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European Network on Microvesicles and Exosomes in Health and Disease (ME-HaD)

A brief history of ME-HaD: In summer 2011 the idea of ME-HaD begun! Lorraine O'Driscoll attended an information session on EU COST Actions in Trinity College Dublin. This seemed to be an ideal opportunity to bring together international knowledge and expertise in extracellular vesicles (EVs). At the time, microvesicles and exosomes (then abbreviated as ME, but now more typically collectively termed extracellular vesicles (EVs)) were attracting much 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 was quickly expanding. There were, however, many associated issues that needed addressing to ensure optimal research performance and collation and cross-interpretation of data. These issues ranged 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 EVs. There was also urgency to identify gaps in EVs knowledge and establish how best collaborative research could address these questions. This plan for ME-HaD –highly complementing the International Society for Extracellular Vesicles (ISEV) – was, therefore, to create a network of experts, fostering an inter- and multi-disciplinary approach to enhance both the basic understanding and translational potential of EV. To achieve this, ME-HaD's ambition was to bring together a critical, yet fragmented mass of academic, clinical and industry expertise from COST and non-COST countries, while developing a forum for free-exchange of concepts and, importantly, training of early career researchers (ESRs).

For successful progress, ME-HaD researchers in this field -from academia, clinical settings and industry- proposed to work together on the following 4 challenges designated as Working Groups (WGs), to amalgamate past findings and observations in this field and progress to working together to logically synergise future studies on EVs:

[1]. Basic science & associated nomenclature
[2]. Physiological relevance of EVs
[3]. Pathophysiological relevance of EVs
[4]. Diagnostic & therapeutic potential of EVs

On 23/09/2011 the ME-HaD pre-proposal was submitted to Brussels as part of a 3 stage evaluation process. A positive outcome from this, lead to an invitation on 12/12/2011 to submit a full proposal for the 27/01/2012 deadline. A subsequent positive outcome from the pre-proposal led to an invitation to present to a large panel of EU experts in Skopje, Macedonia on 05/03/2012. The positive outcome from this presentation and Q&A session was declared on 7/06/2012. Me-HaD's kick-off meeting was held in a very snowy Brussel on 7/12/2012. Lorraine O'Driscoll (School of Pharmacy & Pharmaceutical Sciences, Trinity College Dublin) was elected as Chair; Marca Wauben (Dept. Biochemistry and Cell Biology, Utrecht University) as Vice-Chair; WG leaders were elected as detailed below; the Short-Term Scientific Mission (STSM) Evaluation Panel formed included Irina Nazarenko (Institute for Environmental Health Sciences and Hospital Infection Control Medical Center, University of Freiburg), Lawrence Rajendran (University of Zurich, Switzerland) and Willem Stoorvogel (Dept. Biochemistry and Cell Biology, Utrecht University). Maja Kosanovic (Dept. of Immunochemistry and Glycobiology, University of Belgrade) was elected as Co-ordinator for ESR.

The success of ME-HaD has been substantial. ME-HaD now includes researchers from 27 European countries, Harvard University, University of Colorado at Denver and University of Louisville in the US, and La Trobe University in Australia. As well as academic and clinical researchers, ME-HaD has 7 industry partners. ME-HaD's membership is 300+, with more than 100 ESRs afforded specialised EV training at ME-HaD Training School and/or via placements in collaborating laboratories (i.e. a short-term scientific mission). Management Committee (MC) and WG meetings were held twice every year, for each of the four years; all in different locations within Europe. Other examples of ME-HaD's successes are detailed for each WP below.

WG1: WG1 was led by Willem Stoorvogel (Dept. Biochemistry and Cell Biology, Utrecht University) and Clotilde Théry (Institut Curie, PSL Research University, Paris). The goals of COST ME-HaD WG1, entitled Guidelines-Nomenclature & Analysis, were firstly to reach consensus and support implementation of a uniform nomenclature of EVs and secondly to derive consensus on guidelines for methods of isolation and analysis of isolated EVs. Within this framework, the ME-HaD network continuously interacted with the International Society for Extracellular Vesicles (ISEV), whose Board of Directors included several ME-HaD members (C. Théry, M. Wauben, E. Buzas, J. Lötvall). Together with ISEV, ME-HaD reached consensus on nomenclature that is nowadays supported and applied by most researchers in Europe and the other continents. As a consequence, the generic term « Extracellular Vesicles » is, at least as keyword, incorporated in all publications. EVs are further specified according to their subcellular origin as either exosomes or microvesicles, according to Raposo and Stoorvogel in 2013 (Raposo, Stoorvogel, 2013). Regular meetings of ME-HaD served as a platform for members to show, compare and discuss their results with regard to different available EV isolation methods, with the aim to optimise separation of EV subpopulations, reduce contamination with other constituents of culture media or body fluids, while retaining biological

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activities (Van Deun, et al., 2014; Andreu, et al., 2016; Kowal, et al. 2016). These discussions helped greatly moving the field forward. On this topic, however, ME-HaD as well ISEV accepted that it is inappropriate to propose a single optimal method for EV isolation. Indeed distinct isolation methods may be chosen dependent on the source of extracellular vesicles and the requirements for purity or separation from contaminating constituents. Furthermore, novel isolation methods, molecular ways of intervention with cargo incorporation or EV secretion, and novel markers for EV subclasses are still under development, continuously shifting the demands of protocols for EV isolation, detection and characterisation. Minimal guidelines for EV studies were, however, generated by ISEV and ME-HaD members (Lötvall et al., 2014). Future updates of these guidelines will benefit from the network established through the ME-HaD COST program.

