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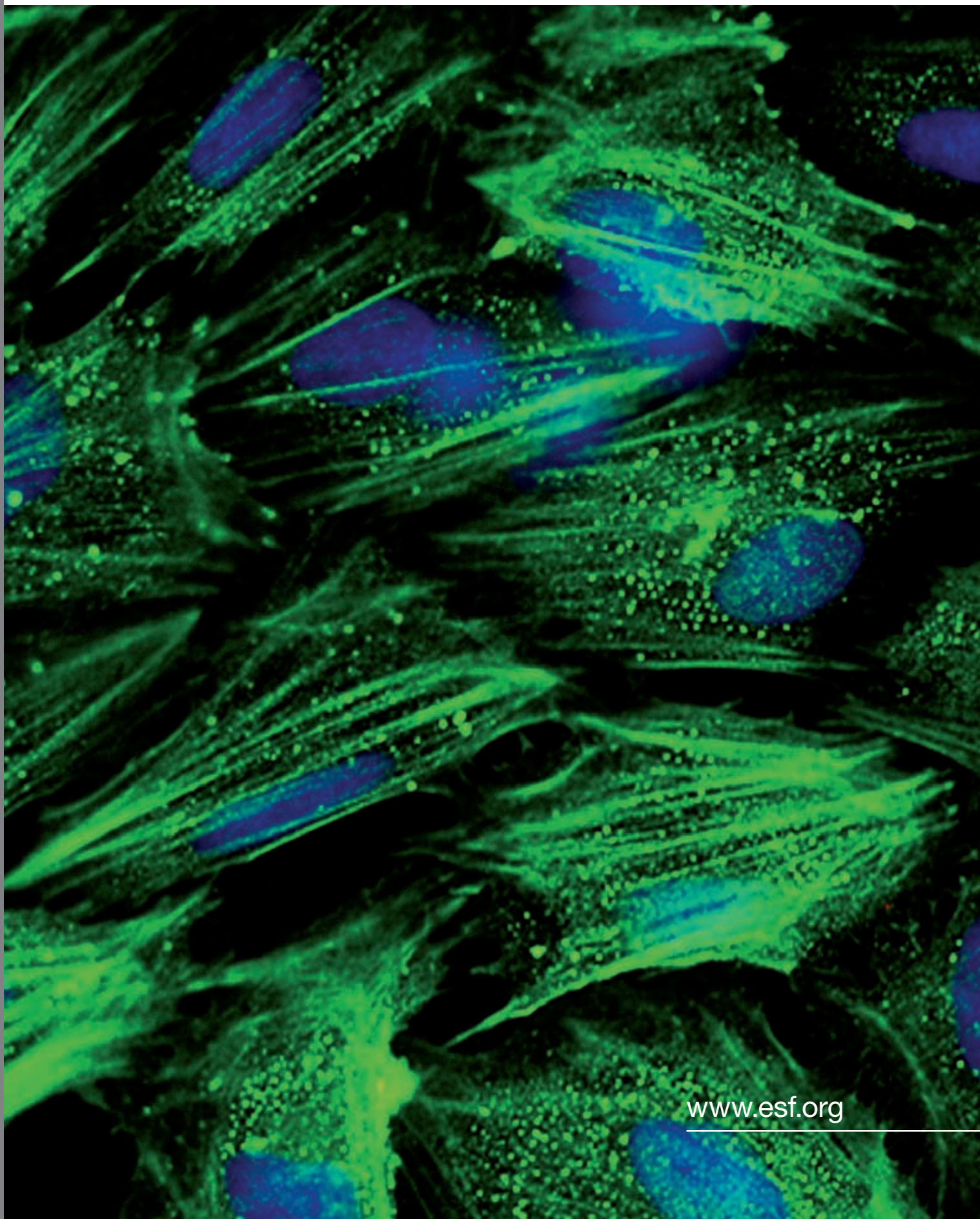
SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme

EuroSTELLS (Development of a Stem Cell Tool Box) Final Report



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Cover Image:
Lazzari G, *et al. Stem Cells* **24**, 2514-2521 (2006)

EUROCORES (European Collaborative Research)

The aim of the European Collaborative Research (EUROCORES) Scheme is to enable researchers in different European countries to develop collaboration and scientific synergy in areas where European scale and scope are required to reach the critical mass necessary for top class science in a global context.
The scheme provides a flexible framework which allows national basic research funding and performing organisations to join forces to support excellent European research in and across all scientific areas.
Until the end of 2008, scientific coordination and networking was funded through the EC FP6 Programme, under contract no. ERAS-CT-2003-980409. As of 2009, the National Funding Organisations provide the funding for the scientific coordination and networking in addition to the research funding.
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Contents

Foreword

EuroSTELLS was one of the first two EUROCORES programmes to be launched by the European Medical Research Councils (EMRC) in 2005, recognising a need for basic stem cell research in Europe. EuroSTELLS was aimed at generating fundamental knowledge on stem cell biology by setting up the bases for comparative analyses of stem cells of different origins and future clinical applications.

Over the course of the past three years, significant scientific achievements were made by the synergistic efforts of three Collaborative Research Projects (CRPs), resulting in the development of common tools and the production of many high-level publications. Advancements were made in understanding the development of the embryonic haematopoeitic or blood system; how paternal genomic imprinting differs from other regions in the genome; and which stem cell pathways control their development and differentiation.

Through the successful conference series, EuroSTELLS fostered innovative and multi-disciplinary collaborations among the project investigators and their laboratories. New project ideas were developed, tools and know-how exchanged, and young postdoctoral and PhD students had the opportunity to present their research to leaders in the field.

EuroSTELLS also had an impact in a global context through the support of an international task force of the International Society for Stem Cell Research (ISSCR) in its preparation of guidelines for the clinical translation of stem cells. In sponsoring such an important initiative, the Project Leaders of EuroSTELLS have helped develop a valuable resource for the international stem cell community.

We hope that this comprehensive report will convince you that, although 36 months is a comparatively short time, EuroSTELLS was a successful and rewarding programme. The collaborations that have started will hopefully continue and strengthen in order to contribute to the international endeavour to address the many remaining questions and challenges in this exciting field.

With this I would like to thank all the Principal Investigators and their research teams, including the three Project Leaders, Professor Cesare Galli, Professor Elaine Dzierzak and Professor Stefan Krauss, for their outstanding contribution and commitment.

Dr. Carole Moquin-Pattey, Head of Unit
European Medical Research Councils (EMRC)

1. Governing Bodies

1.1 Management Committee

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1.2 Scientific Committee

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Professor Stefan Krauss
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(and 2008 where indicated)*

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Dr Angelo Vescovi
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Czech Republic: Czech Science Foundation (GAČR)

Denmark: Danish Agency for Science, Technology and Innovation

Finland: Academy of Finland

France: National Centre for Scientific Research (CNRS)

Iceland: Iceland Centre for Research (RANNIS)

Italy: National Research Council (CNR)

The Netherlands: The Netherlands Organisation for Health Research and Development (ZonMw)

Norway: The Research Council of Norway

Spain: Interministerial Committee on Science and Technology

United Kingdom: Medical Research Council (MRC)

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EUROCORES Communication Officer

• Finance

Mr. David Weber
Director of Finance and Administration

Ms. Philippa Rowe
Finance Controller

2. Description of the EuroSTELLS Programme

2.1 Rationale and Objectives

EuroSTELLS was among the first set of themes to be proposed for a EUROCORES Programme. It was aimed to respond to a need expressed by ESF Member Organisations to address the many unanswered questions in stem cell biology at that time. EuroSTELLS also followed the recommendations set out in a Science Policy Briefing published by the EMRC in 2001, which outlined scientific uncertainties in the field of human stem cell research[1]. It was stated that 'because of the expected demand for stem cells for human medical applications, there is a real need in Europe for supporting research aimed at developing human stem cell lines and their applications'. The aim was to develop a programme that would increase our knowledge of basic features and properties of stem cells, either from embryonic or somatic origin, human as well as from animal models. It was hoped that this knowledge would support the development of stem cells as therapeutic tools for the treatment of severe human diseases such as leukemia, diabetes, Parkinson's disease, and other degenerative diseases.

Developing a common tool box was seen as an effective means of achieving this and a Call for Outline Proposals was launched in early 2004. The scientific priorities identified were (original text from the Call for Outline Proposals):

2.1.1 Propagation and expansion of stem cell cultures

Ability to cultivate and manipulate stem cells *ex vivo* is a critical step towards elucidating their biological properties and developing their biotechnological and therapeutic potential. At present only a handful of stem cell types can be maintained *in vitro*, and of these only Embryonic Stem (ES) cells, certain neural stem cells, mesenchymal stem cells and the recently described Multipotent Adult Progenitor Cells (MAPCs) undergo significant multiplication. Most commonly in stem cell cultures, proliferation is associated with differentiation. There is a pressing need therefore to:

- acquire understanding of self-renewal mechanisms
- develop procedures for expanding stem cells in the laboratory
- optimise and standardise culture protocols.

These goals are critical for future applications of human stem cells but are also important for fundamental investigations in mice and other model organisms. The research programme should therefore include a range of animal stem cells and encourage comparative approaches. Investigations of the genetic, epigenetic and phenotypic fidelity of stem cells during long-term culture are also necessary.

