EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme
European Collaborative Research

EuroSTELLS
Development of a Stem Cell Tool Box

EuroSTELLS Meeting
"Challenges in Stem Cell Differentiation and Transplantation"
30 September-2 October 2007, Milan, Italy

Summary

The aim of this meeting was to discuss some basic issues in stem cell technology which could be relevant to future therapeutic trials. Therefore the focus was on applicability, complementing the first EuroSTELLS workshop held in Montpellier, France (22-24 March 2007), which was mainly devoted to chromatin changes at the molecular level, associated with multipotentiality, reprogramming and cell differentiation. After a general discussion of the state of the art of gene and cell therapy by **Professor Inder Verma** (The Salk Institute, California, USA), Sessions I and II were mainly devoted to the differentiation potential of embryonic stem cells both in humans (Session I) and in large animals (Session II). In Session III some signalling pathways linked to differentiation were examined. Sessions IV to VI dealt with potential clinical applications. In Session IV, the possibility to differentiate ESC into neural cells was examined. In Sessions V and VI the contribution of adult stem cells was discussed. Interestingly, adult stem cells are already used in the clinical framework to rescue tissues such as bone marrow, cornea, skin and bone. In others, studies are progressing fast toward pilot trials of cell therapy; in particular, examples of potential approaches for nervous and skeletal diseases were presented. In the final discussion, the need to start clinical studies was acknowledged, although the participants agreed that they should proceed without too much emphasis on results that are yet to be achieved.

Goals and objectives

The main goal of the meeting was to gather together researchers working in the 21 EuroSTELLS laboratories, which are funded through EUROCORES. The meeting aimed to establish contacts with other international stem cell initiatives, and to develop collaboration between laboratories on stem cell research topics. In addition, the meeting provided an opportunity for young investigators to meet with colleagues of other participating laboratories and with internationally recognised senior scientists. Finally, the last objective was to disseminate the outcome of the conference through the presence of a professional science writer that resulted in three press releases[1].

[1] http://www.esf.org/activities/eurocores/programmes/eurostells/events/challenges-in-stem-cell-differentiation-and-transplantation/media-coverage.html#c21914

For each of the themes that were identified as relevant at this stage of EuroSTELLS, there were one or more invited speakers to meet the goals indicated above. There were representatives of other stem cell initiatives, including experts in gene therapy, embryonic and adult stem cell culture, animal models and regenerative medicine.

The main objectives put forward in the preliminary organisation of the **EuroSTELLS Workshop** "**Challenges in Stem Cell Differentiation and Transplantation"** were the following: i) to get an updated snapshot of stem cell biology and differentiation, ii) to see whether the current state of art of gene therapy can complement stem cell mediated therapy, iii) to re-evaluate the importance of large animal models for stem cell replacement, and to stress on the urgent need for the establishment of embryonic stem (ES) cells from large animals, iv) to bring robust examples on the efficiency of stem cell mediated therapy in preclinical and clinical settings, and v) to encourage interaction and synergy between EuroSTELLS participants, particularly amongst young scientist (Ph.D. students and post docs).

Target audience and participants

The workshop was open to all members of the 21 EuroSTELLS laboratories both at the senior and junior level including Ph.D. students, and to additional researchers interested in the field who had contacts with the EuroSTELLS laboratories. There were in total 89 participants of which two were from the ESF, 1 was a science writer journalist, 62 were outside invited speakers or EuroSTELLS researchers. Among these 62, eleven countries were represented including Sweden (1), United Kingdom (7), Italy (30), Netherland (4), France (4), Finland (1), Czech Rep (3), Iceland (7), Denmark (2), USA (2) and Israel (1).

