European Cooperation in the field of Scientific and Technical Research

- COST -

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COST 025/14

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1404: European Network of Investigators Triggering Exploratory Research on Myeloid Regulatory Cells (Mye-EUNITER)

Delegations will find attached the Memorandum of Understanding for COST Action BM1404 as approved by the COST Committee of Senior Officials (CSO) at its 190th meeting on 14 May 2014.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action BM1404
EUROPEAN NETWORK OF INVESTIGATORS TRIGGERING EXPLORATORY RESEARCH ON MYELOID REGULATORY CELLS (Mye-EUNITER)

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 4114/13 "COST Action Management" and document COST 4112/13 "Rules for Participation in and Implementation of COST Activities" , or in any new document amending or replacing them, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to provide gold standards for the identification, functional analysis, and clinical monitoring of myeloid cells with immunoregulatory activity (= myeloid regulatory cells) in selected human diseases as well as experimental animal models.

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 60 million in 2014 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 section *2. Changes to a COST Action* in the document COST 4114/13.

A. ABSTRACT AND KEYWORDS

In cancer, infection and inflammation, the immune system's function can be dysregulated, contributing to disease pathology. As part of this process, instead of fighting disease, immune cells may suppress beneficial immune responses and increase pathology. Despite their pathophysiological importance, the identity and biology of the so called myeloid regulatory cells (MRCs) is poorly understood. Depending on the MRC subtype and the respective disease, conflicting results have been published. This Action will form a network of researchers and clinicians which aims to establish a gold standard of common protocols and harmonising guidelines for the analysis and clinical monitoring of MRCs. There is also a deficit in the translation of findings from animal models to humans, and Mye-EUNITER will build an analytical mouse-monkey-man correlation line. Standardised and validated tools for MRC analysis will aid the development of cellular biomarkers of disease and guide the design of novel therapies to manipulate the functions of MRCs.

Keywords: Development of standardised analysis tools for MRCs; comparative analysis of MRCs in cancer, infectious and inflammatory diseases in murine, simian, and human models; therapeutic targeting of MRCs; pan-European network of biomedical PhD schools; translational immunology.

B. BACKGROUND

B.1 General background

In a healthy person the immune system is perfectly balanced to provide protection against invading harmful pathogens or malignant cells, whilst maintaining a state of unresponsiveness ('tolerance') to our own body tissues and to harmless substances we eat or inhale. Disbalance in this immune homeostasis can contribute to disease pathology in infections, inflammation and cancer. These diseases cause a major socio-economic burden to the community and in the EU annually at least € 200 billion are spent on their treatment. Despite recent advances in research, there is an urgent need for additional therapeutic options.

The activities of this Action are based on the latest developments in basic and clinical research, which have demonstrated a clear role for myeloid regulatory cells (MRCs; a specialised subset of white blood cells) in infection, inflammation and cancer. The members will harmonise and coordinate their research activities to promote the standardised analysis of the MRC populations thereby enhancing the development of new therapeutic options for major diseases using this

relatively novel immune cell population.
In the context of this Action we are referring to MRCs as a heterogeneous group of myeloid cells which acquire immunoregulatory and/or immunosuppressive activity as a consequence of the disease of the host. The term “regulatory” is used with reference to the far better characterised regulatory T cells, which in contrast to classical T cells are not immune effector cells, but rather downregulate immune responses. Similar phenomena have been observed for myeloid cells; examples include but are not limited to immunosuppressive granulocytes, tolerogenic dendritic cells (DC), regulatory macrophages and the heterogeneous group of so-called myeloid-derived suppressor cells (MDSC).
The full appreciation of MRCs as important players in the pathology of major immune-related diseases is a relatively new finding in the field and only in recent years MRCs have been identified as important mediators of immunoregulation. The latest, exciting findings in the field of myeloid cell immunobiology have resulted in the incorporation of MRC research into a substantial number of individual grants at individual laboratories. However, complementary collaborative research activities, which better coordinate these singular and specific MRC research projects while at the same time focusing on the clinical applications of these activities are lacking. This Action will provide an organisational platform for individual research grant holders to coordinate their activities on a European level.

B.2 Current state of knowledge

Immunity is mediated by a tightly balanced network of specialised tissues, cell types and molecules, which identifies and neutralises harmful pathogenic intruders and also potentially dangerous cancer cells. Insufficient activity of the immune system permits pathogens to cause morbidity and mortality and fails to prevent the progression of single malignant cells to cancer. Compared to T cells (a cell type of the adaptive immune system), the dysfunction of myeloid cells (cell types belonging to the innate immune system) is poorly understood.
For many decades and according to earlier textbook knowledge, myeloid cells have been regarded as anti-microbial and immunostimulatory cells. More recently, additional regulatory and even immunosuppressive functions have been described for the major myeloid cell types, neutrophils, dendritic cells, and macrophages. Recent murine studies have additionally described the induction and pathologic function of so-called myeloid-derived suppressor cells (MDSCs), resulting in the search for these cells in humans.
Not unexpectedly for an emerging field, research on the regulatory function of myeloid cells, their

biology and pathophysiological relevance has developed in a non-coordinated fashion. Nevertheless, this has resulted in important insight into the biology of individual MRC subsets. For example, many features and cell biological properties of MDSC have been uncovered. Similarly, the functional plasticity of macrophages has been elucidated and researchers are trying to identify markers which are useful for identifying or even isolating such macrophage subtypes. The same is true for neutrophils, where anti-microbial, inflammatory and immunosuppressive subsets have been described. It is, however, a particular complication of the field - and a reason for forming this Action - that research on MRCs has evolved in a cell-specific manner, resulting in individual research groups having excellent expertise and technical tools for just one particular MRC subtype, such as MDSC, neutrophils, macrophages or DC. Furthermore, a comprehensive and comparative analysis of MRC subtypes is missing, especially due to the lack of commonly accepted specific markers, which could be utilised by the scientific community to unequivocally identify these cells. This generates controversial and unclear results even among research groups working on the same cell type. Thus, the organised and standardised comparative use of markers and functional assays for all MRC subtypes in one consortium is a major innovative aspect and aim of this Action.