1. Human Stem Cell Research: scientific uncertainties and ethical dilemmas, ESF SPB (p.14, 1st edn. 2001; p.18, 2nd edn. 2002)

2.1.2 Optimisation of stem cells cultures

Non-human materials in cultures bear a risk for interspecies infections, and optimally no such materials should be used. To keep human ES cells undifferentiated, feeder cells have been necessary. When the first cell lines were established, foetal mouse feeder cells were used and, only recently, establishing and culturing these cell lines on human feeder cells have been successful. Optimally no feeder cells should be used, but much research is still needed to identify the factors which are necessary for promoting the growth of stem cells as undifferentiated cells. If cells are used in human cell transplantation, Good Manufacturing Practice (GMP) quality is required. Optimally, the culture techniques have to allow large-scale production of cells which maintain their stem cell characteristics during the process.

2.1.3 Phenotypic and genotypic characterisation

A given candidate stem cell population is as good as the model in which it was characterised. Haematopoietic stem cells have been formerly characterised and purified to homogeneity because reliable, sensitive and quantitative assay systems were available, *in vitro* and especially *in vivo*. Conversely, a major limitation in many current projects related to stem cell research is the lack of appropriate assays. Hence special consideration should be given to projects in which novel stem cell assays will be developed and validated:

- ***In vitro* assays**
  These include the classical two-dimensional culture of dissociated cells but also the development of three-dimensional cultures of either intact or reconstructed tissues. Conditions required to maintain 'stemness' or induce differentiation may include cell-cell and cell substrate interactions, substrate nature, oxygen pressure, medium composition, presence of growth/differentiation factors, optimal cell density.

- ***In vivo* assays**
  Isogenic assays should be conducted mainly in small laboratory animal models. Host conditioning should be determined depending on the stem cell type analysed: irradiation, chemo-, immune or surgical ablation; cell lineage ablation or damage in transgenic mice. Allogenic assays should be conducted mainly in small laboratory animal models. Activities should include at least:
  – definition of the immuno-phenotype
  – characterisation of the host immune response

Xenogenic assays should be developed primarily for human stem cell characterisation taking into account the ethical guidelines concerning human stem cells. Congenitally immuno-deficient mice or rats, early blastocysts or pre-immune foetuses in rodents or larger animals can be used as hosts. Human stem cells to be

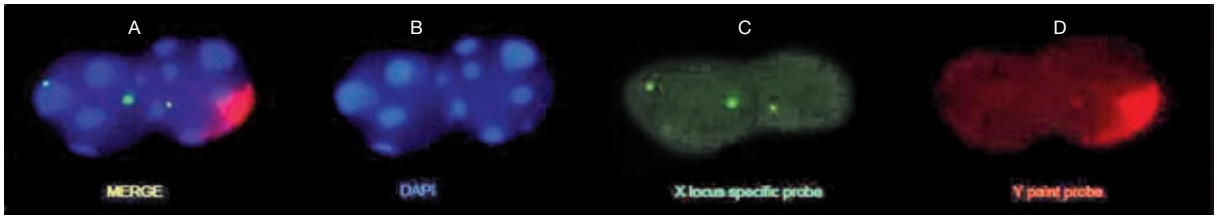

Figure 1. Bi-nucleated hepatocyte from an XX/XY mouse chimera. D) shows the Y chromosome (paint probe, red) and C) shows the X chromosome (locus specific BAC probe, green). A) is a merge showing that one of the two nuclei is XX and the other is XY, indicating that a cell fusion event has occurred. B) shows DNA stained with DAPI (4',6-Diamidino-2-Phenylindole) in blue. Courtesy of Dr. Paolo Vezzoni, Institute of Biomedical Technologies, (CNR), Segrate, Italy.

assayed in tolerant animal hosts can be administered directly to the host target tissue, following appropriate conditioning (see above), or engrafted into human target tissue previously implanted in the animal host. An important point to be taken into account is the marking system used in these experiments to assess chimerism, be it intrinsic (HLA or other markers) or secondarily brought to the cell under study (marker transgene).

2.1.4 Genetic modification of stem cells

Genetic modification of stem cells could pave the way for their successful medical application. In current gene therapy efforts, the most promising methods involve *ex vivo* modification of stem cells, and then auto-transplantation of the modified cells into the patient. Based on this strategy, non-functional or malfunctioning genes in the stem cells of a patient may be replaced by genes properly regulated and producing normally functioning proteins. Thereafter the genetically modified stem cells could be inserted into the patient's body. A successful insertion and propagation of genetically modified stem cells should provide the basis for a new era of curing, for example, heritable, autoimmune or malignant diseases. Before reaching this stage, there are numerous problems to be solved by extensive basic research. The key issues in stem cell-based gene therapy research should include the establishment of efficient and safe methodologies in order to:

- genetically modify stem cells, including the development of efficient and safe gene insertion systems
- avoid unwanted stem cell transformation or differentiation during this procedure
- control differentiation of stem cells towards a desired direction
- allow an efficient re-insertion and long-term survival of genetically modified stem cells in the body and avoid immunological rejection
- avoid any possible malignant transformation of the modified and re-inserted stem cells
- provide a selective advantage of the genetically modified stem cells after their re-insertion
- use stem cells as delivery vehicles and development of appropriate homing assays.

After an international peer review process carefully managed by the ESF, three Collaborative Research Projects (CRPs) were launched in 2005. These three CRPs consisted of 21 individual research projects based in 10 different European countries. By bringing together expertise from a range of backgrounds and countries, the programme aimed at generating fundamental knowledge of stem cell biology, by setting up the bases for comparative analyses of stem cells of different origins, and focusing on their future clinical applications. In Section 3, the three EuroSTELLS research teams highlight the outcomes of their research, both in terms of the programme and in the field of stem cell research in general. It shows that EuroSTELLS has made a significant contribution to this field of research during the past three years, both through the results achieved, but also through networking and dissemination activities, and synergies created for future research activities.

Facts and Figures

Deadline for Applications: 30 April 2004
Funded Collaborative Research Projects (CRPs): Three, consisting of 21 Individual Research Projects in 10 different countries.
Duration of Programme: 2005-2008
Budget for research: 3.2 million Euro
EC Contract Number: ERAS-CT-2003-980409

2.2 List of Projects

• Novel approaches to enhance animal embryonic stem cell research

Professor Cesare Galli (CRP Leader),
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Professor Keith Campbell,
School of Biosciences, University of Nottingham,
Loughborough, United Kingdom
Dr. Robert Feil, Institute of Molecular Genetics
(CNRS), Montpellier, France

Dr. Josef Fulka,
Research Institute of Animal Production,
Prague, Czech Republic
Professor Pasqualino Loi,
Department of Comparative Biomedical Sciences,
Teramo University, Teramo, Italy
Dr. Paolo Vezzoni,
Institute of Biomedical Technologies (CNR),
Segrate, Italy

• Translational stem cell research: from basic biology to regenerative medicine

Professor Stefan Krauss (CRP Leader),
Institute for Cellular and Genetic Therapy,
University of Oslo and Rikshospitalet, Oslo, Norway
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Center for Reproductive Medicine, Academic
Medical Centre, Amsterdam, the Netherlands
Professor Jonas Frisén,
Department of Cell and Molecular Biology,
Karolinska Institute, Stockholm, Sweden
Dr. Thorarinn Gudjonsson,
Faculty of Medical Science, University of Iceland,
Reykjavik, Iceland
Dr. Morten Meyer,
Institute of Medical Biology, University of Southern
Denmark, Odense, Denmark
Dr. Juha Partanen,
Institute of Biotechnology, University of Helsinki, Finland
Dr. Fiona Watt,
Cancer Research UK, London Research Institute,
Cambridge, United Kingdom
Professor Ernest Arenas (AP),
Department of Medicinal Biochemistry and
Biophysics, Karolinska Institute, Stockholm, Sweden
Dr. Patrick Brundin (AP),
Wallenburg Neuroscience Centre, Lund University,
Sweden
Dr. Jonas Muhr (AP),
Ludwig Institute for Cancer Research,
Karolinska Institute, Stockholm, Sweden
Professor Hannu Sariola (AP),
Institute of Biomedicine, University of Helsinki, Finland
Professor Irma Thesleff (AP),
Institute of Biotechnology, University of Helsinki, Finland

• Regulation of hematopoietic stem cell self-renewal in the embryo (HSC-SR)

Professor Elaine Dzierzak (CRP Leader),
Department of Cell Biology and Genetics, Erasmus
University Medical Center, Rotterdam, The Netherlands
Dr. Anna Bigas,
Municipal Institute for Medical Research (IMIM-
Hospital del Mar), Barcelona, Spain

Professor Tariq Enver,
Medical Research Council (MRC), Molecular
Haematology Unit, John Radcliffe Hospital,
Weatherall Institute of Molecular Medicine, Oxford,
United Kingdom

2.3 EUROCORES Quality Assurance

2.3.1 Theme Selection

New and challenging ideas for EUROCORES programmes are invited from the scientific community through an annual Call for Theme Proposals. In addition to criteria including scientific quality, novelty and feasibility, the proposals are evaluated on the basis of the requirement for European collaboration; why it is necessary to conduct the programme at a European level and how the programme will strengthen and advance Europe's scientific position in a global context.