Conference report

As part of the networking activities of EuroSTELLS, two previous meetings were held in 2006 (General Biology of Stem Cell Systems, 19-21 March, Venice, Italy) and 2007 (Exploring Chromatin in Stem Cells, 22-24 January, Montpellier, France). They were mainly devoted to some basic aspects of embryonic and adult stem cells. The present meeting was planned to be complementary to the previous ones, focusing on more clinically oriented and practical applications. Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system, they can theoretically divide without limit to replenish other cells and tissues. When a stem cell divides, each new cell has the potential to either remain a stem cell or to become another type of cell with a more specialised function, such as a muscle cell, a red blood cell, or a brain cell. As a result, stem cells have the potential to provide cures or new treatments for many diseases and injuries, including cancer, diabetes, multiple sclerosis, Parkinson's disease, osteoporosis and spinal cord injury. However, to develop strategies for stem cell based therapies, the basic knowledge on stem cell biology needs to be improved.

The theme of regenerative medicine was introduced by a plenary lecture given by **Professor Inder Verma**, who is a pioneer of gene therapy. He gave an overview of the history of gene therapy and of the problems encountered and discussed the potential of cell therapy alone or combined with gene therapy.

Session I: Embryonal Stem Cells: Differentiation Potential and Therapeutic Prospectives

After the plenary lecture and the coffee break, Session I, entitled "Embryonal Stem Cells: Differentiation Potential and Therapeutic Application", was introduced by the chairperson **Dr. Josef Fulka** (Institute of Animal Science, Prague, Czech Republic, EuroSTELLS). For any prospective study of differentiation and/or transplantation it is necessary to have cells with the right characteristics to work with. The source is important with the view of having different options available depending on the context and the application. The culture conditions and the scale up required for any differentiation or transplantation work are only at an early stage of development and although there are interesting reports, much work needs to be done including production under good manufacturing procedures (GMP) procedures.

The first speaker, **Dr. Keith Campbell** (University of Nottingham, UK, EuroSTELLS) discussed the reprogramming of somatic nuclei into a totipotent state. So far, this has been obtained by the means of somatic cell nuclear transfer (SCNT) as demonstrated by the birth of live animals after transfer of SCNT derived embryos. The mechanism by which this is happening is being unravelled at present and it is known that reprogramming does not involve changes in DNA sequences but rather epigenetic modifications.

This is very important for the production of stem cells from differentiated tissues. It is clear now that the proteins contained into a mature oocyte fulfil all the requirements to re-programme the genome of a somatic cell. However the paucity of human oocytes will make the development of this route unlikely unless different sources of these proteins are found or if manipulations of somatic cell in culture may make this happen. Dr. Campbell reviewed the two approaches currently being developed in his laboratory. He has shown that exposure of somatic nuclei to the cell extract of amphybia is able to alter gene expression profile in somatic nuclei, therefore facilitating in principle the reprogramming at least when such nuclei are used for SCNT. A second approach to facilitate reprogramming of sheep somatic nuclei is the exposure of reconstructed SCNT embryos prior to activation to 10mM caffeine. The gene expression pattern of such embryos is more similar to that of normal embryos; moreover following transfer to recipients this has resulted in reduced loss and better offspring survival. This effect is apparently due to the activity of MAP kinases enhanced by the presence of caffeine.

Dr. Hannu Sariola (University of Helsinki, Finland, EuroSTELLS) presented "Regulation of stem cell fate by GDNF". In his presentation, Dr. Sariola described the importance of glyal derived neurotrophic factor (GDNF) for spermatogonial development and the signalling pathways activated by GDNF, including a cross-talk with c-Met. GDNF knockout mice (KO) have impaired kidney development. The effect of GDNF to maintain long term cultures of spermatogonial stem cells is dose dependent. Lithium and a small molecule glycogen synthase kinase-3 (GSK-3) inhibitor (BIO) have the same effect on spermatogonial maintenance, although lithium is more toxic.