In addition to knowledge on the cell biology of MRCs and their subtypes, an involvement of MRCs in major diseases has been elucidated in recent years. For example, the regulatory function of myeloid cells is detrimental in cancer and infection by preventing effective immunity. On the other hand, MRCs are exploited for therapeutic purposes in diseases with overshooting immunity such as autoimmunity and allergy. This Action will take a new approach by forming a network of basic scientists and clinicians, which will, for the first time, enable researchers to comprehensively compare MRCs from different diseases on an international level and, by employing common protocols and markers, to characterise these cells in experimental animal models and man.

In sum, current research activities have enhanced our understanding on the biology of individual MRC subtypes and provided substantial evidence for the role of specific MRC subtypes in a given disease. However, comparative cell biological studies and a broader understanding of the clinical and disease-related relevance of MRCs are missing. This Action will close this gap by providing tools and standards for the clinical monitoring of these cells and by developing the technical means to describe the common, cell-type specific and disease-specific functional properties of these cells.

B.3 Reasons for the Action

Myeloid cells with regulatory function (e.g. myeloid suppressor cells, regulatory macrophages, or tolerogenic DC) have been shown to be detrimental in infections, inflammation and cancer, but

have become a promising therapeutic tool to treat autoimmune diseases, severe allergies, or to improve transplantation outcomes. A variety of MRC subtypes in the human and mouse immune system have been identified in the last few years, but the lack of consensus on markers as well as protocols and methods to detect them makes it difficult to study and compare these cells in different diseases and experimental models. Although research on MRC subtypes is well-funded by individual grants throughout the European Union, the above mentioned obstacles hamper the rapid development of effective therapies, which either target or utilise immunoregulation through MRCs. This Action will harmonise and coordinate dispersed individual and national research activities in the field on a European level. This Action will enable researchers to form a network of experts in basic, translational and clinical research in order to promote the diagnostic and clinical application of MRCs in socio-economically important diseases.

This being said, Mye-EUNITER will address both European economic/societal needs and will advance scientific knowledge in the field. MRCs are involved in the pathobiology of infectious and malignant diseases, which cause a major socio-economic burden to the European health system. Standardised tools developed by this Action will improve the diagnosis and the development of therapeutic options for these diseases. The latter part will be performed in joint efforts with biotech industry whenever feasible. At the same time, these tools and protocols will enhance the scientific knowledge in the field. This will be achieved by the exchange of technologies, analytical tools, obtained data, and researchers at several levels:

- Data exchange between researchers working on different subsets of MRCs will increase scientific knowledge on the cell biology of these cells.
- Exchange of knowledge, data and tools between researchers working on rodents, non-human primates and humans will promote the development of new pre-clinical models for MRC analysis.
- Exchange between basic researchers and clinicians in this Action will facilitate the prospect of the clinical application of protocols and tools used for diagnosis and monitoring of disease progression/treatment.
- Exchange of know-how with industrial partners in this Action will improve the development of diagnostics and therapeutics.

B.4 Complementarity with other research programmes

Mye-EUNITER is complementary to the following activities:
COST ACTION BM1305. Action to focus and accelerate cell-based tolerance-inducing therapies (A FACTT). The primary focus of A FACTT is on the development of quality control parameters for cell-based products in tolerance-inducing therapies. Mye-EUNITER will contact A FACTT in order to discuss and perform activities aimed at testing MRCs as potential cellular therapeutics in diseases with overshooting immunity. Members of Mye-EUNITER and A FACTT will also exchange candidate biomarkers and standard operating procedures (SOPs), which are applicable in both Actions. Due to a joint interest in immunoregulation yet with each having clearly distinctly differing major objectives, the interaction of Mye-EUNITER and A FACTT will advance the field synergistically. While A FACTT focuses on the controlled use of tolerogenic antigen presenting cells and regulatory T cells as cell-based therapeutics, Mye-EUNITER focuses on the standardised monitoring of diverse MRC subsets in disease pathology and therapy ranging from murine to translational models and to human disease. Mye-EUNITER members have already established preliminary contacts with the A FACTT coordinator who has expressed a mutual interest in performing the activities mentioned above in the case that this proposal is funded.
A cooperative project funded by CORDIS FP7: The ONE Study, A unified approach to evaluating cellular immunotherapy in solid organ transplantation. This cooperative action is focused on identifying distinct populations of hematopoietic regulatory cells and comparatively testing their safety and efficacy in minimising pharmacological immunosuppression in solid organ transplantation.
A cooperative project funded by FP7: TRIAD, Tolerance Restoration in Autoimmune Disease, provides a strategy to autoimmune diseases therapy based on the use of anti-CD28 antibodies in pre-clinical models.
A cooperative project funded by FP7: TARKINAID, Targeting Src-family tyrosine kinases in chronic autoimmune and inflammatory diseases, developing novel strategies to target autoimmune and inflammatory diseases by small-molecule tyrosine kinase inhibitors.
TIMCC: Marie Curie Initial Training Network on Tumour Infiltrating Myeloid Compartment Cells. This ITN investigates a specific aspect of myeloid cells in cancer, which is the regulation of the pro- and anti-tumor activity of tumor-infiltrating myeloid cells.
EUTRAIN: Marie Curie Initial Training Network on the research of chronic inflammation in children. This ITN addresses questions regarding the immune regulation in autoimmune diseases in children and will facilitate the transfer of results obtained in this network to translational and clinical research. In addition, this connection to EUTRAIN links Mye-EUNITER also to the efforts of the EU on rare diseases and supports the goals of the International Rare Diseases Research

Consortium (IRDiRC).
All of the above mentioned research programmes will benefit from the outcomes and deliverables of Mye-EUNITER. Coordinators of Mye-EUNITER will contact coordinators of the above-mentioned research programmes in order to discuss the applicability of Mye-EUNITER marker panels, analytical tools and therapeutic models in these research programmes.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main overall objective of the Action is to provide gold standards for the definition and functional analysis of specific subpopulations of MRCs, to form the first European scientific network investigating and comparing MRCs in several common major immune-related disorders, namely cancer, infection, autoimmunity and inflammation, as well as to examine for the first time the role of MRCs in these diseases in experimental models, translating from mouse and monkey to man.

