ESF EUROCORES on Development of a Stem Cell Tool Box (EuroSTELLS)

Call for Outline Proposals

What is a EUROCORES?

The EUROCORES (ESF Collaborative Research Programmes) is an ESF activity designed to bring together national basic research funding bodies to collaborate on preferably multidisciplinary issues that have European-wide relevance. The aim of the programme is to maintain and develop European science at an internationally competitive level. Participating ESF Member Organisations (national research councils and academies) jointly define a research programme, specify the type of proposals to be requested and agree on the peer review procedure to be followed. Final funding decisions stay with the national research funding agencies.

Further background information on ESF EUROCORES may be found on the ESF website (http://www.esf.org/eurocores).

Research funding opportunities in the field of EuroSTELLS

Following agreement with a group of funding agencies from Europe, the European Science Foundation is launching a first Call for Proposals for research projects to be undertaken within a EUROCORES programme on the *Development of a Stem Cell Tool Box (EuroSTELLS)*. The programme is expected to run for a minimum of **five years** and includes national research funding and a European networking component. Proposals are sought for **Collaborative Research Projects**.

Background

Embryonic or somatic stem cells are seen as promising therapeutic tools for the treatment of number of several severe human diseases such as leukemia, diabetes, Parkinson disease, multiple sclerosis and other degenerative diseases. Embryonic stem (ES) cells have been isolated from the mouse more than twenty years ago, and it is only during the last five years that human ES cells have successfully been isolated and propagated in a very limited number of laboratories mostly in United States, Australia, Israel and Sweden. Somatic stem cells became also highly promising reagents in the past few years when a number of data suggest their potential for efficient differentiation into various cell types. In the haematopoietic system, somatic stem cells (haematopoietic stem cells) have been used for transplantation therapy for a long time. There is also number of studies which indicate that cancer takes place in somatic stem cells. This is particularly true in tissues with high level turnover such as skin, intestine, blood and human breast gland. Striking parallels can be found between stem cells and cancer cells and similar mechanisms may regulate self-renewal in those two cell types.

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. This aim requires that we rapidly increase our knowledge of the basic features and properties of stem cells either from embryonic or somatic origin, human as well as from animal models.

EMRC believes that the EUROCORES programme EuroSTELLS will meet this challenge by fulfilling the following strategic purposes:

- Fill the immediate need for tools, biological materials and protocols in stem cell technology,
- Create a critical mass of expertise in Europe in the field,
- Harmonize definitions, tools, reagents and protocols in stem cell biology,
- Promote and support training and access to European laboratory facilities,
- Set up the bases for comparative analyses between stem cells of different origins, i.e. embryonic vs. foetal and somatic, and between somatic stem cells from various tissues,
- Prepare application of the stem cell technology to therapeutic developments in human.

Although the ultimate goal of research on stem cells will be to develop new therapeutic approaches in human, this specific programme

will aim at developing fundamental knowledge on stem cells and conceptual and technological expertise in the field.

The research projects should not be restricted to human stem cells, as for the moment most of the experimental investigations on these cells have to be performed on animal models of different origins. Moreover, the diversity of the approaches should favour emergence of new concepts and technologies. In that way, the programme, through supporting networked projects in Europe, should allow transferring data collected on animal models in some laboratories to human stem cells studied in other laboratories.

The ESF is well aware that the legal situation concerning stem cell research is differently regulated in its member countries. Nevertheless, for not losing the relevant expertise in Europe, the EUROCORES programme EuroSTELLS aims at allowing as much scientific collaboration as possible. Still, **applicants intending to participate in this EUROCORES have to consider the respective statute on stem cell research in their own country**.

Synergy with other initiatives, such as the International Stem cell Forum (see **Annex 1**) or the EuroStemCell Initiative, the UK Stem Cell Bank and the FP6 project on banking should be looked for by the applicants when applying to the present call.

Scientific priorities

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 are also necessary into the genetic, epigenetic and phenotypic fidelity of stem cells during long-term culture.

Optimization of stem cells cultures

Non-human materials in cultures bear a risk for inter-species 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 have been 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.

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-, immuno- 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:

a/ definition of the immuno-phenotype

b/ 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 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).