Professor Joseph Itskovitz-Eldor (Rambam Medical Centre, Haifa, Israel) gave the last presentation in the session addressing the derivation and culture of human embryonic stem cells (hESCs) in defined conditions. hESCs derived from blastocysts are pluripotent cells capable of differentiating into representative cells of all three germ layers of the embryo and eventually every tissue of the human body. This feature places them as best candidates for tissue repair and regeneration. To achieve this objective an unlimited supply of cells cultured in defined and controlled conditions is required. In the first part Professor Itskovitz-Eldor described various sources and methodologies for the derivation of hESCs. In the second part he described culture conditions that can support the derivation and maintenance of hESCs under serum free and feeder free systems in media supplemented with leukemia inhibitory factor (LIF), transforming growth factor-b (TGFb), basic fibroblast growth factor (bFGF) and serum replacement on a substrate of fibronectin. He is developing a suspension culture system in presence of interleukin 6 receptor (IL6R). Cells grow in aggregates that are mechanically disrupted for expansion of the culture. After 10 days of suspension culture apoptosis appears in the centre of the aggregates. These cells can be re-plated for adherent culture. Addition of PDGF induces differentiation towards smooth muscle actin (SMA) cells, following over 20 passages there is the formation of mesenchymal stem cells (MSC) that express CD105, CD166, CD44, etc. When transplanted to Severe Combined Immunodeficiency (SCID) mice after eight weeks there is the formation of bone and cartilage. This platform technology will make available an unlimited supply of cells for therapy and for industrial applications.

Session II –Large Animal Models

The objective of Session II -"Large Animal Models"- chaired by **Dr. Pasqualino Loi**, (University of Teramo, EuroSTELLS) was to provide a snapshot of the current state of art of the techniques for isolating embryonic stem cells from large animal embryos. Among the speakers allocated to the session, Professor Cesare Galli, and his group, has long dealt with the establishment of totipotent cell lines from ruminant embryos, particularly bovine. The characterisation of adult, multipotent mesenchymal cells was also addressed in the session.

The first talk "Culture and Differentiation of Large Animal Stem Cells**"** presented by **Professor Cesare Galli** (Spallanzani Institute, Cremona, Italy, EuroSTELLS), conveyed the main message that *bona fide* embryonic stem cells are still lacking in large animals (sheep, bovine, pig, and horse to some extend). Despite the large availability of in vitro produced embryos in those species, the inner cell mass cells tend to differentiate after several passages, and none of the protocols established in mice and human ESC makes a difference. This clearly indicates that the signalling pathways necessary to retain a full totipotency of embryonic cell lines are different between species. However, an important finding presented is that the use of culture conditions that favour neural differentiation allow the derivation of neural precursor cell lines from bovine and ovine embryos. In addiction, with an eye to "therapeutic cloning", bovine neural precursor cell lines from nuclear transfer embryos were obtained with the same efficiency of normal embryos. These neural cell lines retained a multipotent potential, being able to differentiate into neural crest derivatives including smooth muscle and cartilage. The second speaker, **Dr. Irena Vacková**

(Institute of Animal Science, Prague, Czech Republic and the Center for Cell Therapy and Tissue Repair, Charles University Prague, Czech Republic, EuroSTELLS), with the talk "Porcine embryonic stem cells: advances and problems" reported 5 years of work on the establishment of totipotent cell lines from pig embryos. She presented her attempts to establish totipotent cell lines from pig blastocyst, and the main finding was essentially a confirmation of Galli's talk, further complicated by that *in vitro* embryo embryology in pigs is less reliable than other large animals, like sheep and cattle. Dr.Vackova's future plans include the establishment of a sort of "conditioned" totipotent embryonic cells through the use of Wnt-catenin pathway inhibitor, like the small molecule GSK-3 inhibitor (BIO). BIO has been produced in her lab, the Institute of Animal Science in Prague, and is currently being tested on pig epiblast cells.

The fundamental importance of large animal as a safety and efficiency pre-clinical controls before the clinical application of stem cell therapies was highlighted in the last talk "Cardiac commitment of embryonic stem cells: experimental cell therapy in large animal models of myocardial infarction" given by **Dr. Michel Pucéat** (Université d'Evry, Inserm, Evry, France). Dr. Pucéat reported on xenografting of bone morphogenetic protein 2 (BMP2)-treated murine ESC genetically modified to express both β-galactosidase and enhanced yellow fluorescent protein (eYFP) under transcriptional control of Nkx2.5 and β-actin in infarcted sheep myocardium. The results in term of re-colonisation of the inducted lesion by the transplanted cells were striking; remarkably, the xenograft was tolerated by the recipient sheep even in the absence of immunosupression of the host[2]. This important achievement further stressed the importance of large animal models for human stem cell transplantation.