C.2 Objectives

The primary objective of this Action is to provide gold standard analytical tools and markers for the analysis and definition of specific subpopulations of MRCs. The provision of these tools is of crucial importance for the comparability of results obtained in experimental and clinical studies across different laboratories and EU countries. Members of this Action will exchange markers, tools, reagents and protocols to develop optimal analytical tools specific for each MRC subtype. Definition of such gold standards plus standardised analytical tools and technical protocols is only possible by a large international and inter-laboratory approach executed within the framework of a structured research network. The activity of this Action will enable researchers and clinicians to reliably monitor the frequency and activity of MRCs in human diseases. This will offer new options in assessing the effect and efficacy of interventional therapies in major immune-related disorders. The deliverables generated by this Action will also provide new insight into the immunopathology of those diseases.
Further objectives of Mye-EUNITER are:

- Use the developed standardised tools for a functional comparison of MRCs in different animal models and from different human disease states. Identify common and divergent markers and functions of MRCs present in the different human diseases.

- Make developed marker profiles and analytical tools available to the scientific community by publication of protocols and scientific results in journals and on the Mye-EUNITER website.
- Exchange of Early Stage Researchers (ESRs) between participating laboratories and involvement of doctoral schools at participating universities. In many European countries biomedical research is primarily carried out by doctoral/PhD students. This is in contrast to the USA, where post-doctoral fellows are more commonly involved. It is therefore of crucial importance to involve doctorates and ESRs in Mye-EUNITER activities. This will enable the members of this Action to train a new generation of ESRs, equipped with the expertise to translate novel findings from the Action into clinical and translational research.
- Develop, prepare and submit applications for multicenter trials within the context of HORIZON 2020 with the goal to further utilise standardised analytical tools and monitoring approaches generated during the course of this Action.
- Promote the active participation of scientists from the new Central-Eastern-European EU members in Europe-wide research activities and coordination.

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

The objective of the Mye-EUNITER Action is to focus and accelerate the field by defining standardised protocols of analysis between different centres, allowing for comparisons between subpopulation of MRCs. In particular, comparative parameters to identify MRCs will be defined and validated. Furthermore, harmonised protocols for the immune monitoring and functional analysis of MRCs in different human diseases and disease stages well as in different animal models of infections, inflammation and cancer will be standardised. Harmonisation and standardisation will be achieved by activities executed within the specialised Working Groups. To this end, Working Groups (WGs) will form task forces, which will suggest markers and functional assays to be included in the Action's activities. These markers and assays will then be subjected to rigorous inter-laboratory testing. After evaluation of the data, the Working Groups will come up with suggestions for marker panels and analytical functional assays. These will then be transferred to the Working Groups specialised on analysis of patient samples and translational animal models for further validation. These Working Groups will subsequently use those standardised assays as

biological read outs in patients and therapeutic clinical trials (see section D for details on the tasks and the interactive nature of the Working Groups).
The expected result of this process is that the implementation of these standardised protocols will greatly facilitate our understanding of MRC biology, will make results between laboratories comparable on an EU level, and pave the way for the clinical monitoring and therapeutic use of MRCs.

C.4 Potential impact of the Action

The expected benefits to stem from this Action are:

- Technically: to develop, implement and standardise biomarkers of MRCs specifically in malignant, infectious and inflammatory diseases in animal and human models.
- Scientifically: to increase the knowledge about an important but still poorly understood group of immune cells, namely MRCs. Specific areas, which will benefit from the research programme carried out in Mye-EUNITER are: differentiation and development of MRCs, mode of action of MRCs, pharmacologic intervention with MRC function.
- Clinically: to enable clinicians to monitor the frequency and activity of an important immune cell type in immune-related pathologies. To translate this knowledge into clinical trials of targeted immunotherapy by manipulation of MRC function.
- Societally: to improve the training of and job opportunities for ESRs and members of the participating doctoral schools. To establish a means of support for the active participation of female ESRs and participants with children. To promote the integration of new Central-Eastern-European members of the EU into the European scientific networks.
- Economically: to avoid redundant and non-coordinated studies on MRCs at different locations in Europe. To harmonise and coordinate the future monitoring of MRCs in large clinical trials in a cost-effective way. To improve immunologic read-outs and the accuracy of clinical trials sponsored by the pharmaceutical industry. Technical and scientific outcomes of this Action may also result in patents filed by members of the respective WGs.
- Public health: as a long-term benefit Mye-EUNITER will accelerate the identification of novel targets for therapeutic intervention in infectious diseases, inflammation, and

cancer. In addition, MRC can be suitable also for cell-based therapies for the cure of autoimmune diseases, allergy or for the prevention of graft rejection. Thus, this Action has the potential to improve patient health and well-being, and reduce the economic burden of chronic and debilitating diseases in Europe and the rest of the world.

C.5 Target groups/end users

The end users of this Action are:

- Academic researchers and clinicians interested and/or involved in MRC biology and their pathological relevance.
- Pharmaceutical industry and biotech companies involved in the development of novel target therapeutics for the treatment of malignancies, infections and inflammatory diseases.
- Patients and clinicians, who will test, exploit and benefit from novel analytical and diagnostic tools developed via this COST Action.
- Doctoral students and ESRs at the participating centers, because i) ESRs carry out research activities in the course of this Action, ii) ESRs will benefit from STSMs iii) the education of ESRs in participating doctoral schools will benefit from the networking activities carried out by Mye-EUNITER.

