EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

**Exploratory Workshop Scheme**

Standing Committee for the European Medical Research Councils (EMRC)

## ESF Exploratory Workshop on

# Around Mesenchymal Stem Cells: Dissection and Exploitation of Secretory Activity of MSC for Regenerative Medicine and Anticancer Therapies

**Bologna (Italy), 11-13 April 2012**

**Convened by:**
***Nicola Baldini① and Massimo Dominici②***

---

# SCIENTIFIC REPORT

---

*① University of Bologna and Istituto Ortopedico Rizzoli, Bologna, Italy*
*② University of Modena e Reggio Emilia, Modena, Italy*

## 1. Executive Summary

The ESF Exploratory Workshop entitled, "*Around Mesenchymal Stem Cells: Dissection and Exploitation of Secretory Activity of MSC for Regenerative Medicine and Anticancer Therapies*" was a two-day workshop held on Thursday, April 12th and Friday, April 13th, 2012 in Bologna, Italy. The Workshop was convened by Prof. Nicola Baldini, University of Bologna, Department of Biomedical and Neuromotion Sciences and co-covened by Prof. Massimo Dominici, University of Modena and Reggio Emilia, Department of Oncology, Hematology and Respiratory Diseases. The Workshop was held in Sala Vasari in the Istituto Ortopedico Rizzoli located approxiatemly ten minutes by bus from the Bologna city centre nestled in the San Michele woods overlooking the city.

The location of the Workshop emphasized the cultural and scientific significance of the Institute Ortopedico Rizzoli, which holds a scientific prominance as a pioneer and a leader in the field of orthopaedics in Italy. Moreover, it is affiliated with the University of Bologna and therefore is a centre for academic training, making it one of the most multifaceted national and international medical facilities in the world. Along with its scientific significance, Istituto Ortopedico Rizzoli also houses some of the most extraordinary architectural and artistic works including Sala Vasari where the Workshop was held. The participants also had the opportunity to tour the Institute grounds and were able to relish in the artisitic ambience that where they engaged in discussions and the exchange of scientific ideas, making it truly a unique experience for them.

The overall atmosphere of the Workshop proved to be quite positive. It should be noted that the there was a balance between particiants who had either worked together on projects in the past and those who are currently collaborating in separate projects as well as participants who met and were able to establish a rapport for the first time due to their encounter at the Workshop.

Accomodations for participants were held in two hotels; Art Hotel Orologio and Art Hotel Novecento, both conveniently located in the city centre with easy access to public transportation. With regards to the social aspect of the Workshop, participants were treated to dinner on the evening of the 12th at Ristorante Biagi, one of Bologna's traditional and well known restaurants. Participants were able to enjoy an array of gastronimcal specialties of the region while continuing their discussions about the Workshop.

As mentioned, the Workshop was comprised of Prof. Nicola Baldini as convenor, Prof. Massimo Dominaci as co-convenor and 18 official participants coming from 13 different countries. Originally there was Dr.ssa Rita Bussolari from the University of Modena and Reggio Emilia scheduled to take part in the Workshop, however, due to unforeseen circumstances, Dr.ssa Bussolari was not able to take part in the Workshop. Although this issue emerged last minute, a replacement was quickly found. Dr.ssa Giulia Grisendi was a

suitable substitute and is also a colleague of Dr.ssa Bussolari in the Department of Oncology, Hematology and Respiratory Diseases Department of Oncology, Hematology and Respiratory Diseases, University of Modena and Reggio Emilia. The original number of 20 participants therefore, remained the same.

The Workshop maintained the standard proportion of participants outlined by the ESF guidelines and conveyed a variety of experts from renown institutions across Europe. The demographic breakdown of participants involved were as follows: 5 participants from Italy; 3 participants from Germany; 1 participant from Sweden; 1 participant from Spain; 1 participant from the United Kingdom; 1 participant from Austria; 1 participant from France; 1 participant from Denmark; 1 participant from Switzerland; 1 participant from Hungary; 1 participant from Portugal; 1 participant from the Czech Republic and 2 participants from the Netherlands.