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 of a new era of curing e.g. 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.

Stem Cell Banking and Registration

A major bottleneck in stem cell research is the availability of and access to validated stem cells, whether of embryonic, foetal or adult tissue origin. This situation effectively precludes many researchers from entering the field.

A **European Stem Cell Repository and a related European Stem Cell Registry** that would curate stem cells and provide unencumbered access to academic researchers would be of enormous benefit. This repository would accept embryonic, foetal and somatic stem cells, both human and non-human. The focus should be stem cells for research purposes rather than therapeutic applications in the first instance.

The minimal criteria for acceptance in the repository would be demonstrated capacity for multiplication *in vitro*, in other words, a stem cell line rather than a primary isolate. The

repository would undertake microbiological and karyotypic quality controls and prepare and curate frozen stocks. Archived cells would be distributed to research groups at cost, along with protocols for culture and, where necessary, specialist training. The success of such an operation will rely on close interaction between depositing scientists and the repository staff. This process will be labour intensive and dedicated funding will be essential for effective transfer of materials and protocols through the repository. There is consequently a need to develop a programme that will facilitate deposition of stem cell lines. This is an essential complement to the isolation of new stem cells.

The International Stem Cell Forum, hosted by MRC, UK, in 2003 launched an international project to invite researchers to examine new and existing stem cell lines, using standardised tools and procedures in order to set international scientific benchmarks on the cell line characteristics in accordance with the respective national legislation. The initial focus of the initiative will be the characterisation of human ES cells, co-ordinated by Professor Peter Andrews (Centre for Stem Cell Biology, University of Sheffield). The data generated will be posted in a new registry of stem cell lines which will be made available on an international web site being developed for the Forum (see **Annex 1**).

Discussion with the agencies involved in the International Stem Cell Forum should be undertaken to determine whether this banking initiative might form the basis for the proposed European repository and registry (at least for human stem cells). Proposals on building such a European stem cell bank and registry have to address the questions how existing stem cell repositories will be used and in which way the scientists involved intend to contribute to their enhancement and extension. Moreover, a detailed description should be provided how the characteristic parameters used to specify cell lines, markers, etc. will be determined and adjusted.

Training opportunities

Current interest in initiating stem cell research either for basic research interests, towards developing stem cell based therapies or for tissue engineering purposes is widespread in Europe. A significant bottleneck, however, is the availability of the necessary expertise for derivation, culture and maintenance, and differentiation of stem cells. The laboratories with experimental protocols running successfully are limited and requests for training in these laboratories often exceeds their capacity to supply training next to their own research commitments.

Biotech companies and academic research groups have started distributing human stem cells, but given the very strict growth conditions required to propagate human stem cells (feeder cell and serum batches, substrate, colony growth) distribution has been slow and not been sufficient to start up all planned human stem cell research programs. Likewise, the skills required to identify and isolate somatic stem cells are often underestimated. Although at present it may seem likely that somatic stem cells provide the best solution to some urgent clinical problems, in others, human embryonic stem cells may represent the best option in the future. Direct comparison of these cells in functional assays or in animal models is essential if this question of the most suitable stem cell source is to receive a scientific answer. At present very few laboratories have the necessary skills to produce good quality somatic and embryonic stem cells for direct comparison. Training would also harmonize research methods and protocols within a core group of researchers within Europe, so increasing input in trouble-shooting and optimizing methodology further.

The training programs proposed here would consist of workshops designed to provide participants with skills in both areas. They would take place every other year (three in total) and would provide training in passage, maintenance, characterization, differentiation and cryo-preservation of human stem cells, in combination with one other somatic stem cell source (bone marrow, cord blood, neural stem cells). These practical workshops for potential

users have to result in efficient transfer of expertise and the results will be evaluated by seeking feedback from participants. Duration of 4-5 days should be sufficient and could include a half-day symposium with expert lectures.

There will be an opportunity for training course participants to apply for travel and subsistence fellowships, should this be prohibitive for taking part. Priority will be given to participants with immediate plans to initiate a stem cell research programme.

Programme structure and management

The programme is expected to run for a minimum of **5 years** with research funding starting in 2005.