WG2: The WG2 of the ME-HaD COST action focused on the physiological roles of EVs and was led by Pia Siljander (University of Helsinki, Finland) and Francisco Sánchez-Madrid (Instituto de Investigación Sanitaria Princesa, Spain) and his substitute member María Yáñez-Mó (Universidad Autónoma de Madrid, Spain). According to the Memorandum of Understanding, WG2 was designated with two major tasks. One task was to integrate and to critically evaluate the physiological roles of EVs. In this task, the available knowledge of the EV biology was collected and summarised to unveil the biological significance of EVs in health, and to identify any substantial gaps in the understanding of EV function under normal, healthy circumstances. Following these principles, writing of a comprehensive review article was coordinated among the ME-HaD participants. The final review comprised the current knowledge of EV composition and the functional roles of each molecular component (proteins, glycosylation, RNA, DNA and lipids). Thereafter, the review summarised the roles of EVs in different body fluids, from urine to uterine fluid. A thorough summary of the proposed functions of EVs in different organs and systems of the body was also included, and the review ended with an overview of EV functions in other organisms such as parasites, bacteria, viruses or plants highlighting the cross-kingdom significance of EVs. This article not only summarised the relevant aspects of physiological EV functions found in the literature, taking special note of citing the original articles describing each role; but the review also identified and highlighted the unknown or less defined aspects of EV function. The final version was impacted by the collective work of 60 EV experts from 20 different countries. To disseminate this extensive amount of expert-filtered information to all interested parties, the review was published with the help of ME-HaD as an Open Access article in the Journal of Extracellular Vesicles, having been so far cited by 214 papers after only a year of its publication (Yañez-Mo and Siljander et al., 2015).

WG2's second task focused on extending research activities to further determine the role of EVs under normal, healthy circumstances. The active exchange of information among the Action participants, both in the WG and MC meetings, as well as by the interchange of researchers through STSM, has broadened the EV research by novel ideas and collaborations. Further, the effort made in training of new researchers in hands-on training schools, combined with the fast advance of technologies spread via ME-HaD interactions, as well as the implementation of new isolation and characterisation techniques for EVs, particularly boosted by the WG1, have all improved both the quality and the design of EV research. During 2012-2016, many aspects of EV biology have been deciphered including the sorting mechanisms of genetic information in EVs. Now, the plasticity of EV release in relation to the (activation) status of the parent cell, specificity of targeting of EVs and their interactions within the cellular microenvironment are also starting to be tackled in the field. Finally, it should be stressed here, that most EU funding instruments are strictly focused on disease-driven research, which approach tends to forget the fact that the most powerful changes in disease treatment have always come from very basic research (e.g. the example of invention of the LASER from a long list). The current funding policies do not favour research of the purely physiological aspects of EV function, which however, would ultimately boost their translational utilization in the near future, as has been beautifully illustrated by the information crowd-sourcing of the WG2 experts within the ME-HaD action.

WG3: WG3 of the ME-HaD COST action focused on the pathophysiological roles of EVs and was led by Edit I Buzás (Semmelweis University, Budapest, Hungary) and Irina Nazarenko (University Medical Center, Freiburg, Germany). According to the Memorandum of Understanding, WG3 was designated with two major tasks. The first task was to integrate and to critically evaluate the available data on the pathophysiological roles of EVs. During the regular meetings, WG3 provided broad opportunity to ME-HaD participants from all over Europe to introduce their most recent findings with respect to the pathophysiological roles of EVs. Given that numerous ME-HaD COST action members were identified to carry out studies on the pathophysiological aspects of EVs which were in line with open H2020 calls, WG3 collected information on proposed contributions to these calls. Furthermore, WG3 shared this integrated information with all participants of ME-HaD to facilitate formation of novel consortia for H2020 applications. This effort has resulted in three submitted applications by consortia formed by members of ME-HaD COST action.