As outlined above, MRCs are now recognised as important cellular regulators in infectious, malignant and immune-related diseases. Consequently, there is a need and an increasing interest to monitor MRCs in clinical trials. For example, large pharmaceutical companies in both Europe and the US have started monitoring MRC subsets in oncological clinical trials. However, technical tools and standards are still poorly developed. Researchers participating in the Mye-EUNITER Action are also involved in the immunological monitoring activities of pharmaceutical companies. Thus, the tools and protocols developed in this Action can directly be implemented into ongoing clinical trials. Furthermore, MRCs are attractive new candidates for cell based anti-inflammatory therapies, e. g. therapy refractory autoimmune disease or graft versus host disease. Analytical tools developed in this network will be a prerequisite for definition of target populations for such novel cell-transfer approaches.

Researchers interested in Mye-EUNITER are members of existing academic research groups and organisations involved in research on myeloid cells. Specifically, the European Phagocyte Group,

an informal group of researchers interested in the biology of phagocytes (with an emphasis on neutrophil biology) has been contacted and some members have expressed their interest or have already joined Mye-EUNITER. Also the European Macrophage and Dendritic Cell Society (EMDS) has been contacted and members have expressed their interest or joined Mye-EUNITER. While this COST Action will focus on a defined work programme to develop MRC-specific research tools and apply these clinically, the above-mentioned societies focus on the academic exchange of novel research findings, mainly organised and executed by one annual meeting. In this way, myeloid cell-related societies and this COST Action are complementary, providing the possibility that novel findings presented at the society meetings can be incorporated into the scientific programme of Mye-EUNITER. Conversely, standardised immunomonitoring studies executed via Mye-EUNITER will be presented at society meetings.

Similarly, some members of Mye-EUNITER are members of CIMT (Association for Cancer Immunotherapy). CIMT promotes research in and the development of cancer immunotherapies. CIMT also runs so-called proficiency panels for the immunomonitoring in cancer patients. Mye-EUNITER will contact CIMT for the discussion on and potential use of markers and assays suitable for the immunomonitoring in cancer patients.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

This Action is pioneering in that it will form the first European scientific network investigating and comparing MRCs in several common major immune-related disorders, namely cancer, infection and inflammation. Furthermore, this Action will examine for the first time the implication of MRCs in these diseases in experimental models, translating from mouse to monkey to man.

For decades, neutrophils, macrophages and dendritic cells have almost exclusively been seen as cells inducing or executing immunity. More recently, these myeloid cells have been shown to have regulatory functions, with MDSCs attracting a lot of attention. However, this research field is still very diverse because standardised markers and protocols are missing.

The structure of the network will allow us to address innovative, poorly understood and clinically important aspects of MRC biology in order to achieve the following research tasks:

- Identification of shared and distinct markers useful for identifying specific MRC subtypes. It is a major task of this Action to facilitate the use and evaluation of such markers “across cell types”. This can only be achieved by the coordinated networking activity of researchers and laboratories otherwise specialised in “only” one particular

cell-type. Based on published literature and own laboratory experience, tested markers will include but not be limited to cell surface molecules, secreted immunoregulatory molecules such as cytokines, chemokines and lipid mediators, intracellular functional molecules stored in the cytoplasm or in subcellular vesicles and organelles, and markers of myeloid cell differentiation and activation. WG 1.

- Implementation and development of standardised and reproducible analytical tools and protocols for the functional analysis of MRCs. Next to the identification and enumeration of MRCs, it is also of pivotal importance to assess their functional activity. Here, as well, the "working across cell borders" approach is a critical and innovative aspect of Mye-EUNITER. Functional assays including but not limited to immunosuppressive, immunoregulatory, inflammatory, T cell-stimulatory and anti-microbial activity will be performed and applied to neutrophils, macrophages, dendritic cells, MDSCs and even eosinophils, basophils, and mast cells. WG 2.
- Identification of shared and distinctive cell biological markers and functional molecules/properties between MRCs present in subjects with cancer, infection, inflammation, and autoimmunity. By incorporating clinical institutions and researchers, the marker panels and standardised functional assays will be applied to clinical samples obtained from patients with selected immune-related diseases. WG 3, based on work performed by WG 1 & 2.
- Application of these (possibly disease specific) marker panels and standardised functional assays to monitor the frequency and functional activity of MRCs in human patients. Monitoring will also be performed to assess the effect of therapeutic interventions on MRCs frequency and function. WG 1-3.
- Design of protocols and tools to manipulate MRC subtype function in pre-clinical animal models of disease with high translational potential. Translation of research from experimental animal models to human diseases is a major challenge. Non-human primates and humanised mice represent well-accepted models for many human diseases. However, research tools for MRCs in these models are particularly scarce. This COST network will bridge the gap between mice and humans in an important area of immunological research by including scientific partners investigating non-human primate models and humanised mice (the mouse-monkey-man translational line). WG 4, based on work performed by WG 1 – 3.

- Identification of candidate target molecules and approaches for the pre-clinical and clinical modulation of MRCs in disease. Possible approaches include the identification of strategies to target and abolish myeloid regulatory function in order to boost anti-viral and anti-cancer immunity, as well as strategies to exploit the immunosuppressive activity of MRCs in diseases associated with overshooting immunity or autoimmunity. While these approaches reflect the current expertise within the network, the final decision will depend on the results of the WGs formed in this Action and on a critical evaluation of these data in WG 5 (modulation of MRCs for therapeutic purposes). WG 5 with other WGs.

In summary, this Action will lead to a better understanding of the pathophysiology of MRCs and to new therapeutic options targeting these cells in a number of important human diseases.

D.2 Scientific work plan methods and means

In order to optimally achieve the overall objectives and tasks of this Action, Mye-EUNITER will form the following Working Groups (WG):
WG 1: Immunomonitoring and definition criteria for MRC subtypes
WG 2: Functional and molecular analysis of MRC; common protocols and signatures
WG 3: Disease-specific MRCs
WG 4: Animal models and translational research
WG 5: Modulation of MRCs for therapeutic purposes.