The programme will be overseen by a *Management Committee (MC)* formed by one science manager from each participating national funding agency. The Secretary of the ESF Standing Committee for the European Medical Research Councils will also attend the Management Committee.

An independent international *Review Panel (RP)* formed of leading scientists in the field, with a mandate from the funding agencies, will oversee the scientific aspects of the programme. Full proposals will be internationally peer-reviewed. Referees will be selected by the ESF from suggestions by the RP based on a pool of international scientists whose names are provided by the participating national funding agencies. Based on the results of refereeing, the RP will recommend and prioritise the best applications for funding by the national funding agencies of the selected applicants. The membership of the RP and the names of referees used will be published after the selection process is complete.

Funding of the proposals recommended by the RP will depend on the total amount of money made available in each country by the national funding agencies supporting the programme. The use of funds in a project will be subject to the rules of each national funding agency supporting that project as well as to the national laws of those countries.

Once the Collaborative Research Projects launched, the ESF will support successful applicants involved by networking them. This is expected to facilitate cross-project communication, exchange of information and presentation and discussion of results. To this aim, scientific workshops, conferences and similar activities will be organised during the programme's lifetime. Web-based resources will also be developed to support the exchange of technical information, reagents and expertise and to promote collaborative interactions between European researchers working in the area.

The networking and coordination aspects of EUROCORES will be funded through the EU 6th Framework Programme. In the present EUROCORES, those parts dealing with human embryonic stem cell research will not be supported in this way. They will be supported by participating agencies.

Guidelines for proposals

Proposals from individual scientists and research groups from the countries participating in the programme will be accepted, subject to the eligibility rules of their national funding agency participating in the EUROCORES programme EuroSTELLS. Priority will be given to applications from individual scientists/groups planning to undertake cross-disciplinary research together with scientists in other European countries participating in the EuroSTELLS programme. **Proposals must, as a minimum, involve 3 eligible groups or individuals from 3 countries (i.e. 1 each) participating in the programme**. Proposals involving larger collaborations will be welcome.

Applicants are asked to clearly identify their proposals as dealing or not with human embryonic stem cells.

The participating agencies are:

Austria – Fonds zur Förderung der wissenschaftlichen Forschung (FWF)
Belgium – Fonds National de la Recherche Scientifique (FNRS)
Belgium – Funds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO)
Cyprus – Research Promotion Foundation
Czech Republic – Akademie věd České republiky
Czech Republic – Grantová agentura České republiky
Denmark – Statens Sundhedsvidenskabelige Forskninsråd (SSVF)
Finland – Suomen Akatemia/Finlands Akademi
France – Centre National de la Recherche Scientifique/Direction des Sciences de la Vie (CNRS)
France – Institut National de la Santé et de la Recherche Médicale (INSERM)
Hungary – Magyar Tudományos Akadémia
Hungary – Orszagos Tudományos Kutatási Alapprogramok (OTKA)
Iceland – Rannsóknarráð Islands (RANNIS)
Italy – Consiglio Nazionale delle Ricerche (CNR)
Lithuania – Lithuanian State Science and Studies Foundation
Netherlands – Nederlandse organisatie voor Wetenschappelijk onderzoek (NWO)
Norway – Norges Forskningsråd
Portugal – Fundaçao para a Ciência e a Tecnologica (FCT)
Romania – National University Research Council (NURC)
Slovakia – Slovak Academy of Sciences
Spain – Conseja Superior de Investigaciones Cientificas (CSIC)
Spain – Oficina de Ciencia y Tecnología (OCYT)
United Kingdom – Medical Research Council (MRC)

The involvement of *Associated Scientists* or *Associated Groups* not eligible to apply to these agencies for funding, and from industry, is acceptable when their added value to a proposal is justified in the scientific case. Their participation must be fully self-supporting and will not be financially supported by the Programme.