The second task of WG3 was extending existing research to advanced elucidation of their role in disease. During the past years several groups of the ME-HaD COST action made significant advances regarding the role of EVs in pathological conditions. WG3 identified that an outstanding research activity within focused on the role of EVs in various types of cancer (including breast-, prostate-, large bowel- and gastric cancers as well as melanoma). Highly successful studies have been carried out demonstrating that EVs represent novel tumor biomarkers (Corcoran et al., 2014; Øverbye et al., 2015; Neeb et al., 2014). Intensive research targets i) EV-mediated interaction of tumor cells with stromal and immune cells, ii) EVs produced by 2 and 3D systems, iii) the nucleic acid (miRNA) cargo of EVs, iv) cancer-derived EV biodistribution and v) the role of hypoxia. A preliminary map of European Cancer Research Groups has been drawn and shared among members of ME- HaD to facilitate future collaborations. Besides cancer, other pathological conditions in which that role of EVs has been studied extensively by ME-HaD COST action members included i) autoimmune inflammatory disorders, ii ARDS, iii) infectious diseases such as leishmania infection, iv) cardiovascular diseases and v) liver damaging conditions (under which metabolically active EVs have been proposed to directly contribute to different pathophysiological processes (Royo et al. 2016). Not only disease progression promoting effects of EVs have been identified by members of the ME-HaD COST action, but also protective EV functions have been discovered in different pathologies (e.g. O'Brien et al., 2015; Tímár et al., 2013).

WG4: WG4 focused on the diagnostic and therapeutic potential of EVs. Initially it was headed by Lawrence Rajendran (University of Zurich, Switzerland) and co-leaded by Stefano Fais (Instituto Superiore di Sanit, Rome, Italy). In September 2014, Lawrence stepped back and Bernd Giebel (University Hospital Essen, Germany) took over the lead of WG4.

According to the original tasks, WG4 focused on the therapeutic and diagnostic potential of EVs. During the funding period an increasing number of international groups have demonstrated therapeutic potentials of EVs in a huge variety of different animal models, in a Graft-versus-Host Disease (GvHD) patient and in the frame of clinical studies in some tumor patients (reviewed in Lener et al., 2015). To date three mayor application fields have emerged, EVs in vaccination trials, EVs as drug delivery vehicles and native EVs in regenerative medicine and immunotherapy. Important issues for translating EVs into the clinics are regulatory guidelines for the EV production and quality analyses processes. As such guidelines were missing, Bernd Giebel and Eva Rohde (Universität Salzburg, Austria) coordinated a position paper as joined effort of ISEV and ME-HaD with a total of 56 different authors, many of them being members of ME-HaD. The paper was published

open access on December 31st 2015 in the Journal of Extracellular Vesicles. It comprehensively reviewed the field, discusses potential regulatory classifications and provides recommendations for the preparation and quality controls of proposed EV therapeutics (Lener et al., 2015).

Regarding EVs as biomarkers, an increasing number of groups qualified EVs as biomarkers for different diseases. Depending on the disease such EVs are harvested from different body liquids with a variety of different purification and detection methods. In a joined effort between many researchers of the ME-HaD initiative, Stefano Fais and Bernd Giebel coordinated a review article summarising studies reporting on the identification of EVs as biomarkers. This article has been published in April 2016 and contains 37 authors (Fais et al., 2016); most of whom are ME-HaD members.

ME-HaD's conclusion: While all good things must come to an end – especially if they are funded for a fixed period of 4 years, as summarised above, this has been a very important successful activity for leaders and ESRs in academia, clinics, and industry who have an interest in progressing the international field of EVs. We collectively are very grateful to EU COST and H2020 for their support of ME-HaD [BM1202].

We are grateful to the EJPS having this opportunity for the SI with Open Access.

The MS presented at this SI will follow the order of the WGs presented above and are listed under.

We hope the readers to find this SI:MeHaD interesting, and create the possibilities to interesting contacts in the field of EV-research in life scinces.

Outline: EU-COST BM-2012
SI: MeHaD (Microvesicles and Exosomes in Helath and Disease)

WG1:

1. Biological reference materials for extracellular vesicle studies (00567)
2. Effect of shear stress in the flow through the sampling needle on concentration of nanovesicles isolated from blood (00502)
3. KeepEX, a simple dilution protocol for improving extracellular vesicle yields from urine (00631)

WG2:

4. Extracellular vesicles in food: Experimental evidence of their secretion in grape fruits (00594)
5. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: yield, physicochemical properties, and pharmacokinetics (00852)

WG3:

6. Metabolically active extracellular vesicles released from hepatocytes under drug-induced liver-damaging conditions modify serum metabolome and might affect different pathophysiological processes (00595)
7. A study of extracellular vesicle concentration in active diabetic Charcot neuroarthropathy (00509)

WG4:

8. Exosome levels in human body fluids: a tumor marker by themselves (00474)
9. Extracellular vesicles as a source for non-invasive biomarkers in bladder cancer progression (00421)
10. Exosomal proteins as prostate cancer biomarkers in urine: From mass spectrometry discovery to immunoassay-based validation (00483)
11. Extracellular Vesicles: Immunomodulatory messengers in the context of tissue repair/regeneration (00512)

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