A key aspect of this workshop was to provide an interactive output-oriented meeting aimed at opening up new directions in research to explore emerging research fields with potential impact on new developments in mesenchymal stromal cell biology and mesenchymal stromal cells-based clinical applications.The main objective of the workshop was to establish collaborative research in the emerging field of cell-free therapy based on the paracrine activity of mesenchymal stromal cells. The workshop focused on defining: 1) potential clinical application of cell-free therapies; 2) major advantages/limitations; and 3) gaps in current knowledge.

Future activities that were put forth during the group discussion included: 1) identification of research issues for clinical translation; 2) strategy development for joint collaborative research; 3) education/research training networks; and 4) preparation of a consensus paper as a preliminary step for grant application. Discussions regarding available funding opportunities were also brought to light and explored such as Horizons 2020; FP/ Marie Curie Actions; COST, European Cooperation in Science and Technology which funds research networks to exchange knowledge and to embark on new European perspectives.

## 2. Scientific Content of Workshop

The morning of the first day of the Workshop commenced with welcome remarks by the Executive Director of the Istituto Ortopedico Rizzoli, Dr. Giovanni Baldi and Deputy Director, of the University of Modena e Emilia Romagna, Prof. Sergio Ferrari.

The Workshop was divided into three sessions. In sessions one and two, each presenter was allotted 20 minutes of speaking time followed by a ten minute discussion period for their presentation. Session three was dedicated to discussions about future activities and perspectives.

The first session, Paracrine activity of MSC, was chaired by Prof. Massimo Dominici from the University of Modena e Reggio Emilia. The first presentation was entitled **"MSC culture and characterization"** and was presented by **Lucy Di Silvio** from King's College London, United Kingdom. Di Silvio summarized current knowledge on mesenchymal stem cell culturing, emphasizing the need for standardized practices in order to obtain reproducible results to be translated into clinical applications. Standardization of regulations for sample storage, sample shipment and use of biobanks were crucial aspects also acknowledged.

The second presentation, **"Mapping of the secretome of stem cells"** was presented by **Helena Skalnikova** from the Academy of Sciences of the Czech Republic, Libechov, Czech Republic. Skalnikova introduced the concept of MSC secretome. In some cases of cell based therapies, not the transplanted cells by themself, but the secreted proteins, such as inflammatory cytokines and growth factors, reduce inflammation and cell death, activate intrinsic stem cells, and promote regeneration. A screening of secretome content by proteomics may constitute the basis for the use of selected proteins instead of cells, with the potential advantage of avoiding side effects and simplifying regulatory procedures.

The third presentation, **"Regulation of players involved in osteogenic differentiation"** was presented by **Serena Rubina Baglìo** representing VU University Medical Centre, Amsterdam, The Netherlands as well as Istituto Ortopedico Rizzoli, Bologna, Italy. Baglio presented an extensive analysis of gene expression profile in differents steps of MSC differentiation, including the key role of miRNA. These, in particular, could also contribute to an efficient, although poorly defined, paracrine and/or juxtacrine effect via encapsulation in microvesicles or exosomes. Besides the potential application for regenerative strategies, this modality of cell interaction is also relevant for MSC based anticancer therapies.

The fourth presentation, **"Replicative senescence of MSC: Population dynamics, genetic and epigenetic changes"** was presented by **Wofgang Wagner** from the Helmholt Institute for Biomedical Engineering, Aachen, Germany. Wagner focused on the feasibility of cell therapy procedures. In particular, he described the effects of culture expansion on senescence of MSC, suggesting a careful consideration for epigenetic changes.

The fifth presentation entitled, **"Exosome-mediated communication"** was presented by **Edìt Buzas** from Semmelweiss University in Budapest, Hungary. Buzas provided a thorough review on the potential role of microvesicles secreted by MSC. In particular, these structures could be responsible for several effects of cell-cell interactions, including increased cell proliferation and modulating differentiation. The striking eterogeneity of microvescicles requires strict standardization of isolation and characterization methods.