Each proposal is sent for **written external assessment** to at least three referees. Based on these reviews, the Science Advisory Board (SAB) recommends which themes are to be further developed; a decision which is then ratified by the Governing Council.

2.3.2 Project Selection

The peer review of the Collaborative Research Project proposals in a EUROCORES programme such as EuroSTELLS is a multistage process, including the establishment of an **international and independent Review Panel** (RP). In response to an open Call for proposals, **outline proposals** of about three pages are submitted by a team of applicants (minimum three from three different countries). At that stage, the RP is responsible for the sifting of outline proposals prior to the invitation of full proposals. At the **full proposals stage**, each proposal is sent for **written external assessments** to at least three referees, including referees from outside Europe. Applicants are given an opportunity to reply to the anonymous referee reports.

Written referees' assessments and replies by applicants are then considered by the RP with scientific quality being the main selection criterion. The RP makes recommendations for funding of Collaborative Research Projects (CRPs), with prioritisation, which ESF communicates to the EUROCORES Funding Organisations (EFOs).

As described in the previous Section 2.1, after such an international peer review process, three Collaborative Research Projects (CRPs) were selected for EuroSTELLS and launched in 2005.

2.3.3 Management Committee

At the time that the Call for Proposals is published, a Management Committee (MC) is established (see Section 1.1 for the EuroSTELLS MC).

- The MC has overall responsibility for the EUROCORES programme within the guidelines of the EUROCORES Scheme;
- The MC can request expert advice from the EUROCORES Scientific Committee, Review Panel or any other ad hoc advisory group;
- MC Members support the EUROCORES review process by nominating the potential Review Panel and external expert referees on behalf of their funding organisation;
- Each MC member is responsible for liaising with their funding organisation, including supervision of the funding process for EUROCORES projects within their organisation;
- Members may attend all meetings of the EUROCORES Programme as observers.

2.3.4 Mid-Term and Final Reviews

Each EUROCORES Programme undergoes two comprehensive reviews to evaluate their progress at the mid- and final stages. The aim is to assess scientific cooperation and interactions among the investigators and for the case of a mid-term review to:

- examine the merits of the EUROCORES Programme and its potential

and a final review to:

- examine the merits of the EUROCORES Programme and the lessons there are to be learned for potential follow-up initiatives.

They are assessed using the following criteria:

- Novelty/Originality: Most innovative/original scientific contribution of each CRP to the programme and to the relevant field of research;
- Multidisciplinary Research: How is each CRP working towards (or achieving) multidisciplinary research;
- Collaborative Research: Results obtained within the CRP during this reporting period that would not have been achieved (or would have taken longer to achieve) in an individual project;
- European added value: a European dimension given to national funding (e.g: building up the European Research Area (ERA); developing a critical mass of expertise; addressing issues of scale and scope). For CRPs involving partners outside Europe: a clear example illustrating their added value to the programme and their contribution to the relevant field of research in Europe;
- Relevance to the Call: Achievement most relevant to the Call.

EuroSTELLS had very positive mid-term and final reviews from the Review Panel, who commented in the final report that *'the three Collaborative Research Projects (CRPs) contributed significantly to progress in stem cell biology, with a significant number of publications, including in high impact journals (*Nature Biotechnology, The EMBO Journal, Journal of Cell Biology and Development*). The major strength of the programme was in establishing collaborations in the various stem cell fields, uniting laboratories from different countries and disciplines, leading to more publications and quality high-profile studies'.*

2.3.5 EUROCORES Acknowledgements

To promote the EUROCORES Programme and the national funding organisations who support it (and previous to 2008, the European Commission), all publications, posters, websites and other dissemination outputs are required to be clearly identified as being programme-funded or co-funded. This is an important indicator for monitoring the outputs of the programmes, particularly peer-reviewed publications.

For EuroSTELLS the acknowledgement was:
The European Science Foundation (ESF) provides scientific coordination and support for networking activities of funded scientists currently through the EC FP6 Programme, under contract No. ERAS-CT-2003-980409. Research funding is provided by participating organisations. EuroSTELLS is managed by the European Medical Research Councils (EMRC) at the ESF.

For other EUROCORES Programmes from 2009 onwards the acknowledgement is:
The aim of the European Collaborative Research (EUROCORES) Scheme is to enable researchers in different European countries to develop collaboration and scientific synergy in areas where European scale and scope are required to reach the critical mass necessary for top class science in a global context.
The Scheme provides a flexible framework which allows national basic research-funding and research-performing organisations to join forces to support excellent European research in and across all scientific areas.
Until the end of 2008, scientific coordination and networking was funded through the EC FP6 Programme, under contract No. ERAS-CT-2003-980409. As of 2009, the national funding organisations provide the funding for the scientific coordination and networking in addition to the research funding.

3. Highlights of the EuroSTELLS Collaborative Research Projects (CRPs)

3.1 Novel approaches to enhance animal embryonic stem cell research (CRP I)

Principal Investigators

Professor Cesare Galli (Project Leader)
Professor Keith Campbell
Dr. Robert Feil
Dr. Josef Fulka
Professor Pasqualino Loi
Dr. Paolo Vezzoni

Funding Organisations

Czech Republic: Czech Science Foundation (GAČR)
France: National Centre for Scientific Research (CNRS)
Italy: National Research Council (CNR)
United Kingdom: Medical Research Council (MRC)

This ambitious project took a comparative and multidisciplinary approach that used non-human mammalian species (cattle, sheep, pig and mouse) as model systems for the derivation, molecular and epigenetic characterisation of and *in vitro/in vivo* differentiation of nuclear transfer stem cell (NTSC) lines. NTSCs are derived from somatic cell nuclear transfer embryos. Nuclear transfer is the process when the nucleus of a somatic cell (such as a fibroblast) is inserted into an egg from which the nucleus has been removed. The somatic cell nucleus is reprogrammed and the embryo begins to cleave.

While this technique represents a possible route for treating patients using autologous (i.e., their own) stem cell therapy, there was, and still is a need to improve reprogramming techniques, develop new ones and understand the mechanisms involved. With these aims in mind, the five Principal Investigators (PIs) took on various aspects of this research during the three years of EuroSTELLS:

1. Somatic cell nucleus reprogramming by the oocyte cytoplasm (lead PI, Josef Fulka, Jr.);
2. *Ex-ovo* reprogramming (both by chemicals or by cell extracts) (lead PIs, Pasqualino Loi and Keith Campbell);
3. Nuclear transfer stem cells (NTSC) derivation and characterisation (lead PI, Cesare Galli);
4. Differentiation and transplantation in mouse models of human diseases (lead PI, Paolo Vezzoni);
5. Epigenetics (lead PI, Robert Feil).

Dr. Fulka's group successfully established a model biological system that enables a comparison of the natural epigenetic changes that occur soon after NT (nuclear transfer). Epigenetics involves heritable changes in gene expression that are not due to changes in DNA sequence, but rather chemical modifications to the DNA or to the proteins that are associated with DNA. This is an important process as dysregulation can lead to syndromes such as Angelman and Prader-Willi Syndromes and can contribute to multi-factorial diseases such as cancer.

Another project examined the role of the mammalian egg nucleolus – a small structure within the nucleus – in the development of the NT embryo. Upon fertilisation the contribution from the egg and the sperm is not equal, with some structures such as the mitochondria – the powerhouse of the cell – coming from the egg. It was demonstrated that the egg nucleolar material is essential for the development of NT embryos and that the same material from the somatic cell cannot provide a substitute (*ref. 16*). This was an important discovery regarding early embryonic development.

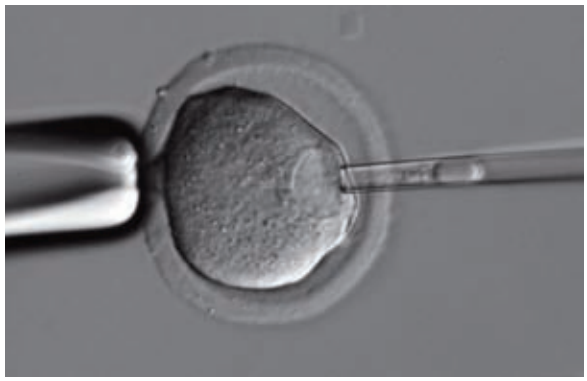

Figure 2. An immature mouse oocyte in the process of enucleolation with a pipette (the nucleolus is in the pipette already).
From: Ogushi S, *et al. Science* **319**, 613-619 (2008).

Professor Loi, who published a number of papers with Dr. Fulka, produced the first ever sheep androgenetic (two paternal genomes) embryos. These types of embryos are a useful model for investigating the contribution of the paternal genome to embryonic development, including epigenetic changes. One notable discovery was that androgenetic and cloned embryos display similar patterns of high methylation, suggesting the probability that placental abnormalities in cloning could be mechanistically similar to those seen in androgenetic embryos. This may explain why somatic cloned embryos have a low efficiency in generating viable pregnancies that go to full term.