Session III: Cell Signalling Pathways

The first day of the meeting ended with Session III entitled "Cell Signalling Pathways". **Dr. Thorarinn Gudjonsson** (University of Iceland, EuroSTELLS) chaired the session which included two talks on molecular regulation of tissue stem cells. Signaling pathways and transcription regulation are key players in stem cell regulation. It is the fine tuning of transcription regulation that determines the fate decision in different stem cell system. In recent years we have witnessed how complex stem cell regulation is and that any imbalance in this regulation can easily facilitate cancer growth. In this session two prominent scientists, **Dr**. **Eirikur Steingrimsson** (University of Iceland) and **Professor Tariq Enver** (The Weatherall Institute, UK, EuroSTELLS), reviewed their work on melanocyte stem cells and hematapoietic stem cells, respectively.

The first speaker of the session, Dr. Steingrimsson, discussed the role of *microphthalmia* associated transcription factor (*Mitf*) gene in melanocyte fate decision. Multiple lines of evidence suggest that the activity of Mitf in melanocytes and melanocyte stem cells is regulated by signaling mechanisms and post-translational modifications resulting in effects on transcription regulation. Dr. Steingrimsson's group applied bacterial artificial chromosome (BAC) transgene

[2] Tomescot A., *et al.* Differentiation in vivo of cardiac committed human embryonic stem cells in postmyocardial infracted rats. Stem Cells, 2007, Sep (25)9, 2200-5.

rescue experiments to investigate the roles of these signaling pathways and post-translational modifications in vivo to determine if, when and where they play a role. BAC recombineering was used to mutate the post-translational modification sites of Mitf in a BAC clone and transgenic mice generated on a *Mitf* mutant background; the BAC clone contains the entire *Mitf* gene, except exon 1A, and was able to rescue the phenotype of the *Mitf* $^{mi\text{-}vga9}$ loss-of-function Mitf mutation. The phenotypes of mice carrying mutant BACs were reported. Studies in Dr. Steingrimsson lab provide new insights into the mechanism of transcriptional regulation by Mitf and will address the role of signaling in melanocytes and melanocyte stem cells.

The second speaker of the session, Professor Enver, discussed the molecular regulation of hematopoietic and leukemic stem cells. Clinically successful hematopoietic cell transplantation is dependent on hematopoietic stem or progenitor cells. One of the major problems in transplantation is the lack of methods to expand hematopoietic stem cells in vitro. Professor Enver and his group have identified a protein called Nephroblastoma overxpressed (Nov) as an essential factor for hematopoietic stem cell expansion. Using umbilical vein cord blood they have shown that knockdown of Nov gene expression in CD34 positive cells abrogated their function in vitro and in vivo. Interestingly, forced expression of Nov or addition of recombinant protein enhance hematopoietic stem cell activity. His work provided strong evidence that Nov is a key player in molecular regulation of hematopoietic stem cells.

Session IV. Stem Cell Differentiation for Therapeutic Aims

The second day of the meeting, chaired by Dr. Campbell, started with Session IV entitled "Stem Cell Differentiation for Therapeutic Aims". Amongst the most studied stem cells there are the neural stem cells both of adult and embryonic origin. The great interest in neural stem cells originates from the devastating degenerative diseases that the loss of such cell types causes in humans. The nervous system is composed of many specialised cell types originating from the same precursor but following many different pathways leading to complex differentiation pathways. Some of the common pathways have already been unravelled in both embryonic and somatic stem cells.