The sixth presentation, **"Detection and isolation of cell-derived microparticles"** was presented by **Michiel Pegtel** from VU University Medical Centre, Amsterdam, the Netherlands. Pegtel introduced the emerging role of exosomes in the pathogenesis of different conditions, including viral infections and cancer. Beside their potential use in diagnosis, when detected in body fluids, exosomes could be exploited for therapy. This, however, should be preceded by a careful analysis of their content and activity in target cells.

The seventh presentation started after lunch and was entitles, **"Acidic tumou pH as regulator of autophagy and exosomes release"** which was presented by **Angelo De Milito** from the Cancer Center Karolinska in Stockholm, Sweden. De Milito showed the importance of microenvironment in exosome release and activity. In fact, acidic pH increases lipid-dependent exosome fusion to target cells.

The eighth and final presentation for session one was entitled **"Exosomes as potential biomarkers in neurodegenerative diseases"** which was presented by **Anja Schneider** from the Medical University Goettingen in Germany. Schneider showed that exosomes are now recognized mediators of neurodegenerative conditions, such as dementia and Parkinson's disease. Moreover, based on their ability to carry small molecules at sites of brain tissue damage, they are being studied as new therapeutic tools.

The Workshop maintained it's intended schedule for the day and session two commenced on time. Session 2 was entitled MSC-based therapy: state of the art in different clinical settings was chaired by Prof. Nicola Baldini. Once again, each presenter was allotted 20 minutes of speaking time followed by a 10 minute discussion period for their presentation.

The first presentation, **"MSC secreatome and applications in regenerative medicine"** was presented by **Antonio Josè Salgado** from the University of Minho in Braga, Portugal. Salgado summarized his successful results on the use of MSC derived from umbilical cord to enhance the recovery of glial and neuronal cells, suggesting the seeding of cells in an artificial resorbable scaffold for controlled release.

The second presentation was entitled **"MSC-based therapy for bone repair and regeneration"** by **Moustapha Kassem** from the University Hospital of Odense in Denmark. Kassem outlined the potential applications of MSC based therapies for systemic bone conditions, such as osteoporosis, after careful genomic and proteomic analysis.

The third presentation, **"MSC and angiogenesis"** was presented by **C James Kirkpatrick** from Johannes Gutenberg University in Mainz, Germany. Kirkpatrick reported on the importance of paracrine activity of MSC on circulating or resident endothelial precursors for bone engineering strategies.

The fourth presentation**, "MSC in arthritis: the ADIPOA experience"** presented by **Christian Jorgensen** from INSERM U844 in Montpellier, France. Jorgensen showed the role of MSC as anti-inflammatory effectors through the inhibition of Th17 differentiation, suggesting their use for osteoarthitis, as investigated in the ADIPOA FP7 Project.

The fifth presentation, **"Stem cells for tooth regeneration"** was presented by **Thimios Mitsiadis** from the University of Zurich, Switzerland. Mitsiadis addressed the challenging aim of engineering a complex structure, such as the tooth. He described the genetic basis for dentin and enamel morphogenesis and the multipotentiality of pulpar MSC.
The sixth presentation, **"MSC for kidney repair"** was presented by **Benedetta Bussolati** from the University of Torino, Italy. Bussolati presented her successful preclinical findings on the use of MSC-derived microvesicles for acute tubular damage healing.

The seventh and final presentation for the first day of the Workshop was **"Stem cell therapy for diabetes mellitus"** by **Bernat Soria** from Andalusian Centre for Molecular Biology and Regenerative Medicine in Seville, Spain. Soria introduced another successful application of MSC based therapy. Peripheral arterial disease associated with diabetes mellitus were healed by regional administration of MSC acting on the microvascular system.

Session 2 continued with presentations, starting the day off with **"MSC-based therapy in cancer"** which was presented by **Giulia Grisendi** from the University of Modena e Reggio Emilia. Grisendi showed the influence of MSC on tumor microenvironment and, based on the peculir tropism of MSC, suggested the use of MSC as a "Trojan horse" to selectively carry death signals as a novel tool for anticancer therapy.