As the generation of embryos by nuclear transfer is very low, one key project in Professor Campbell's laboratory focused on improving the reprogramming of ovine (sheep) somatic cells by using egg and oocyte extracts from one species of frog (*Xenopus*). This resulted in some interesting epigenetic effects and the number of pregnancies that went to full term was higher compared to the control group. However, the high rate of mortality suggests that the epigenetic changes induced could

be unfavourable to development. Further experiments to understand the timing and specificity of the changes are necessary.

It is also notable that Professor Campbell, who worked with Sir Ian Wilmut on the creation of 'Dolly', the first cloned sheep, was jointly awarded the Shaw Prize by the foundation of the same name in Hong Kong. The other recipients were Sir Ian Wilmut (Scottish Centre for Regenerative Medicine, University of Edinburgh, UK) and Professor Shinya Yamanaka of the Institute for Frontier Medical Science at Kyoto University, Japan, for their work at the forefront of stem cell research.

Professor Galli's group worked on NTSC derivation and characterisation in other large animal models including bovine and porcine models. The work concentrated on a model of interspecies nuclear transfer (i.e. the nucleus of one species into the oocyte of another) and confirmed that such interspecies embryos are not viable, therefore it will be complicated to derive embryonic stem cells from such type of embryo (*ref. 17*). One paper published in *Stem Cells*, characterised neural crest stem cells lines derived from *in vitro* fertilisation and cloned bovine embryonic cells (*ref. 1*). This not only provided a system to understand the early steps of mammalian nervous system development but also demonstrated the possibility of obtaining stem cell derivatives for potential cell therapy and tissue engineering applications. The control of differentiation will be a crucial step and might rely upon efficient techniques for genetic modifications (*refs. 2, 3*). Following on from this research, an *in vitro* human model of neuronal development was developed, and the results should be published in early 2009.

Also in 2008, Professor Galli was awarded the Simmet Prize for Assisted Reproduction from the International Congress of Animal Reproduction for his contribution to the field of large animal reproduction and cloning by nuclear transfer.

Using animal models of human diseases, Dr. Vezzoni's group focused on the use of murine embryonic stem cells as a possible therapeutic strategy. Among the models he used was the *oc/oc* mouse mutant, a model of human autosomal recessive osteropetrosis (arOP); recessive meaning that two defective copies of the gene are necessary for disease pathology. arOP is a severe bone disease with a fatal outcome, generally within the first decade of life. This study demonstrated that hematopoietic stem cells (HSC) from adult bone marrow and foetal liver could rescue the *oc/oc* mouse mutant when delivered prenatally (*results are submitted*).

Finally, Dr. Feil, who heads the Genome Imprinting and Development Laboratory at the Montpellier Institute of Molecular Genetics, investigated a process known as genomic imprinting in embryonic stem cells. This is an essential mechanism in mammalian development, whereby certain genes are expressed from only one of the two parent alleles. It has been demonstrated that *in vitro* culture and manipulation of embryonic stem cells can affect this genomic imprinting, comprising the embryo's growth and development.

Dr. Feil's group made a number of significant discoveries including the demonstration that parentally imprinted regions carry different modifications from other regions in the genome (*refs. 8,9*) and how imprinting is regulated by histone methylation in the murine placenta (*refs. 10,10a*). Such fundamental studies provide insights into a process that is only beginning to be understood. Another study showed that a large part of the development abnormalities associated with somatic cell nuclear transfer procedures can be explained by abnormal development and proliferation of extra-embryonic membranes. This has important implications for the development of therapies using SNCT techniques and understanding the role of imprinting in human disease pathology.

Selected Publications

1. Lazzari G, Colleoni S, Giannelli SG, Brunetti D, Colombo E, Lagutina I, **Galli C**, Broccoli V. Direct Derivation of Neural Rosettes from Cloned Bovine Blastocysts: A Model of Early Neurulation Events and Neural Crest Specification In Vitro. *Stem Cells* **24**, 2514-2521 (2006).
2. Lombardo A, Beausejour CM, Genovese P, Colleoni S, Lee Y-L, Kim KA, Ando D, Urnov F, **Galli C**, Gregory PD, Holmes MC, Naldini L. Gene editing in human stem cells using zinc finger nucleases and integrase – defective lentiviral vector delivery. *Nat. Biotechnol.* **25**, 1298-1306 (2007).
3. Santoni de Sio FR, Gritti A, Cascio P, Neri M, Sampaolesi M, **Galli C**, Luban J, Naldini L. Lentiviral Vector Gene Transfer is Limited by the Proteasome at Post-Entry Steps in Various Types of Stem Cells. *Stem Cells* **26**, 2142-2152 (2008).
4. Cruz NT, Wilson KJ, Cooney MA, Tecirlioglu RT, Lagutina I, **Galli C**, Holland MK, French AJ. Putative imprinted gene expression in uniparental bovine embryo models. *Reprod. Fertil. Dev.* **20**, 589-597 (2008).
5. Maalouf WE, Alberio R, **Campbell KH**. Differential acetylation of histone H4 lysine during development of *in vitro* fertilized, cloned and parthenogenetically activated bovine embryos. *Epigenetics* **3**, 199-209 (2008).
6. Bowles EJ, Lee JH, Alberio R, Lloyd RE, Stekel D, **Campbell KH**, St John JC. Contrasting effects of *in vitro* fertilization and nuclear transfer on the expression of mtDNA replication factors. *Genetics* **176**, 1511-1526 (2007).
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9. Pannetier M, Julien E, Schotta G, Tardat M, Sardet C, Jenuwein T, **Feil R**. Pr-Set7 and Suv4-20h regulate H4 lysine-20 methylation at imprinting control regions in the mouse. *EMBO Reports* **9**, 998-1005 (2008).
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11. Palmieri C, **Loi P**, Ptak G, Della Salda L. Review paper: a review of the pathology of abnormal placentae of somatic cell nuclear transfer clone pregnancies in cattle, sheep, and mice. *Veterinary Pathology*, **45**, 865-880 (2008).
12. **Loi P**, Beaujean N, Khochbin S, **Fulka J Jr**, Ptak G. Asymmetric nuclear reprogramming in somatic cell nuclear transfer? *BioEssays* **30**, 66-74 (2008).
13. Matsukawa K, Turco MY, Scapolo PA, Reynolds L, Ptak G, **Loi P**. Development of Sheep Androgenetic Embryos Is Boosted following Transfer of Male Pronuclei into Androgenetic Hemizygotes. *Cloning Stem Cells* **9**, 372-81 (2007).
14. Palmieri C, **Loi P**, Reynolds LP, Ptak G, Della Salda L. Placental abnormalities in ovine somatic cell clones at term: a light and electron microscopic investigation. *Placenta* **28**, 577-84 (2007).
15. Fulka H, Barnetova I, Mosko T, **Fulka Jr J.** Epigenetic analysis of human spermatozoa after their injection into ovulated mouse oocytes. *Human Reproduction* **23**, 627-634 (2008).
16. Ogushi S, Palmieri C, Fulka H, Saitou M, Miyano T, **Fulka Jr J**. The maternal nucleolus is essential for early embryonic development in mammals. *Science* **319**, 613-616 (2008).
17. **Fulka Jr J**, Fulka H, St John J, **Galli C**, Lazzari G, Lagutina I, Fulka J, **Loi P**. Cybrid human embryos –warranting opportunities to augment embryonic stem cell research *Trends in Biotechnology* **26**, 469-474 (2008).
18. Sobacchi C, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, **Vezzoni P**, Villa A, Helfrich MH. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat. Genet.* **39**, 960-962 (2007).
19. Marrella V, Poliani PL, Casati A, Rucci F, Frascoli L, Gougeon ML, Lemercier B, Bosticardo M, Ravanini M, Battaglia M, Roncarolo MG, Cavazzana-Calvo M, Facchetti F, Notarangelo LD, **Vezzoni** P, Grassi F, Villa A. A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome. *J. Clin. Invest.* **117**, 1260-1269 (2007).
20. Guerrini MM, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, **Vezzoni P**, Villa A, Frattini A. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am. J. Hum. Genet.* **83**, 64-76 (2008).

3.2 Regulation of haematopoietic stem cell self-renewal in the embryo (CRP II)

Principal Investigators

Professor Elaine Dzierzak (Project Leader)
Dr. Anna Bigas
Professor Tariq Enver

Funding Organisations

The Netherlands: the Netherlands Organisation for Health Research and Development (ZonMw)
Spain: Interministerial Committee on Science and Technology
United Kingdom: Medical Research Council (MRC)

The haematopoietic system, including the tissues and organs involved in the production of blood, is made up of many different specialised cells such as red blood cells, platelets, macrophages and granulocytes. In the adult, the blood cells must be replenished continuously; on average a person needs about one hundred billion new cells each day. Hematopoietic Stem Cells (HSCs) – robust, self-renewing cells and mainly located in the bone marrow – are the source of these new blood cells. The HSC numbers are kept constant through self-renewing cell divisions; one daughter differentiates, one remains a stem cell. At the time of the proposal submission, the molecular mechanisms involved in the regulation of stem cell fate and division, both in the adult and developing embryo were poorly defined. So, Professor Dzierzak, together with Professor Enver and Dr. Bigas, began a collaborative project using their differing expertise to unravel the signalling pathways controlling HSC self-renewal.