The first speaker of the session, **Professor Ernest Arenas** (Karolinska Institute, Sweden, EuroSTELLS), presented new data on interaction between Wnts, Dkks and the receptor components frizzled and LRP with a focus on precursor development in the ventral midbrain[3]. Current therapeutic strategies aim at differentiating stem cells into dopaminergic neurons for transplantation or for drug development and testing. A particular focus of the presentation was on the consequence of the dose of Wnt 3a, and Wnt 5a on the activation of canonical versus non-canonical Wnt signaling. He suggested that low levels of Wnt 3a lead to a direct activation of canonical Wnt signaling through LRP, while high levels of Wnt 3a activate canonical Wnt signaling in a process that requires the LRP and frizzled receptor, and Dsh. In contrast high levels of Wnt 5a would recruit frizzled to a speculative receptor2 that retains Dsh from activating canonical Wnt signaling. These factors are currently being applied to diverse type of stem cells including neural stem cells and embryonic stem cells.

[3] Bryja V., *et al.* Wnt-5a induces Dishevelled phosphorylation and dopaminergic differentiation via a CK1-dependent mechanism. Journal of Cell Science, 2007, 120, 586-95.

The second speaker of the session, **Dr. Lorenz Studer** (Sloan Kettering Institute, New York, USA), discussed the applications of hESCs in neural development and disease. He presented data on a new technique based on BAC transgenesis to genetically mark and purify specific neural derivatives from mouse and human ESC. Other techniques critical in the human ESC field include the use of highly purified cell populations in high-throughput screening (HTS) assays to systematically identity compounds that affect stem cell fates. He provided an example on how human ES cells can be adapted to an HTS platform and how such assays could be developed in the future for drug discovery. Dr. Lorenz identified and characterised an early neural stem cell stage termed rosette neural stem cells (R-NSCs) derived from human or mouse ESCs that is susceptible to developmental patterning cues. His data suggest that R-NSCs have specific signaling requirements and genetic make up compared with developmentally later more restricted NSC stages. Applications based on the isolation, patterning and differentiation of R-NSCs include the derivation of midbrain dopamine neurons and spinal motorneurons for the treatment of Parkinson's disease or ALS respectively. All together these two presentations are leading the way towards translational research.

Session V: Adult Stem Cell Therapy

In Session V, chaired by Professor Dirk de Rooij (Center for Reproductive Medicine, Academic Medical Center, Amsterdam, The Netherlands, EuroSTELLS), the meeting started examining the possible consequences of adult stem cell technology for clinical applications. **Dr. Marina Cavazzana Calvo,** (Necker Hospital for Children, Paris, France), discussed the usefulness of bone marrow transplantation (BMT) in primary immunodeficiencies (P.I.). P.I. are among the genetic diseases which benefit most from BMT, and the Necker Hospital has a large series of P.I. patients and has contributed towards the identification of many genes responsible for these pathologies. She reported that BMT is an already established and widely accepted procedure which is routine for patients having an at least partially matched donor. As pointed out by Professor **Verma** in the discussion, the French group was the first to obtain good results in a gene therapy trial involving human subjects, although the appearance of a few patients who developed leukaemia as a consequence of viral vector integration in the proximity of an oncogene is a major problem to solve.

Professor Michele De Luca (University of Modena and Reggio Emilia, Italy) reported on the usefulness of adult stem cells in two tissues, the cornea and the skin. It is now possible to produce an entire cornea from a thin rim of stem cells by growing these cells in vitro. Likewise, it is possible to growth large pieces of skin in vitro. This ability could also form the basis for gene modification of human skin stem cells. Professor De Luca discussed at length the only patient for whom he has received the authorization to treat with gene transfer technology[4]. This patient was affected by a mutation in a gene encoding the basement membrane component laminin 5 (LAM5), which causes junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder. Epidermal stem cells from an adult patient affected by LAM5-beta3-deficient JEB were transduced with a retroviral vector expressing LAMB3

[4] Mavilio F., *et al*. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nature Medicine, 2006, Dec 12(12), 1397-402.

cDNA (encoding LAM5-beta3), and used to prepare genetically corrected cultured epidermal grafts. Nine grafts were transplanted onto surgically prepared regions of the patient's legs. Engraftment was complete after 8 d. The grafts showed a better morphology than the homologous region on the other leg and survived for more than one year (so far). These data suggest that growing skin stem cells ex vivo could be a useful technique which allows in principle the implementation of both cellular and gene therapy approaches.