The following presentation was **"In vivo tracking of MSC"** presented by **Mariann Pavone-Gyöngyösi** from the Medical University of Vienna, Austria. Gyöngyösi focused on the relevant issue of MSC biodistribution after systemic administration, showing that cells selectively accumulate in reservoirs such as liver, spleen and bone marrow, as shown by different imaging techniques. She also raised the issue of detecting the biodistribution of secretome products.

The concluding Workshop presentation was **"Saftey, quality control, storage and banking for cell-free therapy"** presented by **Lorenza Lazzari** from the Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena in Milano, Italy. Lazzari outlined the regulatory aspects of cell and cell-free biological therapies, including quality control tests, toxicology and tumorigenicity.

Session 3 provided participants with two hours of open discussion dedicated to the following features of MSC-based therapies:

1. Technical aspects in the collection procedure of exosome/microvesicles produced by MSC: cell culture conditions, harvest time, detection methods, artifacts bias;

2. Identification and classification of the following players of MSC paracrine activity, including soluble factors, exosomes, microvescicles, and apoptotic bodies;

3. Biologic content of exosome/microvesicles: proteins, mRNA, miRNA, lipids, other. This also includes consideration to animal-xenogenic elements due to in vitro culture and other viral/bacterial derived molecules;

4. Mechanism of action of exosome/microvesicles: extracellular interaction with extracellular matrix, interparticles interactions, and receptor interaction;

5. Pharmacokinetics of exosome/microvesicles: half life in vivo injection, catabolic activity (site and effectors);

6. Cell source specificity by comparative studies in different sources and species;

7. Modification of secreatome potential to potentiate the release of wild type factors or products from gene modified cells. Manufacturing of artificial exosomes;

8. Preclinical regulatory aspects of secreatome, including identity, purity, potency, toxicology and choice of preclinical models.

**3. Assessment of the results, contribution to the future direction of the field, outcome:**

Following the Workshop an informal networking was established that was used as a platform to define the future actions.These can be summarized as follows:

- To circulate a summary file among Workshop participants in order to elaborate a position paper to illustrate the emerging field of cell based therapies exploiting the secretory activity of MSC for regenerative medicine and anticancer strategies (Cytotherapy or European Cells & Materials)

To suggest EMRC the writing of a Science Policy Briefing on this subject

- To start collaborations and search for funding to establish bilateral collaborations between the participating groups under the FP/Marie Curie Actions or other sources in order to advance forward the final objective

- To present proposals in the FP7 call Cooperation Health, expected to come out in July (HEALTH.2013.1.4-1. Controlling differentiation and proliferation in human stem cells intended for therapeutic use)

- To maintain networking in order to get ready for the coming Horizon 2020 initiative.

## 4. Final Programme

**Wednesday, April 11, 2012**

Evening *Arrival of participants in Bologna*

**Thursday, April 12, 2012**

09.00-09.10 **Welcome** **Nicola Baldini** (Bologna, I) and **Massimo Dominici** (Modena, I) Welcome remarks by the University of Bologna, the University of Modena e Reggio Emilia, the Istituto Ortopedico Rizzoli

09.10-09.30 **Presentation of the European Science Foundation (ESF)** **Isabel Varela Nieto**, ESF Standing Committee for the European Medical Research Councils (EMRC)

**09.30-15.00 Session 1: Paracrine activity of MSC *Chaired by Massimo Dominici - University of Modena e Reggio Emilia, Modena, Italy*** *Each talk will be 20' plus 10' discussion*

09.30-10.00 **Presentation 1 "MSC culture and characterization"** **Lucy Di Silvio** (King's College London, London, UK)

10.00-10.30 **Presentation 2 "Mapping of the secretome of stem cells"** **Helena Skalnikova** (Academy of Sciences of the Czech Republic, Libechov, CZ)

10.30-11.0 **Presentation 3 "Regulation of players involved in osteogenic differentiation"** **Serena Rubina Baglìo** (Istituto Ortopedico Rizzoli, Bologna, Italy and VU University Medical Center, Amsterdam, NL)