WG 1: Immunomonitoring and definition criteria for MRC subtypes
Main objective: define a unifying and commonly accepted panel of markers for the identification of MRC and MRC subtypes.
Methods and means:

- Survey of the literature and definition of the current state-of-the-art markers.
- Establishment of a technical exchange panel with the aim to plan inter-laboratory testing and harmonisation of marker panels.
- Inter-laboratory testing and evaluation of published markers by Mye-EUNITER technical exchange panel participants.

- WG meeting to exchange data, discuss the value of existing markers and propose useful marker combinations for MRC subtype definition.
- Development of a “consensus flow cytometry protocol” for further testing and for the identification of the major MRC subtypes.
- Development of a flow cytometry SOP for analytical purposes.
- Cooperation with WG 2 to link immunophenotype and markers with function.
- Writing of position and consensus papers on state-of-the-art knowledge and novel definition criteria generated by activities of Mye-EUNITER. Suggest outcomes of this Action will be disseminated to the wider scientific community.

WG 2: Functional and molecular analysis of MRC; common protocols and signatures

Main objective: define common protocols for the functional analysis of MRCs; identify common and cell-type specific molecular signatures of MRCs.

Methods and means:

- Survey of the literature and critical evaluation of the current protocols used for functional MRC analysis. Definition of strengths and weaknesses of current state-of-the-art analytical tools and methods.
- Broad inter-laboratory testing of available functional assays in order to expand the portfolio of available assays and data derived thereof.
- Development of a defined but comprehensive set of functional assays for MRC analysis.
- Harmonisation and standardisation of the technical performance of these assays. Identification and outlining of important variables influencing the outcome of the selected assays. Development of a SOP.
- Implementation of large scale genomic (DNAse 1 foot printing, epigenetic analysis) and gene expression profiling approaches (miRNA, lncRNA arrays and NGS RNA-seq). Epigenetic studies on myeloid cells have already been initiated and will be conducted in collaboration with the EU-funded BLUEprint project aiming to decipher the epigenome of blood cells. These data will lead to the discovery of novel MRC-related genes and their regulation and function. Together with the gene expression and epigenetic signatures they will be implemented into the activities of WG 1 and WG 2.

- An important aspect of this WG is the “Learning from each other’s approach”. This means that specialised research groups compare and exchange isolation protocols and functional assays. As a result a major assay in the MDSC field may be adopted by neutrophil researchers or a molecule regulating dendritic cell function will be tested in macrophages or MDSCs and so forth.
- Writing of position and technical consensus papers in order to distribute and suggest the results of this WG to the international scientific community.
- Write scientific opinion papers on the functional relationship of MRC subtypes.

WG 3: Disease-specific MRCs

Main objective: Comparative analysis of MRCs obtained from patients with different diseases in order to define common and disease-specific features of MRCs. This task will be performed in close collaboration with and by using results obtained in WG 1 and WG 2.

Methods and means:

- Generation of a task force with the aim to identify diseases and patient cohorts for the analysis of disease-specific (and common) features of MRCs.
- Contact with WG 1 and WG 2 on the selection of markers and analytical tools.
- Definition of SOPs for the collection, storage and analysis of data (together with members from WG 1 and WG 2).
- Comparative analysis of MRCs according to the main objective of this WG 3.
- Based on the expertise and composition of the Action during the planning phase, WG 3 will start with material from cancer patients and patients with viral infection. The programme of WG 3 remains flexible so as to be able to incorporate new scientific developments and incoming network participants.
- Validated guidelines and SOPs will be implemented into clinical immunomonitoring protocols operative at the partner sites.

WG 4: Animal models and translational research

Main objective: Bridge the gap between conventional rodent models and human patients by the incorporation of non-human primate models and models employing humanised mice.

Animal models allow the discovery of new immunoregulatory mechanisms, and test for the safety

and efficacy of novel therapeutic strategies. Several immune cell subsets, receptor expression repertoires as well as innate responses have a high degree of similarity between the human and non-human primate immune system, which is in contrast to rodents. Due to the difference at the level of innate immunity in particular, we propose using humanised mice models. Non-human primates are the closest relatives to humans and, for some human diseases, the only available pathophysiological model. This makes non-human primates an indispensable *in vivo* model that can yield far more relevant data for translational studies into humans as well as for many other inflammatory, transplantation and autoimmunity studies. However, presently the analytical tools for non-human primates are limited and these will be developed within the context of this Action.
Methods and means:

- Selected, well-defined models of infection, inflammation and cancer in non-human primates and humanised mice will be used. Based on the results from WG 1-3, markers and tools for immunophenotyping and functional analysis of MRCs will be selected, tested, validated and applied.
- Subsequent SOPs for MRC analysis in non-human primate and humanised mice models will be developed.
- Study of specific MRCs, selected as described above, in blood and tissue samples from non- human primate models of inflammatory and infectious pathologies to analyse their association with disease.
- Depletion of MRC subsets in humanised mice to test for their pathophysiological relevance.
- Writing of a scientific paper on the phenotypic and functional relationship between MRC subtypes in humans vs. non-human primates.

Disease specific models for non-human primates are difficult to develop, cost-intensive and require specialised centers and expertise. Partners at a major European center for infectious diseases models are included in this call have already developed outstanding platforms for monitoring diseases, treatments and prevention in relevant non-human primate models of human infections. Thus, Mye-EUNITER will provide the national and international scientific community with a highly competitive infrastructure for pre-clinical research.

WG 5: Modulation of MRCs for therapeutic purposes

Main objective: development of new options for the manipulation and therapeutic application of

MRCs.

Methods and means:

This WG aims at translating achievements from WG 1-3 to preclinical models and ultimately into clinical studies with the help of WG 4.