Session VI: Practical Applications

Session VI was divided into two parts. In the first, chaired by Professor Arenas, **Dr. Luigi Naldini** and **Dr. Alessandra Biffi** (Telethon Institute for Gene Therapy, TIGET of Saint Raphael Hospital, Milan, Italy) reported on their results obtained in animal models of metachromatic leukodystrophy. This is a rare, fatal, inherited, autosomal recessive, lysosomal storage disorder, characterised by severe and progressive demyelination affecting the central and peripheral nervous systems. It is caused by the deficiency of the lysosomal enzyme arylsulfatase A (ARSA) and can be considered a lysosomal storage disorder with predominant neurological involvement. The disease is characterised by myelin degeneration in both the CNS and peripheral nervous system, associated with the accumulation of sulfatide in glial cells and neurons. Despite the fact that the enzymatic deficiency is systemic, disease manifestations are restricted to the nervous system. Children affected by MLD display progressive neurologic symptoms, including ataxia, seizures, and quadriplegia, culminating in decerebration and eventual death early in infancy. Since the most affected cells seem to be in the microglia compartment, reconstitution by hematopoietic stem cells (HSCs) has been suggested as potentially beneficial. Dr. Naldini and Dr. Biffi demonstrated extensive reconstitution of well-differentiated microglia in the CNS by the transgene-expressing progeny of transplanted HSCs in the mouse *Arsa*$^{-/-}$ knockout model. Taking advantage of lentiviral vectors to achieve efficient gene transfer into HSCs and long-term overexpression of the ARSA gene in their cellular progeny, they were able to prevent the development of major disease manifestations in mice treated at the presymptomatic stage. In addition, they showed that complete normalisation of established behavioral abnormalities and neuropathological alterations of *Arsa*$^{-/-}$ mice can also be obtained after HSC gene therapy. Thus, these results pave the way for future practical applications in humans. In a further methodological development, Dr. Naldini reports on a novel viral vector based on the new technology of the zinc finger approach to target vector insertion at specific, predetermined genome sequences, which could lead to precise integration of the vector in the mutated gene with high efficiency. Importantly, these vectors are able to confine transgene expression between closely related states of therapeutically relevant cells (dendritic cells, hematopoietic and embryonic stem cells), and their progeny.

Dr. Maddalena Mastrogiacomo (University of Genova, Italy) focused on bone reconstitution. She tested resorbable porous ceramic constructs, based on silicon-stabilized tricalcium phosphate (Si-TCP), by implanting them in critical-size defects of sheep tibias, either alone or after seeding with bone marrow stromal cells

(BMSCs)[5]. Only BMSC-loaded ceramics displayed a progressive scaffold resorption, coincident with new bone deposition. With increasing implantation time, scaffold thickness significantly decreased while bone thickness increased. MicroCT data evidenced that all scaffolds showed a uniform density distribution before implantation. Areas of different segregated densities were instead observed, in the same scaffolds, once seeded with cells and implanted in vivo. A detailed microX-ray diffraction analysis revealed that only in the contact areas between deposited bone and scaffold, the TCP component of the biomaterial decreased much faster than the hydroxyapatite (HA) component. This event did not occur at areas away from the bone surface, highlighting coupling and cell-dependency of the resorption and matrix deposition mechanisms. Moreover, in scaffolds implanted without cells, both the ceramic density and the TCP:HA ratio remained unchanged with respect to the pre-implantation analysis. Histology confirmed a better integration between new bone and scaffold in the Si-TCP composites in comparison to 100% HA composites where new bone and scaffold phases remained well distinct. Therefore Si-TCP could be a more suitable scaffold for bone regeneration.