11.00-11.20 *Coffee / Tea Break*

11.20-11.50 **Presentation 4 "Replicative senescence of MSC: Population dynamics, genetic and epigenetic changes"** **Wolfgang Wagner** (Helmholtz Institute for Biomedical Engineering, Aachen, DE)

11.50-12.20 **Presentation 5 "Exosome-mediated communication"** **Edit Buzas** (Semmelweis University, Budapest, HU)

12.20-12.50 **Presentation 6 "Detection and isolation of cell-derived microparticles"** **Michiel Pegtel** (VU University Medical Center, Amsterdam, NL)

12.50-14.00 *Lunch*

**14.00-15.00 Session 1 continued: Paracrine activity of MSC *Chaired by Massimo Dominici, Modena, Italy***

14.00-14.30 **Presentation 7 "Acidic tumor pH as regulator of autophagy and exosomes release"** **Angelo De Milito** (Cancer Center Karolinska, Stockholm, SE)

14.30-15.00 **Presentation 8 "Exosomes as potential biomarkers in neurodegenerative diseases"** **Anja Schneider** (Medical University Goettingen, Goettingen, DE)

**15.00-18.50 Session 2: MSC-based therapy: state of the art in different clinical settings *Chaired by Nicola Baldini, Bologna, Italy*** *Each talk will be 20' plus 10' discussion*

15.00-15.30 **Presentation 1 "MSC secreatome and applications in regenerative medicine"** **Antonio Josè Salgado** (University of Minho, Braga, PT)

15.30-16.00 **Presentation 2 "MSC-based therapy for bone repair and regeneration"** **Moustapha Kassem** (University Hospital of Odense, Odense, DK)

16.00-16.20 *Coffee / tea break*

16.20-16.50 **Presentation 3 "MSC and angiogenesis"** **C. James Kirkpatrick** (Johannes Gutenberg University, Mainz, DE)

16.50-17.20 **Presentation 4 "MSC in arthritis : the ADIPOA experience"** **Christian Jorgensen** (INSERM U844, Montpellier, FR)

17.20-17.50 **Presentation 5 "Stem cells for tooth regeneration"** **Thimios Mitsiadis** (University of Zürich, Zürich, CH)

17.50-18.20 **Presentation 6 "MSC for kidney repair"** **Benedetta Bussolati** (University of Torino, Turin, IT)

18.20-18.50 **Presentation 7 "Stem cell therapy for diabetes mellitus" Bernat Soria** (Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, ES)

20.30 *Discussion continued at Workshop Dinner (Biagi Ristorante, Via Savenella, 9)*

**Friday, 13 April 2012**

**09.00-10.30 Session 2 continued from previous day: MSC-based therapy: state of the art in different clinical settings *Chaired by Nicola Baldini, Bologna, Italy*** *Each talk will be 20' plus 10' discussion*

09.00-09.30 **Presentation 8 "MSC-based therapy in cancer" Giulia Grisendi** (University of Modena e Reggio Emilia, Modena, IT)

09.30-10.00 **Presentation 9 "In vivo tracking of MSC" Mariann Gyöngyösi** (Medical University of Vienna, AT)

10.00-10.30 **Presentation 10 "Safety, quality control, storage and banking for cell-free therapy"**

**Lorenza Lazzari** (Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena, Milan, IT)

10.30-10.50 *Coffee / tea break*

**11.00-13.00 Session 3: Future activities and perspectives**

- Development of strategies to implement joint collaborative research projects
- Identification of working groups
- Identification of basic research issues
- Identification of research issues that are most promising for translation into the clinic
- Development of networks for people exchange
- Planning for preparing the consensus paper on the main issues of the workshop

13.00-13.15 Concluding remarks

13.15 Lunch

## 5. Final List of Participants

Participants are listed in alphabetical order, followed by their affiliation, city and country.
Please see Annex 1 for signed attendance forms for both days of the Workshop.