Based on results from WG 1-4 this WG will:

- Define monitoring parameters and endpoints suitable for qualitative and quantitative measures of MRC modulation *in vivo*.
- Discuss and define the suitability, applicability and the translational value of models analysed in WG4.
- Discuss and suggest early clinical trial protocols for therapeutic modulation of MRC function in patients.
- Seek advice from and closely communicate with regulatory authorities.

Specific considerations with respect to the work flow in this Action

The scientific programme and work plan of Mye-EUNITER is primarily organised within WGs and carried out by the laboratories participating in those WGs. A typical work flow of this Action will start with the formation of task forces (as part of WG 1/2) responsible for the immunophenotyping and functional analysis of MRCs, respectively.

- Each task force will write state-of-the-art/position papers and will identify areas which yield the most benefit in the technical standardisation and harmonisation approaches.
- Task forces will join with further members of WG 1 and WG 2 to set up technical exchange panels to execute the experiments planned for these WGs.
- Experiments will be discussed through the electronic exchange of data, followed by face-to-face workshops.
- Subsequently, a second round of tasks will be performed to develop common technical protocols and SOPs.
- Face-to-face workshops will finalise common technical protocols and SOPs.
- Results (marker panels, analytical tools) from WG 1 and WG 2 will be made available to WG 3 and WG 4 for further testing and validation in patient material and pre-clinical animal models.

- Follow-up position papers suggesting common technical protocols and SOPs to the scientific community will be written.

Working Groups and STSMs

In addition to the development of standardised panels of consensus markers and functional analytical assays, SOPs and position papers for dissemination to the scientific community, WG 1-4 will organise 5 Short-Term Scientific Missions (STSM) each per year. A brief technical and scientific report on each STSM will be published on the website of the Action. STSM between basic research and clinical participants will be especially encouraged. WGs will also jointly organise an annual ESR symposium with a satellite training school. The topics of the symposium will reflect the major objectives of the WGs of the consortium.

E. ORGANISATION

E.1 Coordination and organisation

Under the auspices of the Management Committee (MC), the Steering Group (SG) and Dissemination and Development Management (DDM) team will be elected at Mye-EUNITER's 1st MC meeting. Five WGs will be formed to execute the programme of this Action.
Whereas the participating institutions of this proposed COST Action are well able to finance and develop their own laboratory research and fund ESR stipends through national and/or other grants, Mye-EUNITER currently lacks the means to cover the necessary coordination and international European exchange of these activities. During the history and preparation of this proposal Mye-EUNITER participants became aware of the fragmentation and even confusion on certain markers and tools utilised in their research. To achieve a coordinated research activity on MRC at the European level Mye-EUNITER will implement the following organisational means:

Management Committee (MC)

The MC will coordinate the Action according to common COST Action organisational standards for the proposed duration of four years. Currently, annual meetings of the MC are planned, while the MC will have an additional second annual web-based (webinar) meeting. The MC will encourage female researchers to participate and gender balance will be a standard item on the agenda of all MC meetings.

Steering Group (SG)

For each Working Group (WG) Steering Group (SG) members consisting of one WG leader and a

deputy WG leader will be nominated by the MC. The SG is responsible for the coordination, organisation and supervision of their WG's meetings and progress. Each will assign local organisers for every meeting. The WGs will meet once a year for the duration of the Action and will remain in close contact, whereas the SG will meet physically once a year with web-based (webinar) conferences six months later to exchange their latest findings.

Dissemination and Development Management (DDM)

A Dissemination and Development Management (DDM) team consisting of two academic managers will be responsible for the maintenance of public and private web sites and the generation of all publications. The web-based platform will feature an integrated knowledge management tool for information and communication with central databases and an active online forum where the results from different laboratories can be openly discussed. Further responsibilities include the general coordination and management of this Action, organisation of the meetings and workshops as well as supporting the Action's long-term development towards forming an eventual Marie Sklodowska Curie Innovative Training Network (ITN). The DDM shall be the liaison contact partners for both the expert researchers and the ESRs, and shall coordinate the regular exchange of information between the doctoral schools. The DDM members should be observing participants at the meetings of the MC and SG to ensure the informed dissemination of information and results arising from this Action. All DDM activities are subject to MC approval.

Task Forces

Specific individual members of the Action will be selected by the MC as a Task Force responsible for nurturing the following aspects for the duration of this COST Action: STSMs, ESRs, Gender, and Clinical Expert Liaison.

Early Stage Researchers (ESRs)

Since among this Action's main aims are to share latest findings with ESRs, to train the next generation of Mye-EUNITER experts, and to promote interdisciplinary exchange between the existing doctoral schools within the COST Action, annual ESR Symposia and workshops are an integral part of this network. Here, the ESRs will have the opportunity of presenting their research to a critical peer audience which will lead to the emergence of bottom-up collaborations across Europe, further strengthening this Action at the ESR level. Parallel to the symposia, Training Schools focusing on the acquisition of newly developed laboratory techniques will be held to train the ESRs in the correct use of common protocols and SOPs arising from this Action. Participants will be mainly, but not exclusively, young researchers involved in COST Actions and will also cover appropriate re-training as part of life-long learning. This will significantly augment the interactive nature of the WG activities. Furthermore, the results of this COST Action will be

implemented into the curricula of its PhD programmes.

Especially promising collaborations will in turn be backed by Short-Term Scientific Missions (STSMs).

A web-based platform will be created to facilitate the exchange of graduates and ESRs between participants.

Milestones

Whereas the organisational milestones for Mye-EUNITER may be regarded as a fixed framework, the research-related milestones are subject to flexibility, allowing the WGs to adapt to and incorporate the latest developments and state-of-the-art knowledge into their working schedules. Especially the milestones of those WGs depending on the outcomes, data and findings of other WGs within this Action should be regarded as calculated estimates.