In the second part of Session VI, chaired by Dr. Magnus Karl Magnusson (Landspitali-University Hospital, Iceland, EuroSTELLS) further practical clinical applications were discussed. **Dr. Gianvito Martino** (Stem Cell Research Institute, Saint Raphael Hospital, Milan) analysed some issues in adult neural stem cell biology and their use for clinical applications, with particular focus on multiple sclerosis[6]. Adult multipotent neural stem/precursor cells (NPCs) have the capacity to self-renew and generate functional differentiated cells (e.g. neurons, astrocytes or oligodendrocytes) within discrete tissue-specific germinal niches, such as the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus. Due to their intrinsic plasticity, NPCs can be considered an essential part of the cellular mechanism by which the central nervous system (CNS) tries to repair itself after an injury and, as a consequence, they also represent an attractive therapeutic tool for the treatment of neurological disorders. Recent evidence shows that transplantation of neural stem/precursor cells may protect the central nervous system from inflammatory damage through a 'bystander' mechanism that is alternative to cell replacement. This novel mechanism, which might improve the success of transplantation procedures, is exerted by undifferentiated neural stem cells, the functional characteristics of which are regulated by important stem cell regulators released by CNS-resident and blood-borne inflammatory cells. The author discussed at length this alternative bystander mechanism. He concluded that, in his opinion, pilot clinical studies of neural cell administration in multiple sclerosis are warranted.

Dr. Giulio Cossu (Telethon Institute for Stem Cell Research, Saint Raphael Hospital, Milan, Italy) reported on his studies on cell therapy for muscular dystrophy[7]. It is a severe genetic disease including more than 20 different forms, among which the most

[5] Marcacci M., *et al.* Stem cells associated with macroporous bioceramics for long bone repair; 6- to 7-year outcome of a pilot clinical study. Tissue Engineering, 2007, May (13)5, 947-55.
[6] Pluchino S., *et al.* The therapeutic plasticity of neural stem/precursor cells in multiple sclerosis. Journal of the Neurological Sciences, 2008 Feb 15;265(1-2):105-10. Epub 2007 Aug 17.
[7] Morosetti R., *et al.* Isolation and characterisation of mesoangioblasts from facioscapulohumeral muscular dystrophy muscle biopsies. Stem Cells, 2007, Dec; 25(12):3173-82. Epub 2007 Aug 30.

known is Duchenne's dystrophy. In the last few years Dr. Cossu's group has reported that bone marrow contains progenitors able to differentiate into skeletal muscle following bone marrow transplantation into lethally-irradiated recipient mice. They identified cells that are physically associated with the embryonic dorsal aorta in avian and mammalian embryo and can grow extensively in vitro. They termed these cells "mesoangioblasts". When transplanted in vivo, mesoangioblasts give rise to multiple differentiated mesoderm phenotypes such as smooth and skeletal muscle, cartilage and bone. Their ability to extensively self-renew in vitro, while retaining multipotency, qualifies mesoangioblasts as a novel class of stem cells. The fact that mesoangioblasts emerged as an unexpected source of progenitors for skeletal muscle and a variety of other mesoderm-derived tissues, made them suitable for trials of cell therapy in animal models of muscular dystrophies. Indeed, when both wild type or dystrophic, genetically corrected mesoangioblasts were delivered intra-arterially to dystrophic muscle of gamma-sarcoglycan knock out mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype. Interestingly, when donor mesoangioblasts under various regimens of immune suppression were injected in a large animal (dog) model of muscular dystrophy, a great improvement in symptomatology and survival was obtained. These data constitute strong evidence for a potential usefulness of mesoangioblast to treat human dystrophies. As a result, Dr. Cossu's team has applied for their use in humans.

Poster session

The posters (19) were displayed for the duration of the conference and visited by the participants in a dedicated poster session and during coffee breaks. The topics of the poster covered all the 3 EuroSTELLS Collaborative Research Projects (CRPs) and presented preliminary findings of the work funded in EuroSTELLS. They covered several clinically oriented studies together with some investigations on the ability to differentiate embryonal stem cells into tissues, with particular emphasis on neural stem cells.

Outcome of the conference

It can be concluded that that all the aims of this mutidisciplinary workshop have been successfully achieved. The first, and still, major players, of human stem cell isolation, biology and clinical application, were successfully brought together: Professor Itskovitz- Eldor; the gene transfection/therapy pioneer Professor Inder Verma; and Dr. Keith Campbell, the scientific father of the first cloned animal, which actually opened up the way for stem cell transplantation therapy.