It was known that Gata-2, a transcription factor, and Notch-1, a signalling protein, are important regulators of HSC fate and/or self-renewal in the embryo. It was thought that these proteins work together to provide HSC function, but it was not fully understood how they interacted. Using mouse mutant models, it was demonstrated that Jagged-1 activates Notch-1 and in turn activates Gata-2, which turns on the expression of genes essential for normal haematopoiesis in the mouse embryo (Figure 3). This research resulted in a joint publication by the three PIs (*ref. 1*).

Figure 3

Jagged 1 ⇨ Notch 1 ⇨ Gata 2 ⇨ Haematopoiesis

Further studies have identified downstream targets of Gata-2, and now a framework is emerging for understanding how it regulates the cell cycle, survival and differentiation of HSCs and their progeny. These results

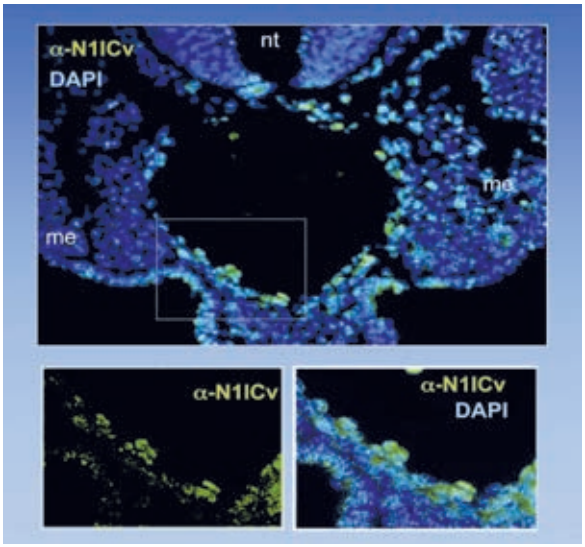

Figure 4. Heterogeneous expression of Notch family members within the aortic haematopoietic (AGM) region in 10.5 day mouse embryos. The figure shows immunofluorescence staining of anti-N1Icv for cleaved Notch-1 in green overlayed with DAPI (4',6-Diamidino-2-Phenylindole) in blue.
From: Robert-Moreno A, *et al. EMBO J*, **27** 1-10 (2008).

serve as a general paradigm for understanding how transcription factors regulate stem cell fate.

Understanding the process of haematopoeisis – the development of the various types of blood cells from a stem cell precursor – not only provides fundamental insights into development pathways but is also essential for understanding defects in disease states such as anaemia, leukaemia and thrombocytopenia.

In addition to the outlined results, many tools were developed for stem cell research in order to:

Elucidate biological properties of stem cells;
– New quantitative and vital imaging methods for HSCs in embryos
– *In vitro* HSC expansion cultures (tissue explants)

Set up bases for comparative analyses of hematopoietic stem and progenitor cells;
– Controlled differentiation HSCs *in vitro* culture systems
– Time course transcriptional profiling
– RNAi/shRNA validation of transcription factor targets

Develop biotechnical potential;
– Lentiviral delivery to tissue explants
– Transcriptional profiling and target gene analyses

Future clinical applications;
– Manipulation of transcription factor expression/targets for the self-renewal and expansion of HSCs for hematologic transplantations.

Selected Publications

1. Robert-Moreno A, Guiu J, Ruiz-Herguido C, López ME, Inglés-Esteve J, Riera L, Tipping A, **Enver T**, **Dzierzak E**, Gridley T, Espinosa E, **Bigas A**. Jagged1-dependent activation of Notch is a requisite for intraembryonic hematopoiesis. *EMBO J* **27**, 1-10 (2008).
2. Robert-Moreno A, Espinosa L, Sanchez MJ, de la Pompa JL, **Bigas A**. The Notch Pathway Positively Regulates Programmed Cell Death during Erythroid Differentiation. *Leukemia* **21**, 1496–1503 (2007).
3. Rodrigues NP, Boyd AS, Fugazza C, May GE, Guo Y, Tipping AJ, Scadden DT, Vyas P, **Enver T**. GATA-2 regulates granulocyte-macrophage progenitor cell function. *Blood* **112**, 4862-4873 (2008).
4. Fukushima H, Nakao A, Okamoto F, Shin M, Kajiya H, Sakano S, **Bigas A**, Jimi E, Okabe K. The association of Notch2 and NF-kB accelerates RANKL-induced osteoclastogenesis *Mol. Cell Biol.* **28**, 2402-2412 (2008).
5. Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, Rodrigues NP, Crockford TL, Cabuy E, Vindigni A, **Enver T**, Bell JI, Slijepcevic P, Goodnow CC, Jeggo PA, Cornall RJ. DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* **44**, 686-690 (2007).
6. Robin C, Ottersbach K, Durand C, Vanes L, Tybulewicz V, Dzierzak, E. An unexpected function for IL-3 in the embryonic development of HSCs. *Developmental Cell* **11**, 171-188 (2006).
7. Durand C, Robin C, Bollerot K, Baron MH, Ottersbach K, **Dzierzak E**. Embryonic stromal clones reveal developmental regulators of definitive HSCs. *Proc. Natl. Acad. Sci. USA* **104**, 20838-20843 (2007).

3.3 Translational stem cell research: from basic biology to regenerative medicine (CRP III)

Principal Investigators

Professor Stefan Krauss (Project Leader)
Dr. Thorarinn Gudjonsson
Dr. Morten Meyer
Dr. Juha Partanen
Professor Dirk de Rooij
Dr. Fiona Watt

Associated Partners

Professor Ernest Arenas
Dr. Patrik Brundin
Professor Jonas Frisén
Dr. Jonas Muhr
Professor Hannu Sariola
Professor Irma Thesleff

Funding Organisations

Denmark: Danish Agency for Science, Technology and Innovation
Finland: Academy of Finland
Iceland: Iceland Centre for Research (RANNIS)
The Netherlands: the Netherlands Organisation for Health Research and Development (ZonMw)
Norway: The Research Council of Norway

The largest CRP of the three, this project aimed at elucidating the mechanisms that control self-renewal and differentiation in a wide variety of stem cell systems and animal models; understanding the basic biology of stem cells is a pre-requisite for their therapeutic use. A multitude of stem cell systems were used comparatively, including haematopoetic, neural, breast, tooth, epithelial, spermatogonial, and embryonic stem cell populations. Murine and bovine models were among the animal models used.

Among the research highlights were a novel understanding of the decision points in important signalling pathways involving the Wnt proteins. A large family of secreted proteins, Wnts are responsible for controlling important processes including embryogenesis and stem cell self-renewal. One such pathway is the Wnt/Frizzled pathway, a crucial element in cellular communication that, if deregulated, can result in cancer and developmental defects. One paper revealed the role of a multifunctional protein – beta-arrestin – in this pathway, opening up the possibility that Wnt may signal through many other additional and as yet unknown pathways (*ref. 17*).

Professor Krauss, the Project Leader, worked on signalling in neural stem cells and in transgenic animal models. One of the papers from his group described how a changing gradient of Wnt signalling in the developing brains of embryonic transgenic mouse models controls the start of neurogenesis – the creation of neurons – and cellular identity *(ref. 2*). The long-term aims of these types of studies are: (i) to contribute to the understanding of the mechanisms that control cortical development and evolution, (ii) to use the knowledge to grow neural stem cell populations of high purity and viability for therapeutic approaches, and (iii) to study how neural stem cells can be activated *in vivo*.

Many other papers were published on discoveries relating to stem cell pathways of differentiation. One was co-authored by Dr. Thorarinn Gudjonsson, which provided evidence of a stem cell hierarchy in the human breast (*ref. 4*). Prior to this paper, although it had been hypothesised that there were stem and progenitor cells in the human mammary gland, neither the location nor the cells had been identified or characterised. The term for where stem cell populations reside is 'niche' – a specific place that ensures that the stem cells are not depleted or divide too rapidly. One example of a stem cell niche is the bone marrow, providing the supply of new blood cells during the course of our lifetime. Similarly, a stem cell niche was identified in the breast, together with four types of epithelial cells which are hierarchically connected such that only one cell type can give rise to all the others. This fundamental knowledge may shed light on how breast cancer develops, as there is evidence to suggest that abnormal stem cells may give rise to cancer.

Stem cells niches also exist in the lower layer of the human epidermis, which is continuously renewed during our lifetime, ensuring that as skin cells are shed, new ones are there to take their place. Within the epidermis several types of differentiation can be discerned, including formation of the interfollicular epidermis (IFE), hair follicles (HF) and sebaceous glands (SG), and it has been shown that they are maintained by their own discrete populations of stem cells. Dr. Fiona Watt co-authored a paper investigating progenitors from SGs in an *in vitro* model, demonstrating that they could differentiate into both sebocytes and interfollicular epidermis, and that a protein – Myc – stimulated the sebocyte differentiation. Understanding this and other pathways that regulate epidermal stem cell renewal is of particular importance to cancer research, as non-melanoma cancer is the most common type of cancer in the world and arises from epidermal keratinocytes.

Another promising area of research explored by Professor de Rooij's group was that of male germ stem cells, which have the potential to restore male fertility after therapy for cancer. Although these cells can be readily transplanted to recipient testes in murine models, at the time of proposal submission, *in vitro* expansion of spermatogonia (precursor of mature sperm) was difficult.