Besides relevance to the EuroSTELLS programme and overall scientific quality/excellence of the proposal, the following criteria will also be taken into consideration:

- Originality/Novelty
- Feasibility (in particular compliance with the statutes on stem cell research in the participating countries)
- Level of international collaboration (including European added value) particularly between the participating groups
- Level of multidisciplinarity
- Qualifications of the proponent(s)
- Budget

It will be assumed that arrangements for the handling of IPR (Intellectual Property Rights) will be in place within projects, following the applicable national legislation and national funding agency rules. Applicants are strongly urged to have such arrangements in place, covering all research groups (including any *associated groups*) before the start of any project. It is expected that the results of the projects supported by this EUROCORES programme will be placed in the public domain and published in accordance with normal academic practice.

The application procedure will take place in two steps:

- the first for outline proposals
- the second for invited full proposals

Outline proposals

As a first step, outline proposals are invited by the close of **30 April 2004**. A short application form, with a scientific rationale of up to 1500 words (3 pages) and details of the collaboration, must be completed. All intended partners (e.g. national, international, industry, university) and who is doing what within the collaboration should be clearly indicated. A *Project Leader (PL)* (contact person) should be designed for each proposal. A *Principal Investigator (PI)* should be designed for each research group. Outline proposals should include a one-page curriculum vitae for the PL and each PI including a list of their 5 most important publications in the field. Any equipment necessary should be specified and an estimate of the total project costs (in euros) to be financed by the EuroSTELLS programme (i.e. excluding costs of Associated Groups) should be included. Application guidelines for outline proposals will be available on the ESF website *(http://www.esf.org/eurostells)*.

An international Review Panel (RP) to the programme will screen the outline proposals. The RP may give recommendations concerning the further development of a proposal such as suggesting joining forces with teams in other countries. It may also reject proposals that are considered either not to fit within the scope of the programme or non-viable. Successful applicants will be invited to submit full proposals.

Full proposals

As a second step, full proposals will be invited. The deadline for full proposals will be expected in **autumn 2004**. Full proposals must include a well-argued scientific case, a list of participants, a detailed budget and other supporting information. To integrate multi-national applications, a single, common scientific case must be submitted; however, the budget requested from each national funding agency has to be clearly and separately specified.

Funds requested within the EUROCORES programme EuroSTELLS should be for the additional costs of participating in the project and can include items like salary for temporary scientific and technical staff, equipment, travel and project networking costs, fellowships, etc, according to the rules of the participating national funding agencies. Major items of expenditure will require justification in the proposal.

Opportunities for parallel funding e.g. of fellowships for young researchers, may exist in participating funding agencies.

Application guidelines for full proposals will be available on the ESF website *(http://www.esf.org/eurostells)*.

A second Call for Proposals

It is anticipated that a second, more focused Call will be made in 2006/7 to cover the final years of the programme.

Outline proposals should be sent by email (in one attachment only) in pdf format by 30 April 2004 to:

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Annex 1

International Initiative to Characterise Human Embryonic Stem Cells Conducted under the Auspices of the International Stem Cell Forum

The International Stem Cell Forum has planned an international initiative to characterise human embryonic stem (HES) cell lines. The initiative will conduct a comparative study of the different HES isolates worldwide, to reach a consensus on the key criteria that should be used to identify HES cells and to establish the degree of heterogeneity that may arise because of different genotypes, different isolation and culture protocols, or because of long term adaptation to culture. The initiative will also aim to establish an inventory of key features of the established lines, including differentiation potential, karyotype, DNA fingerprint and microbiological testing, as well as any consistent variations from the common phenotype of HES cells. Prof Peter Andrews (Sheffield) with the UK Stem Cell Bank, has drawn up the specification for the initiative which will be organised on a 'hub-and-spokes' principle. The hub will prepare and provide standard reagents, protocols and tests. The participating laboratories (spokes) will provide DNA, RNA and protein, plus xenograft tumour specimens and fixed cells for immunostaining according to a specified protocol to a central reference laboratory. These studies may also include immunostaining *in situ* and sorting of cells for surface antigen expression, prior to RNA analysis. Data from the specified assays will be collated centrally and discussed at an international workshop in the summer of 2004. The accredited data will be deposited in a new human ES cell line registry that will be developed by the UK MRC as part of the Forum's new web pages; this will mirror the existing NIH cell line registry. The central costs of the initiative will be funded jointly by participating Forum agencies and each participating country will fund the work of its contributing research groups; the UK MRC has made a post available to Prof Andrews to begin co-ordinating activities.

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