The workshop has actually been a forum where basic science on stem cell biology and gene targeting was closely paralleled by research carried out in preclinical and clinical settings, with large animal models playing a major role. The scientific content of the talks was outstanding, with a crescendo of breathtaking communications until the very end of the meeting. Many innovations and advancements in the field of embryonic and adult stem cell mediated therapy were presented in the conference. From the scaling up of human ESC culture through suspension culture (Professor Itskovitz-Eldor), which is a fundamental step to provide enough cells to be grafted; to the remarkable progress achieved in the design and production of the third generation

lentiviruses for gene therapy, which are able to be integrated into proliferating and quiescent cells, into most majors organs (Professor Verma). A robust example on the application of lentivirus mediated therapy was provided by Dr. Naldini and his co-worker Dr. Biffi. Mouse models of metachromatic leukodystrophy (MLD) treated with haematopoietic stem cells transfected with a lentiviral vector coding for the missing enzyme (arylsulfatase A) did not develop clinical symptoms. These highly positive results have prepared the path for the first clinical trial in MLD, which will start in a few months. Dr. Naldini's group also presented a revolutionary approach to target specific cells through the generation of miRNA responsive vectors, which should allow safer and more effective applications of gene/stem cell based therapy.

Other strong examples of overlap between the basic and applied research presented at the meeting were the communications of Professor Arenas on dopaminergic differentiation of neural stem cells, and the following given by Dr. Martino on the therapeutic plasticity of neural stem cells. The talk given by Professor Arenas reported on five years of studies devoted to understanding the molecular players which drive differentiation of dopaminergic neurons during brain development in the fetus. Members of the Wnt family of proteins were found to have a critical role in dopaminergic neuron development, implying positive effects of the use of these factors in neural stem cell differentiation for therapy. Dr. Martino extended these studies further giving sound evidence of the therapeutic potential of neural stem cells in clinical application. Dr. Martino presented the evidence that inflammation in MS is aimed not only at destroying and phagocytosing damaged cells, but also at promoting tissue regeneration or tissue repair via scar formation.

Two more reports used large animal models for cell transplantation therapy, both with extraordinary outcomes; Dr. Mastrogiacomo's presentation on engineered scaffolds on bone marrow stromal cells; and the talk by Dr. Cossu on the repair role of mesangioblasts in muscular dystrophies. Dr. Mastrogiacomo described the effectiveness of new resorbable porous ceramic constructs, based on silicon-stabilised tricalcium phosphate, seeded with bone marrow mesenchimal cells, basically replaced major bone ablation in sheep tibias. Dr. Cossu provided evidence of an unexpected differentiation plasticity of mesoangioblasts, which were easily converted into skelectal muscle progenitors. Moreover, genetically corrected dystrophic mesangioblasts delivered intra-arterially to dystrophic muscle of β-sarcoglycan KO mice induced a significant ameliorement.

Finally, two practical and effective examples of adult stem cell therapy were brought by Dr. Cavazzana Calvo who presented a multicentric clinical trial on bone marrow transplantation in primary immunodeficiencies; and by Professor De Luca, who described impressive clinical cases based on the use of human epithelial stem cell for the treatment of severe corneal degeneration. Other topics covered by the EuroSTELLS and external invited speakers were nuclear reprogramming, contributed by Professor Campbell (EuroSTELLS), and mechanism of self renewal of spermatogonial and melanocyte stem cells (Professor Sariola, and Dr. Steingrimsson). All these talks were of the same, excellent standard, as detailed in the report of the single sessions.

Overall, the outcome of the conference has been highly positive, as also indicated by the comments of EuroSTELLS members and importantly external speakers and participants. A further positive note was the opportunity the workshop offered to enforce the co-operation between EuroSTELLS participants, and to stimulate the establishment of new collaborative links in transversal areas of common interest, particularly between young scientists.