foundation to advance upon in follow-on collaborative efforts exploring the role of these vesicles under normal, healthy circumstances.

ME, for example, have emerged as potent vehicles for cell-cell communication since the discovery that they contain functional RNA, protein (and possibly DNA) molecules that can functionally interact with target (acceptor) cells. The content of ME, it seems, is not always simply a straightforward reflection of the content of the cell of origin. Specific populations of RNAs are selectively packaged into exosomes, indicating the existence of an as-yet unknown mechanism that controls the sorting of specific RNAs into exosomes. The information contained in ME can influence or even direct the fate of the target cell, for example triggering its activation, migration, differentiation, or promoting apoptosis, with the functional consequences of this transfer of genetic information depending on the origin and status of both the donor and recipient cells. The transfer of material via ME to target cells can occur both in a cell-cell contact dependent manner e.g. via specialised junctional structures such as synapses or at longer distances.
These findings have revealed important, novel cell-cell communication modes and point to new ways to modulate cell-cell interactions and their responses. It is timely that this information be brought together in an organised and open manner, evaluated and progressed upon through collaborative European research efforts.

This will be achieved through:

Task-4. Integrating and critically evaluating available data on physiological roles of ME

- Collecting current knowledge in ME biology and summarising the state-of-art biological significance of ME in health and identifying substantial gaps in our understanding of ME under normal, healthy, circumstance;
- Subsequently making this information available, through ME-HAD, to all interested parties.

Task-5. Extending existing research to further determine the role of these vesicles under normal, healthy circumstances:

- Ensuring that subsequent analysis of the physiological relevance of ME, during the course of ME-HAD’s 4 years and beyond, are properly designed and performed (e.g. optimal technologies, learning from WG1, adequately powered, etc.) to result in meaningful outputs;
- Exploring (i) the mechanisms regulating ME-mediated transfer of genetic information and proteins from cell-to-cell; (ii) the plasticity of ME release in relation to the (activation) status of the producing cell, target cell and microenvironment;
- Defining important future research topics and publication of a joint White paper.
WG3 (Tasks 6-7): Pathological importance of ME

In a similar vein to determining the physiological relevance of ME, data already generated by Action members on MEs in disease and deemed suitable for integrating and/or directly comparing will be co-assessed in order to determine the breadth of our collective understanding of the pathophysiological role(s) of ME. Again, subsequent studies will leverage from both the positive data and also data deemed to be negative (learn from mistakes and build on our collective strengths) to more comprehensively understanding the relevance of ME in diseases. Based on the expertise of the current participants and being realistic with timing (we cannot address all issues together!), the initial diseases considered will be cancer, allergy, autoimmunity and neurodegenerative diseases, and although an open-policy is intended – both to learn from and to contribute to research arising from MEs in other diseases.

This will be achieved through:

Task-6. Integrating and critically-evaluating available data on pathophysiological roles of ME

- Collecting and summarising current knowledge in ME pathophysiological and identifying substantial gaps in our understanding of ME in disease;
- Making this information available, through this COST Action, to all interested parties.

Task-7. Extending existing research to advanced elucidation of their role in disease

In relation to neurodegenerative diseases, Action participants have shown that b-amyloid peptides, causatively linked to Alzheimer’s disease, are released into the extracellular milieu and can contribute to the propagation of amyloids in the brain and formation of extracellular amyloid plaques. During this Action, the cellular aspects of the release of exosomes and the mechanism by which amyloid proteins are loaded onto the exosomal vesicles, as well as trafficking of exosomes through the brain and how exosomes contribute to the formation of amyloid plaques, will be investigated through collaborative efforts involving scientists, clinicians and industry.
In relation to cancer, ME-HAD participants have shown evidence that dysregulation of vesicle trafficking in cancer cells contributes to tumorigenesis and that cancer cell-derived MEs can transfer “aggressive” characteristics to recipient cells or serve as cancer cell immune-tolerising entities. During this Action, mechanisms involved (e.g. Rab GTPases) in regulating ME release from cancer cells; how exosomes instruct the local and distant environment, including metastatic niches; and the contents (mRNA, miRNA, lncRNA, proteins) of those MEs will be explored in *in vitro*, *in vivo* and *ex vivo* model systems through collaborative efforts including ME-HAD scientists, clinicians and industry.