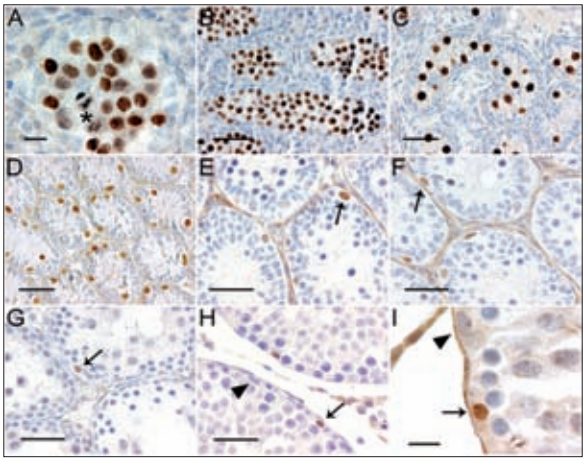

Figure 5. Immunohistochemical localisation of the pluripotency marker undifferentiated embryonic cell transcription factor 1 (UTF1) in a subset of spermatogonia, including spermatogonial stem cells, in testes of rat of several ages. A: 16dpc (days post coitum), B: 18dpc, C: 4dpp (days post partum), D: 9dpp, E: 13dpp, F: 17dpp, G: 24dpp, H: 42dpp, I: 56dpp, J: 70dpp. Arrows: UTF1 positive spermatogonia; arrowheads: UTF1 negative spermatogonia, asterisk: dividing foetal germ cell.
From: van Bragt M, *et al.* Reproduction **136**, 33-40 (2008).

Research involved improving the culturing conditions and understanding characteristics such as differentiation, self-renewal and plasticity of spermatagonial cells *in vitro*. One paper described the importance of a protein, coded for by the gene *Stra8*, in a critical stage in the production of sperm (*ref. 10*). Furthermore in recent years, it has been shown that mouse spermatagonial stem cells (SSCs) can differentiate to multipotent cells, offering new possibilities for studying the mechanisms of stem cell differentiation. In humans this could be a possible route for obtaining ES-like cells for use in regenerative medicine. Two papers on the formation of multipotent germ stem cells from the bull and human will be published early in 2009.

This section has highlighted some of the key results of the PIs of this CRP and for more information please refer to the selected publications below.

Selected Publications

1. Faedo A, Tomassy GS, Ruan Y, Teichmann H, **Krauss S**, Pleasure SJ, Tsai SY, Tsai M J, Studer M, Rubenstein JL. COUP-TFI coordinates cortical patterning, neurogenesis, and laminar fate and modulates MAPK/ERK, AKT, and beta-catenin signaling. *Cereb Cortex.* **18**, 2117-2131 (2008).
2. Machon O, Backman M, Machonova O, Kozmik Z, Vacik T, Andersen L, **Krauss S**. A dynamic gradient of Wnt signaling controls initiation of neurogenesis in the mammalian cortex and cellular specification in the hippocampus. *Dev. Biol.* **311**, 223-237 (2007).
3. Solberg N, Machon O, **Krauss S**. Effect of canonical Wnt inhibition in the neurogenic cortex, hippocampus, and premigratory dentate gyrus progenitor pool. *Dev. Dyn.* **237**, 1799-1811 (2008).
4. Villadsen R, Fridriksdottir AJ, Rønnov-Jessen L, Gudjonsson T, Rank F, LaBarge MA, Bissell MJ, Petersen OW. Evidence for a stem cell hierarchy in the adult human breast. *J. Cell Biol.* **177**, 87-101 (2007).
5. Sigurdsson V, Fridriksdottir AJ, Kjartansson J, Jonasson JG, Steinarsdottir M, Ogmundsdottir HM, & Gudjonsson T. Human breast microvascular endothelial cells retain phenotypic traits in long-term finite life span culture. *In Vitro Cell Dev. Biol. Anim.* **42**, 332-340 (2006).
6. Anwar MR, Andreasen CM, Lippert SK, Zimmer J, Martinez-Serrano A, **Meyer M**. Dopaminergic differentiation of human neural stem cells mediated by cocultured rat striatal brain slices. *J. Neurochem*. **105**, 460-470 (2008).
7. Andersen R K, Johansen M, Blaabjerg M, Zimmer J, **Meyer M**. Neural tissue-spheres: A microexplant culture method for propagation of precursor cells from the rat forebrain subventricular zone. *J. Neurosci. Methods* **165**, 55-63 (2007).
8. Saarimäki-Vire J, Peltopuro P, Lahti L, Naserke T, Blak AA, Vogt Weisenhorn DM, Yu K, Ornitz DM, Wurst W, **Partanen J**. Fibroblast growth factor receptors cooperate to regulate neural progenitor properties in the developing midbrain and hindbrain. *J. Neurosci.* **27**, 8581-8592 (2007).
9. **de Rooij DG**, Mizrak SC. Deriving multipotent stem cells from mouse spermatogonial stem cells: a new tool for developmental and clinical research. *Development* **135**, 2207-2213 (2008).
10. Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, **de Rooij DG**, van Pelt AMM. *Stra8* and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc. Natl. Acad. Sci. USA* **105**, 14976-14980 (2008).
11. **Watt FM**, Frye M, Benitah SA. MYC in mammalian epidermis: how can an oncogene stimulate differentiation? *Nat. Rev. Cancer* **8**, 234-242 (2008)
12. Torres J, **Watt FM**. Nanog maintains pluripotency of mouse embryonic stem cells by inhibiting NFkappaB and cooperating with Stat3. *Nat. Cell Biol.* **10**, 194-201 (2008).
13. Giangreco A, Qin M, Pintar JE, **Watt F M**. Epidermal stem cells are retained *in vivo* throughout skin aging. *Aging Cell* **7**, 250-259 (2008).
14. Lo Celso C, Berta MA, Braun KM, Frye M, Lyle S, Zouboulis CC, **Watt FM**. Characterization of bipotential epidermal progenitors derived from human sebaceous gland: contrasting roles of c-Myc and beta-catenin. *Stem Cells* **26**, 1241-1252 (2008).
15. **Arenas E**, Koltzenburg M, Charnay P, El Manira A, Ibañez CF, Ernfors P. Histone H2AX-dependent GABA(A) receptor regulation of stem cell proliferation. *Nature* **451**, 460-464 (2008).
16. Parish CL, Castelo-Branco G, Rawal N, Tonnesen J, Sorensen AT, Salto C, Kokaia M, Lindvall O, **Arenas E**. Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in parkinsonian mice. *J. Clin. Invest.* **118**, 149-160 (2008).

17. Bryja V, Gradl D, Schambony A, **Arenas E**, Schulte G. Beta-arrestin is a necessary component of Wnt/beta-catenin signaling *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA* **104**, 6690-6695 (2007).
18. **Sariola H**, Immonen T. GDNF maintains mouse spermatogonial stem cells *in vivo* and *in vitro*. *Methods Mol. Biol.* **450**, 127-135 (2008).
19. Kuure S, Popsueva A, Jakobson M, Sainio K, **Sariola H**. Glycogen synthase kinase-3 inactivation and stabilization of beta-catenin induce nephron differentiation in isolated mouse and rat kidney mesenchymes. *J. Am. Soc. Nephrol.* **18**, 1130-1139 (2007).
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21. Pummila M, Fliniaux I, Jaatinen R, James M, Laurikkala J, Schneider P, **Thesleff I**, Mikkola ML. Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of BMP activity and induction of Shh expression. *Development* **134**, 117-125 (2007).
22. Holmberg J, Hansson E, Malewicz M, Sandberg M, Perlmann T, Lendahl U, **Muhr J**. SoxB1 transcription factors and Notch signaling use distinct mechanisms to regulate proneural gene function and neural progenitor differentiation. *Development* **135**, 1843-1851 (2008).
23. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, **Frisén J**, Arner P. Dynamics of fat cell turnover in humans. *Nature* **453**, 783-787 (2008).
24. Barnabé-Heider F, Meletis K, Eriksson M, Bergmann O, Sabelström H, Harvey MA, Mikkers H, **Frisén J**. Genetic manipulation of adult mouse neurogenic niches by *in vivo* electroporation. *Nat. Methods* **5**, 189-196 (2008).

4. Networking and Dissemination Activities

Networking and Dissemination activities are key characteristics of a EUROCORES Programme such as EuroSTELLS. Their aim is to encourage and facilitate scientific collaboration and diffusion across the Collaborative Research Projects (CRPs) within a given domain or if appropriate across different domains and programmes. These activities are flexible and can be tailored to the needs of a given programme.

Networking activities

These are collaborative activities bringing together scientists from EUROCORES Programmes and colleagues from other relevant programmes in order to discuss, plan and implement future collaboration and interaction.

Typical examples are:
- Working group meetings, seminars, workshops, symposia, conferences;
- Summer schools (targeted for the members of academia, the private sector, and governmental or non-governmental organisations);
- Training programmes and specialised courses (graduate-level and continuing-education);
- Short visits.