Organisational milestones:

Milestones	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Establishment: MC, SG, DMM, WGs	X															
Kick-off Meeting and Inaugural Conference	X															
Website set-up		X														
Dissemination up-dates		X		X		X		X		X		X		X		X
MC Meetings	X		X		X		X		X		X		X			X
SG Meetings	X		X		X		X		X		X		X		X	
DDM Meeting	X				X				X				X			
WG Annual Meetings	X				X				X				X			
ESR Symposium with satellite Training School			X				X				X				X	
STSM	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mid-Term Conference and preparation of International Project Application								X								
Final Conference																X
International Project Application															X	

Scientific milestones:

Milestones WG 1 & 2	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Establishment, survey, definition of markers/assays	X															
Technical panel, testing, harmonising		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluation of data			X		X		X		X		X		X			X
SOPs/position papers			X			X				X				X		X

Milestones WG 3	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Comparative analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Definition of features, evaluation of data			X		X		X		X		X		X		X	
Implementation of SOPs							X	X	X	X	X	X	X	X	X	X

Milestones WG 4	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Selection of animal models, markers and tools	X	X	X	X												
Testing, validating, application		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluation of data			X		X		X		X		X		X			X
SOPs/position papers			X			X				X				X		X

Milestones WG 5	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Definition of measures	X	X	X	X	X	X										
Translational model testing							X	X	X	X	X	X	X	X	X	X
Planning and start of clinical trials											X	X	X	X	X	X
SOPs/position papers										X			X			X
Evaluation of data							X		X		X		X			X

E.2 Working Groups

All researchers participating in the Action will be encouraged to join at least one WG. WGs will

address specific subtopics as outlined in section D. For members of WG 4 & 5 the participation in one of the WGs 1-3 is mandatory in order to optimise transfer of results between the WGs. Both experienced researchers and ESRs will be members of the WGs, consequently greatly facilitating discussion between these groups. The MC will appoint coordinators of WGs (Steering Group, SG). SG will organise the activities of their respective group according to the aims and objectives outlined in the Memorandum of Understanding (MoU). SG will organise one annual meeting of their board and will ensure appropriate exchanges between laboratories by STSMs. SG will also ensure continuous contact with associated WGs of this Action in order to ensure the fulfilment of the collaborative tasks as outlined in Part D.2. SG will report on the progress and activities of the WG to the MC and the Action Chair. The MC is responsible for monitoring and evaluating the activities of WGs in order to ensure the optimal and on-schedule achievement of Action tasks.

E.3 Liaison and interaction with other research programmes

Mye-EUNITER will be open to interacting with other research programmes not yet involved in the preparation of this application. Mye-EUNITER will be especially interested in clinical research programmes willing to apply marker panels and analytical tools developed within this Action. Mye-EUNITER will ensure proper interaction with all programmes mentioned under Section B4 by inviting the respective programme chairs (or substitutes) to scientific Mye-EUNITER meetings. MC members of Mye-EUNITER will express vice-versa interest to participate in scientific meetings of those complementary research programmes.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.
Whereas the initial participants of this Action have been approached on the basis of their pioneering scientific expertise in the field of myeloid regulatory cells, wherever possible a female counterpart has been sought. Thus, at the launch of this Action, Mye-EUNITER's proposed MC will have a gender balance of 35,5 % females, which is well above the latest European average of 15,8 % (source: *European Commission, Women on boards – Factsheet 2. Gender equality in the Member States, March 2013*). Should this Action be approved, it is its aim to readdress the current

imbalance to reach an ideal 1:1 gender ratio at all levels (MC, SG, WG, ESR) by the end of the four-year COST funding period. To achieve this, the following measures will be taken:

- Since, at the date of submission, a number of the COST Countries participating in this Action have only one nominated member for the MC, they will be encouraged to select a second MC nominee who is female, possibly even a competent representative stemming from their country's ESR ranks.
- At the Action's kick-off meeting, where the members of the WGs and SG will be determined, both gender equality and ESR involvement will be considered when selecting candidates for these positions.
- Female researchers will be particularly encouraged to participate in STSMs to extend their professional network and widen the scope of their career opportunities.
- Once the fundamental structures have been established, and as the Action develops, the increasing involvement of women and ESRs in all of Mye-EUNITER's activities at every decisive level will be a priority item on the agenda.
- Capacity building of a new gender-balanced generation of ESR Mye-EUNITER experts is planned by striving towards a common curriculum for COST participating doctoral schools which additionally will further international exchange and broaden the geographical job market open to young researchers in this field within the European Research Area.
- ESRs will be offered their own independent networking opportunities at the ESR Symposia and Training Schools.
- ESRs will be a well-represented contingent at all of Mye-EUNITER's conferences. For professional profiling outside the Action's network, they will be encouraged to actively participate at other significant international conferences in the field organised by external societies or organisations.
- Important meetings will be planned around the major school holiday seasons. If an Action member is physically unable to attend an important discussion round or other event due to family reasons, every attempt at audio-visually and/or digitally linking them to the event in real time will be made upon request.
- A standard part of Mye-EUNITER's conference organisation will be the consideration of local childcare facilities, information about which will be passed onto the participants

if required. Guidelines on family-friendly policies as set down in the document on "*COST Strategy towards increased support of early stage researchers***"** will be followed.

The MC and DDM will oversee and evaluate the development of gender balance and the integration of ESRs at yearly intervals, suggesting corrective strategies for the forthcoming year in order to ensure its goals are fulfilled by the end of the Action period.

F. TIMETABLE

	Year 1				**Year 2**				**Year 3**				**Year 4**			
Kick-off Meeting	X															
Inaugural Conference	X															
MC Meeting	X		X		X		X		X		X		X			X
SG Meeting	X		X		X		X		X		X		X		X	
DDM Meeting	X				X				X				X			
Website set-up		X														
Website up-date/ Newsletter		X		X		X		X		X		X		X		X
WG 1 Meeting	X				X				X				X			
WG 2 Meeting	X				X				X				X			
WG 3 Meeting	X				X				X				X			
WG 4 Meeting	X				X				X				X			
WG 5 Meeting	X								X				X			
ESR Symposium with satellite Training School			X				X				X				X	
STSM (5/year: WG 1-4)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mid-Term Conference and preparation of International Project Application								X								
Final Conference																X
International Project Application															X	

G. ECONOMIC DIMENSION

The following COST Countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CH, DE, ES, FR, HR, HU, IE, IL, IT, NL, PL, 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 60 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?