**WG4 (Tasks 8-10): Diagnostic & therapeutic potential of ME**

Translation of preclinical findings into medical applications is of upmost socio-economical impact but generally suffers from poor communication and exchanges between researchers, clinicians and industries. This Action will provide the necessary platform to facilitate the transfer of knowledge between these groups, advancing on successes from appropriate research performed by individual groups scattered throughout Europe. Working closely with WGs1-3 and, again, bringing together “like-minded” researchers with a particular interest in ME as either biomarkers for a given disease and/or as therapeutic delivery vectors, will help ensure that future studies, facilitated by ME-HAD, are not duplications of disparate and small under-powered studies, but collaborative efforts that can lead to meaningful outcomes. Furthermore, it is realistic to envisage that when the therapeutic potential of ME has been further explored by ME-HAD researchers, this Action will act as the foundation, even beyond its 4 years, for designing inhibitors of ME release as potential therapies for conditions where such an approach would be relevant.

WG4 will include the following:
Task-8. Advancing our collaborative research on ME as minimally-invasive biomarkers

- Results from a number of studies by Action Participant and involving a range of diseases including cancer and Alzheimers indicate that both ME quantities and contents (proteins, nucleic acids) have relevance as minimally-invasive biomarkers. As for all other WGs, former suitable data will be collated, evaluated and used as a foundation for more extensive collaborative studies on relevantly-sized cohorts of cases for more extensive characterising and quantification of ME isolated from human body fluids, in order to identify/validate disease-specific biomarkers (and to set standards for patient-specific biomarkers required for personalized therapies).

Task-9. Progressing research on ME as therapeutic delivery vectors

- The demonstration in preclinical studies, performed by Action participants, that ME can deliver molecules into secondary/recipient cells and that it is possible to pay-load ME or synthetic ME-like vesicles with desired functional siRNA or other therapeutic molecules, supports these vesicles being considered as “biomimetic” vectors for therapeutic delivery in, theoretically, any diseases. Advancing on previous successes, ME-HAD researchers will accelerate scientific progress in this space though fostering cooperation across WGs and disciplines. Of course the chances that, in the future, these findings will be translated to the clinic highly depend on the level of communication between basic, pre-clinical and clinical scientists and industry. This Action will substantially facilitate progress here by enabling collaborative progress, synergism and valorisation of output, through the extensive interaction with the European research groups active in this and relate fields.
Task-10. Designing inhibitors of ME release as therapy

- COST Action researchers have established that in neurodegenerative diseases, inhibition of exosomes could ameliorate amyloid pathology. A similar approach may be very relevant in prevention of cancer spread. Through the Actions academic/industry collaborations, inhibitor of ME release will be designed and screened via miniaturised exosomes-based assays, starting during the 4 years of this Action and progressing beyond this to larger scale application.

E. ORGANISATION
E.1 Coordination and organisation

Increased scientific exchange and improved co-ordination of European research activities in the area of microvesicles and exosomes will enable exchange and direct comparison of procedures and data, accelerate technology progress, scientific knowledge and translation to clinical application which benefits society. The main focus of the Action will be the interaction between basic scientists, clinicians, and associate companies. Although all 3 of these parties are currently undertaking research activities in this field, the exchange between them is very limited, due to the lack of appropriate structures. An unprecedented organisation will be created, in the form of ME-HAD, to fill this gap.

This will be implemented through the following organisational structure including scientists and clinicians representing academics/hospitals/companies where feasible to do so:

The Management Committee (MC):

The Chair, Vice-Chair and MC members will be responsible for the overall strategy, allocation of funds, and the Action reports; meeting at least once/year. The Chair, Vice Chair, Working Group Leaders will be held at the first MC meeting to be held at the start of the Action. Each COST National Coordinator (CNC) will appoint up to two MC members, wherever feasible one of these two persons shall be an Early-Stage Researchers.
To ensure that two way flow of information between MC and all the COST Action participants and to ensure that all important issues are tabled and discussed, at the initial MC meeting, an Early-Stage Career Coordinator, a Gender Balance Coordinator, a Website Coordinator; and a Knowledge Transfer Coordinator will be nominated from among the members. Other areas where a specific Coordinator would be of benefit to ensure the success of the Action will be considered; at this and at subsequent MC meetings.

_The Steering Committee (SC):_

The Chair and WG leaders (co-opting others e.g. local organisers of workshops, as necessary). SC’s key role will be ensuring co-ordination of the WGs activities; meeting twice/year.

_The Working Groups (WGs):_

The Chair and all Action Members with an interest in the WG activities to progress the associated Tasks; meeting twice/year.

Every effort will be made to ensure strong representation of Early-Stage Researchers (ESRs) and also gender balance on all Committees/WGs.

An Action Meeting will be held during each of the 4 years. Short-Term Scientific Missions in Year 1 will foster early-stage training and information exchange. A Summer School will be held in Years 2 & 4, to provide knowledge exchange and harmonisation and to train young researchers.

Every effort will be made to organise meetings as satellites to other international conferences, to ensure that all interested parties can attend. Other consultations will include teleconferences, email.