Dissemination activities

These are all the activities with an aim of raising awareness of and diffusing results of the EUROCORES Programme.
- Leaflets, posters, publications, books, exhibition booths or stands at conferences;
- Invited sessions at larger conferences (when the EUROCORES Programme is not directly involved in the conference as a main or co-organiser of the event);
- Dissemination travel grants, to support an active participation at conferences (organised outside the EUROCORES Programme), while promoting the EUROCORES Scheme in general and disseminating the achievements of the Programme in particular.

For EuroSTELLS the principle networking activity was a series of four conferences focused on various aspects of stem cell research. Among the dissemination activities were publication of a brochure, presentations by the PIs as invited speakers at conferences and press releases. This section provides an overview of the main networking and dissemination activities of EuroSTELLS.

Figure 6. Istituto de Scienze, Lettere ed Arti sede de Palazo Franchetti, Campo Santo Stefano, Venice, Italy.

First EuroSTELLS Conference 'General Biology of Stem Cell Systems'

Venice, Italy, 19-21 March 2006

The goal of this conference was to establish a stable European forum of stem cell researchers by fostering synergy with other European and international initiatives in the stem cell field. To this end, keynote speakers included the Director of the UK Stem Cell Bank, representatives of the EuroStemCell consortium, the US National Institutes of Health, the Baltic and International Stem Cell Initiatives, biotechnology companies Cellartis and Invitrogen as well as the Project Leaders of the EuroSTELLS projects.

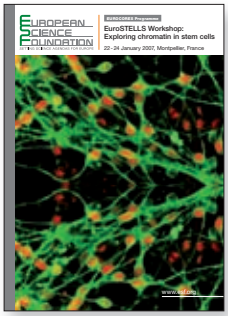
Press Releases*

• *Clues to breast cancer hidden inside stem cells*
Stem cells and how to boost them is hot on the research agenda. But stopping them could be critical too, as evidence implicating stem cells in cancer is mounting.

• *Testes to incubate stem cells*
Sperm-producing stem cells found in testicles could be extracted, grown in the lab and frozen for future use. A team in the Netherlands has successfully harvested spermatogonial stem cells from cows and cultured them inside mouse testes. The hope is that the same thing could be done for men. These findings were announced at the recent EuroSTELLS conference in Venice.

Second EuroSTELLS Conference 'Exploring Chromatin in Stem Cells'

Montpellier, France, 22-24 January 2007



This conference provided an overview of the latest developments in exploring epigenetics and chromatin in various stem cell systems. There were six sessions on topics such as 'Exploring stem cells and pluripotency' and 'Embryonic stem cells as epigenetic model systems', with poster sessions and selected oral presentations. Attended by over 100 participants, this was a lively and informative meeting where researchers had an opportunity to meet specialists in this rapidly expanding field and learn about the latest technical insights and breakthroughs.

Press Releases*

• *Cancer is a stem cell issue*
There is an urgent reason to study stem cells: stem cells are at the heart of some, if not all, cancers. Mounting evidence implicates a clutch of rogue stem cells brandishing 'epigenetic' marks as the main culprits in cancer. Wiping out tumours for good, some biologists believe, depends on uprooting these wayward stem cells.

• *Liposuctioned fat stem cells to repair bodies*
Expanding waistlines, unsightly bulges: people will gladly remove excess body fat to improve their looks. But unwanted fat also contains stem cells with the potential to repair defects and heal injuries in the body. A team led by Philippe Collas at the University of Oslo in Norway has identified certain chemical marks that allow him to predict which, among the hundreds of millions of stem cells in liposuctioned fat, are best at regenerating tissue.

• *Epigenetics to shape stem cell future*
Everyone hopes that; one day, stem cell-based regenerative medicine will help repair diseased tissue. Before then, it may be necessary to decipher the epigenetic signals that give stem cells their unique ability to self-renew and transform them into different cell types.

Third EuroSTELLS Conference 'Challenges in Stem Cell Differentiation and Transplantation'

Milan, Italy, 30 September-3 October 2007

This conference provided an overview of recent advances in stem cell biology, focusing on differentiation, transplantation and safety issues, as well as some of the practical hurdles that need to be overcome for a successful therapeutic application of stem cells. There were six sessions: I) Embryonal Stem Cells: Differentiation and Therapeutic Prospectives; II) Large Animal Models; III) Cell Signalling Pathways; IV) Stem Cell Differentiation for Therapeutic Aims; V) Adult Stem Cell Therapy; and VI) Practical Applications. Presentations featured comparative analyses of different tissues including neural, muscle, bone and skin. The plenary speaker was Professor Inder Verma (The Salk Institute, USA), who provided an overview of the challenges of gene and stem cell-mediated therapy of human diseases.

Press Releases*

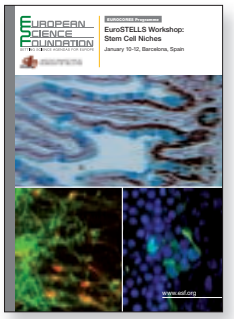
• *Stem cell research marches on*
Stem cell research proceeds apace, but many challenges lie ahead. Significant strides are being made in fundamental stem cell research in laboratories across the world, but many hurdles remain to be overcome before stem cells are routinely used to treat diseases.

• *New stem cells by re-programming*
By 'de-programming' existing specialised cells it might be possible to create cells which resemble embryonic stem cells, bypassing many of the ethical and moral objections to using human embryos. Researchers are discovering new ways to help 'de-program' specialised cells so that they can be re-programmed to form a range of different types of tissue, an international meeting of stem cell biologists was told.

• *An eye for an eye: using stem cells to treat damaged eyes and a rare skin disorder*
Stem cells can be used to grow new corneal tissue and, together with gene therapy, treat a rare genetic skin disorder. Doctors and scientists in Italy have shown how stem cells can be used to treat damaged eyes and, in combination with gene therapy, a rare and debilitating skin disease.

Fourth EuroSTELLS Conference ‘Stem Cell Niches’

Barcelona, Spain, 10-12 January 2008



The fourth and final conference provided a global view of stem cell regulation in different niches and featured the latest developments in this rapidly expanding field. Co-sponsored by the Spanish Ministry of Education and The Bellvitge Institute for Biomedical Research (IDIBELL), this high-profile event attracted over 130 participants from across the three CRPs and the wider stem cell community in Europe, Israel and the United States. Among the distinguished speakers was the Spanish Minister for Health, Professor Bernat Soria, himself a distinguished stem cell researcher; Professor Scott Armstrong, The Children’s Hospital Boston, USA; and Professor Tariq Enver (EuroSTELLS PI), The Weatherall Institute, UK.

Press Releases*

• *Minister predicts role for stem cell biologists in re-shaped pharmaceutical industry*
Stem cell research should have a bright future and could play an important role in tomorrow’s pharmaceutical industry, Spain’s Minister for Health told an international conference of stem cell biologists on January 11.

• *Stem cell research aims to tackle Parkinson’s disease*
Scientists in Sweden are developing new ways to grow brain cells in the laboratory that could one day be used to treat patients with Parkinson’s disease, an international conference of biologists organised by the European Science Foundation (ESF) was told.

• *Stem cells give clues to understanding cancer; make breakthrough in childhood leukaemia*
Scientists in Switzerland are uncovering new clues about how cancer cells grow – and how they can be killed – by studying stem cells, ‘blank’ cells that have the potential to develop into fully mature or ‘differentiated’ cells, and other scientists in UK have made a breakthrough in understanding the cause of the most common form of childhood cancer, acute lymphoblastic leukaemia (ALL).

* Full press releases available at **www.esf.org/media-centre**

5. Outreach Activities

5.1 ISSCR Guidelines for Clinical Translation of Stem Cells

The International Society for Stem Cell Research (ISSCR) is an independent, non-profit organisation to foster the exchange of information on stem cell research[2]. Recognising the lack of clear reference materials to assist patients and patient advocacy groups in navigating the landscape of stem cell-based therapies, the ISSCR brought together a diverse, international task force of top stem cell researchers, clinicians, bioethicists, and regulatory leaders to draft professional guidelines relating to the clinical translation of stem cell research in 2007. To support them in this commendable effort, the Scientific Committee of EuroSTELLS agreed to financially sponsor the task force, together with the Alzheimer's Research Foundation, The Ellison Medical Foundation and the Juvenile Diabetes Research Foundation Beta Cell Replacement Advisory Committee.

The task force, headed by Professor Olle Lindvall of the University of Lund in Sweden, comprised top stem cell researchers, clinicians, bioethicists and regulatory leaders from 13 countries, including France, Italy, Spain, Sweden and the United Kingdom[3]. Convened for the first time in November 2007, they presented the draft guidelines at the 6th ISSCR Annual Meeting in Philadelphia, 11-14 June 2008, which were released for public comment in September 2008. The resulting feedback was incorporated, and the final version of the *ISSCR Guidelines for the Clinical Translation of Stem Cells* was announced on 3 December 2008[4]. Information is also provided for patients and their doctors evaluating a stem cell therapy in Appendix 1: *Patient Handbook on Stem Cell Therapies*. These guidelines define a roadmap for medical researchers and doctors, outlining what needs to be accomplished to move stem cells from promising research to proven treatments for patients and will accelerate the translation of stem cell research into practice while addressing associated scientific, clinical, regulatory, ethical and social issues.