Research on all MRC subtypes is well funded by individual grants throughout the European Union. However, there is a lack of consensus on markers, tools and methods, making it difficult to study and compare these cells in different diseases and experimental models. This hampers the rapid development of effective therapies targeting or utilising immunosuppression through MRCs. Thus, to combat these issues, the main target audiences of the outreach of this COST Action are:

- Researchers of MRCs in inflammatory and infectious diseases, autoimmunity and cancer. These include the partners in this COST Action as well as other research institutes/university researchers involved in the diagnosis and treatment of these pathological conditions. This target audience will be very relevant for recruitment of new partners to the Action and to establish links and synergies.
- ESRs – Special input will be given to ESRs, given the relevance of having a critical mass of young researchers with a specialised knowledge on MRC in infection, inflammation and cancer. The Action’s latest findings will be used as text book knowledge for ESR doctoral school curricula and the exchange of bottom-up knowledge at annual ESR symposia and Training Schools will further strengthen this network.
- Clinicians - Assays will be developed to define the role of MRCs in certain diseases and therapeutic targeting of MRCs will be developed with industrial partners. Clinicians are imperative for any clinical trials arising from the findings of this Action.
- Pharmaceutical and biotechnology companies working on closely related fields, mainly focused in generation/manipulation of diagnostic and therapeutic strategies. Industrial partners will be actively contacted in order to attract interest in the exploitation of results. Companies in the European biotechnology/bioengineering industry will benefit from progress made by the Action. Given the importance of the European market in this

field, this Action may contribute to a competitive advantage of European biotech companies.

- Patients - Clinical use of drugs manipulating MRCs: Patients (individuals and associations) and their families are the target audience of this Action, as it aims to accelerate the translation of therapies into real therapeutic approaches, moving towards a personalised medicine, improving quality of life of citizens with autoimmune diseases, infection, and malignancies.
- Society at large, for the direct impact of these therapies on patients and general public.

H.2 What?

The dissemination of the results will be provided to the following groups via the listed means:

Scientific public

- Publication of common technical protocols and SOPs, reports of proceedings of Meetings, Conferences, Workshops, Training Schools and STSMs.
- Publications of original research and reviews in peer reviewed journals.
- Participation in scientific divulgation events (local institutions, local scientific organisations, local press and radio), presenting the aims of the Action and the relevance of MRC as advanced therapies for malignancies, inflammation and infections.
- Workshops, seminars and training schools at the conferences and symposia organised by Mye-EUNITER as well as at European and other international conferences.
- A password-protected interactive website for Mye-EUNITER participants posting working documents and other relevant information and documents related to the execution of the Action.

Clinical public

- Lectures and Training Schools on MRC-based therapy
- International interdisciplinary exchange programme
- Active mentoring of ESRs by experienced colleagues

ESRs

- Lectures, ESR Symposia and Training Schools on MRC-based research and therapy
- International interdisciplinary exchange programme with state-of the-art doctoral curricula and regular STSMs
- Online, password-protected access to common technical protocols and SOPs
- Active mentoring of ESRs by experienced colleagues

Industrial partners

- Pharmaceutical and biotech companies; generation/manipulation of diagnostic and therapeutic strategies can benefit from the results obtained through this action and set up clinical trials.

Patients and the general public

- Interlinked public (open) websites in lay languages organised by fellow Mye-EUNITER members will explain new therapies to patients and their families. Links to clinical networks and patient organisations will facilitate dissemination of results obtained in this network directly to the patients.

H.3 How?

The Mye-EUNITER website will inform the target audiences mentioned above by providing information about the latest findings, publications, conferences, activities and breakthroughs. Furthermore, a password-protected modern digital collaboration and learning platform - a prototype of which is already running at one of the participating institutions – will be adapted to offer Action members:

1. internal access to Mye-EUNITER communication and colleagues
2. an information commons, a knowledge database containing scientific methods, recommended biomedical learning resources and collaboratively written shared manuals
3. an overview of the distributed available facilities and expertise and manage their access easily
4. the facilitated exchange of graduates and ESRs between participants.

Moreover, this Action aims to develop a Wikipedia document for the attention of the general public to explain its research results and how and why they are relevant.
Various members of Mye-EUNITER are in contact with pharmaceutical and biotech companies

engaged in the treatment or clinical testing of therapeutics for cancer, infection, inflammation and autoimmunity. As soon as those become available, Mye-EUNITER members will contact company officials to discuss the applicability of markers, tools and scientific insight evolving from this Action with regard to their incorporation into sponsored clinical trial protocols.

Dissemination at the national and international level

The primary means by which results will be disseminated to other researchers working in the field in each COST Country will be through publications and oral/poster presentations at conferences and invited seminars. This will be supported by posting of summarised research findings on the local institutional websites as well as in the newsletters. Where appropriate, the national institutions will contact local/national media to help disseminate scientific discoveries to other scientific groups, industry, and general public.

The DDM team will explore and establish useful profiles for Mye-EUNITER in the social media (LinkedIn, ResearchGate, Twitter, Facebook etc.).

Peer reviewed scientific journals and international conferences will be the main platforms for communication within the international scientific community in this field. The Mye-EUNITER final conference will be organised independently to these conferences and will target the health care industry, and health professionals such as clinicians and researchers interested in MRC-based therapy in immunology, infectious diseases, autoimmunity and cancer.

The dissemination plan will regularly be discussed, updated and adapted by the DDM, MC and SG.