The Action will intensify collaboration with different international partners. Students and ESRs will become efficient “team players”, as well as “team leaders”, who are able to create synergies by collaboration.
E.2 Working Groups

According to the 4 objectives listed in Sections C&D, 4 highly-interdependent Working Groups (WGs) will be formed at the start of the Action to update, discuss and bring forward those important topics in ME research, as detailed in the associated Tasks. WG Co-ordinators will be elected at the initial meeting of each WG, to organise the WG meetings, to lead the scientific discussion, and to provide the Chair will a brief yearly written report. All participants of the Action will be invited to join at least one of the WGs, depending on their research interests. The WGs will meet twice per year, if funds allow, and the first meeting every year will be combined with the MC meeting. Additionally meetings, when relevant, will be through teleconference, Skype or a similar means of communication. The Action will combine these WG meetings as participants will typically work on more than one aspect (i.e. more than one WG) and isolated meetings would hinder scientific exchange. The WGs operated by the Action will be: WG1: Guidelines for nomenclature and analysis; WG2: Physiological importance of ME; WG3: Pathological importance of ME; WG4: Diagnostic & therapeutic potential of ME.

E.3 Liaison and interaction with other research programmes

Members of research programmes related to the activities of this Action as well as future programmes that could benefit from this Action will be invited to participate in Workshops, give seminars, exchange information, and interact with member of the Action. This includes organising conferences, one-day workshops (like the EMBO meetings), and inter-disciplinary meetings (clinicians, drug discovery experts). Due to the open structure of this Action and it’s complementarily with the newly established International Society for Extracellular Vesicles (ISEV), interested research groups can be integrated and every participant is invited to suggest additional participants. The Knowledge Transfer Coordinator will take responsibility for the continuous interaction with existing and newly formed European research groups of relevance. In addition, this Action will set up a technology transfer platform to encourage and facilitate commercialisation of early stage findings from academic laboratories.
Every effort will be made to organise meetings as satellites to other international conferences, to help ensure that all interested parties can attend and also to demonstrate the Action’s achievements and the potential for expansion in this area.

So far, ME research has not been implemented in any European programme. A goal of this Action is, therefore, to create a module for a future Framework Programme Call. Invitations will thus be extended to European scientific leaders and top Framework Programme scientific administrators to emphasise and underscore the importance of this area and to lobby for inclusion in Framework Programme funding opportunities to leverage of the success of the COST Action.

**E.4 Gender balance and involvement of Early-Stage Researchers**

*Gender Balance:*

The COST Action will respect an appropriate gender balance in all its activities and the Management Committee (MC) will place this as a standard item on all of it MC Agendas. (please note: already 44% of the Participants are female). In order to achieve an equitable gender balance, this Action will particularly encourage females to participate in the Action overall; to serve on MC; to lead Working Groups (WGs), etc. Each listed participant is committed to encouraging and supporting the career progression of females already in their team and to favourably view applications, from females, for all of the Actions activities.

*Early-Stage Researchers:*

The Action is also committed to substantially involve early-stage researchers, including PhD/MD student in the final years and early-stage researchers at Post-Doctoral level. Again, this item will be included as a standard item on all MC Agendas. This Action will include STSMs and Summer Schools to engage early stage investigators and to develop the next generation of researchers to create and maintain a lasting tradition of European excellent in this field. Particular attention will also be given to young researchers and to gender balance when selecting speakers and Chairs/Co-Chairs of the Workshop sessions.
As part of the Organisational Structure, both a Gender Balance Co-ordinator and an Early-Stage Researchers Co-ordinator will be nominated at the beginning of this Action. The annual report from each WG will include a section detailing the gender balance and participation of early-stage researchers in its activities. Further efforts will also be made to build and maintain diversity in this Action by nationality and race.

F. TIMETABLE

The duration of this COST Action is four years.

The Action will begin with a Kick-off Meeting and during this meeting the MC, as well as Coordinators of the WGs will be elected. A website will be established for information and communication in which all essential information concerning this Action will be implemented and which will be updated on a regular basis (at least twice/year).

The Action will be closed with a final conference combined with all WG meetings.

TIMETABLE

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<thead>
<tr>
<th>EVENT</th>
<th>Yr-1</th>
<th>Yr-2</th>
<th>Yr-3</th>
<th>Yr-4</th>
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<tr>
<td>Final report</td>
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</table>
G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: BE, CH, DE, ES, FR, IE, IT, NL, SE, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 40 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The main target audiences for the dissemination will be:

- The academic, clinical, industry-based partners in this COST Action;
- The Early-Stage Researchers of the partner groups;
- Other researches in the microvesicles and/or exosomes field outside the Action;
- Research institute and industry working on closely related fields;
- Diagnostics companies ;
- Pharmaceutical and biotechnology companies ;
- Policy makers ;
- The general public.
H.2 What?