EuroSTELLS is proud to have supported this initiative, which also raised the profile of the programme and the overall EUROCORES Scheme to a wider research community. It is hoped that the guidelines will prove to be a valuable resource for stem cell researchers and patients, both in Europe and world-wide.

For more information, see
www.isscr.org/clinical_trans/index.cfm

2. www.isscr.org
3. www.isscr.org/clinical_trans/members.html
4. www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf

5.2 European Science Open Forum (ESOF) 2008

The European Science Open Forum (ESOF) is a biennial event which aims to showcase European achievements across the scientific spectrum. The mission of the Euroscience Open Forum (ESOF) is to provide both the European and the international science and business communities with an open platform for debate and communication. It presents and profiles Europe's leading research trends in the natural sciences, humanities and social sciences. It is an opportunity to discuss and influence the future of research and innovation in Europe by involving all main stakeholders: scientists, business executives and policy-makers. ESOF 2008, hosted in Barcelona on 18-22 July, built on events in Stockholm, 2004 and Munich 2006.

As a collaborator, the ESF was invited to submit abstracts for sessions, and as one of the themes was 'Engineering the Body', an abstract entitled 'Stem Cells – From Bench to Bedside' was selected for presentation on July 19. Three high-profile speakers, listed below, addressed developments in personalised stem cells, the perspective from the potential end users, the patients, and the main ethical issues involved in using stem cell-based treatments. It was a lively and informative session, which was followed by an open session at the ESF exhibition booth, where other conference participants had an opportunity to ask questions.

Session Programme

The Science and Future of Stem Cell Research,
Professor Keith Campbell, Animal Development, School of Biosciences, University of Nottingham, United Kingdom (*EuroSTELLS PI*)

Patients' Perspective on Stem Cell Research,
Mr. Alastair Kent, Director, Genetic Interest Group, London, United Kingdom

Bioethical Issues,
Dr. Kate Millar, Director, Centre for Applied Bioethics at the School of Biosciences at the University of Nottingham, United Kingdom

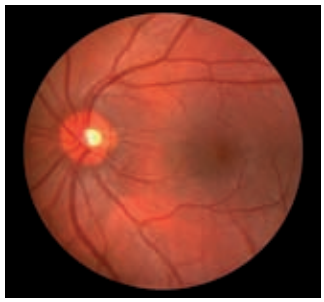
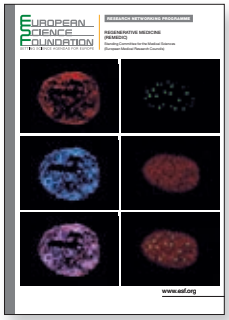


Figure 7. View inside the human eye showing a healthy retina, optic nerve, and macula. Courtesy of iStock (www.istock.com).

6. Related ESF Activities

This section provides a summary of two activities in the field of stem cell research that are currently ongoing at the ESF.

6.1 Research Networking Programme, REMEDIC



Research Networking Programmes are another ESF instrument that are networking activities of four to five years to lay the foundations for nationally funded research groups to address major scientific and research infrastructure issues. They can involve:

- science meetings (workshops, conferences or schools)
- grants for short and exchange visits
- publication of information brochures and leaflets
- creation and maintenance of scientific databases at the European level.

REMEDIC is a cross-disciplinary Research Networking Programme aimed at identifying frontiers and future needs in regenerative medicine. REMEDIC aims:

- To facilitate the exchange of ideas and know-how across disciplines in the area of mesenchymal (stromal) and other stem cells;
- To review the regulatory laws, rules and standards governing the use of regenerative medicine;
- To map the unmet population, academia and company needs in regenerative medicine and the current R&D resources (e.g. instruments, analysis techniques);
- To assist researchers in the field of regenerative medicine in preparing cross-disciplinary coordinated research projects.

To promote links between the REMEDIC and EuroSTELLS, information is disseminated between them, including for example the Call for Short Term and Exchange Visit Grants that was launched for REMEDIC in November 2008. Open to young scientists who are based in one of the 14 countries that financially support REMEDIC, meaning that most of the young PhD and postdocs of EuroSTELLS are also eligible.

The running period of the ESF REMEDIC Research Networking Programme is five years from May 2008 to May 2013 (07-RNP-128).

www.esf.org/remedic

6.2 Science Policy Briefing on Stem Cells and Regenerative Medicine

In 2001 and 2002 the ESF published its 14th Science Policy Briefing (1st and 2nd editions) on 'Human Stem Cell Research: scientific uncertainties and ethical dilemmas'[5]. The aim was to provide a scientific and ethical overview of this rapidly advancing field and as a follow-on from this SPB, described in the brochure foreword, the EuroSTELLS programme was developed. Among the ten recommendations from the SPB, was the following:

'The ESF recognises that the position differs between countries and that there will be continual debates on this sensitive issue. The ESF will ensure that this paper is updated regularly to reflect scientific and regulatory changes in the future'.

In 2007 the EMRC Office initially undertook an update of the regulations on the use of human stem cells in research in Europe, which was validated in the spring of 2008. In a second step an expert group was convened to prepare an updated edition of the SPB, and reflecting recent scientific advances, modified the title to encompass regenerative medicine.

It is expected that this edition will be published in spring 2009.

5. EMRC/ESF Science Policy Briefing 14, Human Stem Cell Research: scientific uncertainties and ethical dilemmas, June 2001; 2nd Edition, EMRC/ESF Science Policy Briefing 18, August 2002. www.esf.org/research-areas/medical-sciences/publications.html

Expert Group

Professor Outi Hovatta, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden

Professor Urban Lendahl, Department of Cell and Molecular Biology, Karolinksa Institute, Stockholm, Sweden

Professor Frank Luyten, Division of Rheumatology, Department of Musculoskeletal Sciences, at the University Hospitals Leuven, Director of the Laboratory for Skeletal Development and Joint Disorders, K.U.Leuven, Belgium

Professor Heike Mertsching, Head of Department of Cell and Tissue Engineering, Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany

Professor Miodrag Stojkovic, Deputy Director and Head of Cellular Reprogramming Laboratory, Centro de Investigación Príncipe Felipe, Valencia, Spain

Professor Ruud ter Meulen, Centre for Ethics in Medicine, Bristol, United Kingdom

Professor Paul van der Saag, formerly Netherlands Institute for Developmental Biology, the Netherlands

Dr. Gerjo van Osch, Department of Orthopaedics and Department of Otorhinolaryngology, Erasmus University Medical Center, the Netherlands

6.3 Exploratory Workshops

Each year, ESF supports approximately 60 Exploratory Workshops across all scientific domains. These small, interactive group sessions are aimed at opening up new directions in research to explore new fields with a potential impact on developments in science. The workshops, which usually last up to three days, have a wide participation from across Europe and involve mature scientists as well as young, independent researchers and scholars with leadership potential. Their relatively small scale (in terms of people involved) provides an ideal platform for focusing on a specific topic and for all participants to contribute to discussions and plan follow-up collaborative work. Interdisciplinary topics are greatly encouraged.

In 2006 the Humanities Unit of the ESF supported an Exploratory Workshop 'Stem Cell Cultures: Exploring the social and cultural background to European debates about human embryonic stem cells'. Held over two days (10-12 March) in Nottingham, United Kingdom, the aim was to provide insights into the diversity of knowledge and understanding of research involving human embryonic stem cells (hESCs) in a variety of European countries and new European member states in order to reveal how different social groups and the mass media use cultural tools to assess the implications of this new technology.

Read a final scientific report of the workshop at **http://www.esf.org/activities/exploratory-workshops/humanities-sch.html?year=2006&domain=SCH**

7. Conclusions

The EuroSTELLS Programme was successful in implementing the recommendations expressed in the Science Policy Briefing 'Human Stem Cell Research: scientific uncertainties and ethical dilemmas' to support research on stem cell lines and their applications. Common tools were made available through networking activities which brought together members from within the network of 22 research laboratories of EuroSTELLS and from the wider research community across Europe and the United States. The three CRPs generated fundamental knowledge on stem cells by exploring novel approaches to enhance animal embryonic stem cell research (CRP1), elucidating the pathways of haematopoesis (CRP2), and investigating the mechanisms of self-renewal and differentiation in a wide variety of stem cell systems (CRP3).

This is supported by the International Review Panel, which stated in the final consensus report that:

'The three Collaborative Research Projects (CRPs) have contributed significantly to progress in stem cell biology, with each CRP producing a significant number of publications, including in high impact journals (Nature Biotechnology, The EMBO Journal, Journal of Cell Biology and Development). The major strength of the EuroSTELLS Programme was in the establishment of collaborations in the various stem cell fields, uniting laboratories from different countries and disciplines, leading to more publications and increased quality of high-profile studies.'

By doing so, the EuroSTELLS Programme attracted international collaborations with organisations such as the International Society of Stem Cell Research (ISSCR), based in the United States, and was also instrumental in delivering the message to the general public at the 2008 European Science Open Forum in Barcelona on how stem cells are used in regenerative medicine.

The ESF will continue to support research into stem cells and regenerative medicine through its ongoing REMEDIC research networking programme, and the publication of the 3rd edition of the Science Policy Briefing in spring 2009 will contribute to the ongoing debate, highlighting areas of scientific priority in this important field.

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