**Convenor**
Prof. Nicola Baldini – University of Bologna, Istituto Ortopedico Rizzoli, Bologna, Italy

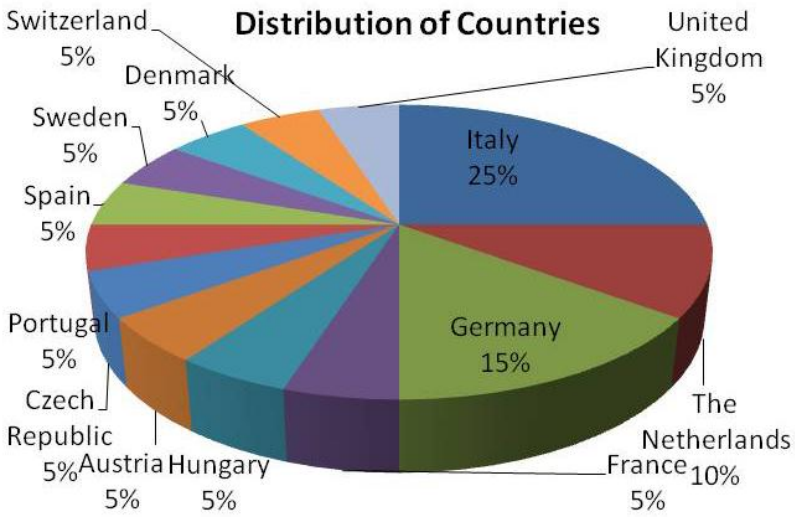
**Co-convenor**
Prof. Massimo Dominici – University of Modena e Reggio Emilia, Modena, Italy

**Participants**

1. Dr. Serena Rubina Baglìo – Istituto Ortopedico Rizzoli, Bologna, Italy and VU University Medical Center, Amsterdam, NL
2. Prof. Benedetta Bussolati – University of Torino, Turin, Italy
3. Prof. Edit Buzas - Semmelweis University, Budapest, Hungary
4. Dr. Angelo De Milito – Cancer Center Karolinska, Stockholm, Sweden
5. Prof. Lucy Di Silvio – King's College London, London, UK
6. Dr. Giulia Grisendi - University of Modena e Reggio Emilia, Modena, Italy
7. Prof. Mariann Gyongyosi – Medical University of Vienna, Austria
8. Prof. Christian Jorgensen – INSERM U844, Montpellier, France
9. Prof. Moustapha Kassem - University Hospital of Odense, Odense, Denmark
10. Prof. C.James Kirkpatrick – Johannes Gutenberg University, Mainz, Germany
11. Dr. Lorenza Lazzari – Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena, Milan, Italy
12. Prof. Thimios Mitsiadis – University of Zürich, Zürich, Switzerland
13. Dr. Michiel Pegtel – VU University Medical Center, Amsterdam, The Netherlands
14. Dr. Antonio Josè Salgado – University of Minho, Braga, Portugal
15. Dr. Anja Schneider – Medical University Goettingen, Goettingen, Germany
16. Dr. Helena Skalnikova – Academy of Sciences of the Czech Republic, Libechov, Czech Republic
17. Prof. Bernat Soria – Andalusian Center for Molecular Biology and Regenerative Medicine (CAMIBER), Seville, Spain
18. Prof. Wolfgang Wagner - Helmholtz Institute for Biomedical Engineering, Aachen, Germany

## 6. Statistical information on participants

**Age Range**
Participants were within the age range of 30 to 65 years of age with a majority of participants over the age of 40.




**Geographical and Distribution**

| COUNTRY | NUMBER OF PARTICIPANTS |
|---|---|
| Austria | 1 |
| Italy | 5 |
| The Netherlands | 2 |
| Germany | 3 |
| United Kingdom | 1 |
| France | 1 |
| Denmark | 1 |
| Hungary | 1 |
| Portugal | 1 |
| Switzerland | 1 |
| Sweden | 1 |
| Czech Republic | 1 |
| Spain | 1 |

**Gender distribution**

| Male | Female |
|---|---|
| 11 | 9 |