Dissemination methods will target either all audiences or selected target groups, as relevant. The progress of this COST Action and the results of its evaluation will be taken into consideration in the dissemination plan during the course of ME-HAD. On all occasions appropriate advertising and publicity will be attempted and the COST support making this possible will be acknowledged.

Website:

- The development and maintenance of ME-HAD’s website is of primary importance for the dissemination of information about this COST Action. The MC will consider the website to be the main method to highlight and promote the activities and results of the Action. It will be established in line with the COST Office requirements and it will include information about the members and the activities of the Action (including members contact details, future meetings, workshops, summer schools, Short-Term Scientific Missions, conferences and other related events). The website will contain and disseminated important scientific information, for example template for best practice including “Guidelines for nomenclature and analysis”, “Principles for ME large-scale production” as they are drafted; protocols/procedures; list of publication from participants (updated at least once per year by the response person), and the results of the Action. The regular access level will include information and conclusions aimed at non-expert audiences. The website will also contain a password-protected section for internal information, shared among members e.g. Agendas and Minutes arising from the MC and WG meetings etc;
- A dedicated section of the website will be direct specifically to Early-Stage Researcher to encourage networking among young researchers in Europe. It will also include and a careers and jobs section, to support their mobility and career development/enhancement;
- The website will also inform interested researchers on the activities of the Action and provide details on how to become a participant.
Scientific literature:

- Members of the Action will publish original and review articles in international peer-reviewed journal. In the event that participants home institutions do not provide open access to certain scientific literature, the Action will support dissemination of these articles. The Action’s participants will also regularly disseminate their most important findings through periodicals, bulletins, and the local press.
- The Action has the option to publish a “Handbook of Terminology & Techniques in Microvesicles & Exosomes Research.”
- Mid-to-long-term key aspects for future scientific goals in ME research as a final outcome of the Action will be published as a joint White Paper.

Meetings and Conferences:

- The internal MC, Steering Group, and WG meetings as well as the open comprehensive research conferences represent the fastest ways to exchange scientific information about the Action’s achievements, both internally and externally. Furthermore, these conferences represent an ideal opportunity to draw Policy Maker’s and the general public’s attention to the latest results arising from ME research and how they could benefit from this.

Short-Term Scientific Missions:

- In addition to improving the scientific education of Early-Stage Researchers, STSMs will very effectively accelerate the exchange of scientific data and technical knowledge. In fact, these often lead to novel scientific ideas and concepts that would not have been achieved by single research groups working in isolation in an academic, clinical or industry setting.
Contacts with Industry:

- In addition to industry-based and clinical-based researchers already actively involved in this Action, researchers from the diagnostic, pharmaceutical and biotechnology industry as well as clinicians will be invited to participate in meetings in order to exchange experiences and ideas on ME research and their translational application; to initiate now collaborations; and to partner in applications for relevant joint funding opportunities.

Contacts with Educational Staff:

- The members of this Action will be encouraged to introduce this topic in the educational plans of their Institute. This Action will be included as an example of best practice in research methods to undergraduate and postgraduate students. This topic will also be included as an example, where possible, in local institute Out-Reach workshops aimed at primary- and high school children to instigate in them an interest and excitement about scientific research.

Contact with other Networks:

- An important issue for the on-going development and evolution of this Action will be its interaction with other networks that are identified as in some way of relevance to the area of ME research. A very open-mind will be kept on this – to include links with the range from relevant EU consortia that may develop, international organisations, respective local and national charities and societies.
H.3 How?

The Action’s participants consider dissemination of the Action’s activities to be an important organisational task. Therefore, at the initial MC meeting, the Website Coordinator and the Knowledge Transfer Coordinator (as well as the Early-Stage Career Coordinator and Gender Balance Coordinator) be nominated. The website will be maintained by the Website Coordinator who will take responsible for the technical aspects of the website. All members of the Management Committee and other Action members, as appropriate, will provide information that need to be available through the website. The Knowledge Transfer Coordinator will support the website by serving as contact person especially for the external scientific environment, including new Action partners, related research programmes, pharmaceutical companies, clinicians, policy makers and the press. At the yearly MC meetings, dissemination will be placed as a standard item on the Agenda. The website will provide full flexibility to acquire additional contents or functions that become necessary as the Action progresses. As appropriate, seminal findings by the Action's participants will also be disseminated by press releases.

This Action is well-suited to COST as all participants have secured funding for ME research. COST support for networking will ensure that this research is synergistic and accelerated, richly benefiting European research in this field.