# 1. PUBLISHABLE SUMMARY

## 1.1. Project Objectives

The **overall objective** of OptiStem is to leverage European scientific excellence through a collaborative project in order to develop new strategies that will enhance the efficacy of clinical trials with adult, tissue stem cells for degenerative deseases of epithelia and skeletal muscle. This synergistic approach will ensure that ongoing and future clinical trials have a greater chance of success. To this end, key actions have been identified :

1. Phase I and potentially Phase IIa cell therapy trials for patients with muscular, skin or ocular disorders
2. In-depth optimisation of cell therapy studies in large animal models
3. Systematic analysis of stem cells fate in these large animals to address *(i)* differentiation, *(ii)* molecular control and *(iii*) transplantation efficacy
4. Identification of novel therapeutic strategies based on clinical trials results and small animal modeling
5. Characterisation of *(i*) regulatory, *(ii)* transcriptional and *(iii)* signalling pathways that control stem cell activity *in vivo*
6. Stimulation of tissue regeneration through activation of angiogenesis, a key success factor in efficient cell therapy
7. Immunological studies both *in vitro* and *in vivo* in order to determine *(i)* any adverse or beneficial effect and *(ii)* factors that can modulate the immune response.

To achieve these stated goals, the programme of work focuses on six interrelated projects in which the partners bring in specific expertise and resources which are collectively leveraged.

The primary focus is on clinical trials (CT) with epithelial and muscle stem cells. CT represent the core of the OPTISTEM project : they will benefit from discoveries during preclinical trials in large animals and their results will lead to the identification of new therapeutic approaches through further studies in small animals. These clinical trials will move towards the treatment of *(i)* muscular dystrophies using allotransplantation of normal mesoangioblasts and intra-muscular transplantation of systemically deliverable stem cells, *(ii)* ocular disorders through an innovative, simultaneous transplantation of corneal and conjunctival cells and *(iii)* skin stem cells deficiencies *via* transplantation of cultured oral mucosal stem cells.

The second focus is on preclinical large animal models. It relies on results produced in small animals and constitutes a key translational interface between stem cell biology and the implementation of clinical protocols. In this regard, dystrophic dogs will be used to test long-term efficacy of wild-type and dystrophic mesoangioblasts transplantation using *(i)* lentiviral vectors or *(ii)* engineered small nuclear RNAs. Pigs will also be used as a model in experimental ophthalmology and we will develop single cell analysis and transplantation of transduced stem cells.

The third project focusses on developing mouse models for cell therapy including immune-deficient animals for human cell transplantation. The injection of human keratinocytes into immune-deficient mice will provide a quantitative assay for the effects of different signaling pathways on epidermal lineage selection. These xenografts of modified human epidermal cells will be used in conjunction with different transgenic mice in order to *(i)* modulate the Notch pathway involved in determining epidermal or hair follicle fate in the skin and *(ii)* to define the optimal conditions of neo-dermis formation.

The fourth focus is on the factors that control stem cell activation and renewal at a mechanistic level. Using cutting-edge molecular and cellular approaches we are studying the different signalling pathways and transcription factors that control *(i)* satellite cell behaviour and repair of skeletal cell muscle, *(ii)* muscle interstitial cells and *(iii)* mesoangioblasts.

The fifth project relates to tissue remodelling and engraftment of stem cells. Besides cells, modifications of the muscle environment is crucial for the successful engraftment of donor cells. Here we shall aim to *(i)* unveil the mechanisms that control the induction of angiogenesis and permeability *(ii)* increase stem cell homing through pharmacological approaches and *(iii)* improve fiber survival by reducing fibrosis using plasminogen activation and pharmalogical depletion of fibrinogen.

Finally, we cover the immunological aspects that result from stem cell engraftment. The regulation of the immune response is a key success factor for stem cell therapy. Here we shall determine *(i)* to what extent mouse and human stem cells, prior or after manipulation, elicit or not an immune response, *(ii)* how this response is modulated and *(iii)* how we can induce tolerance.

### 1.2 Work performed since beginning of the project

The work performed since the beginning of the project covers all the areas mentioned above. The first clinical trial started in June 2009 while preclinical studies have already produced their first results. It is important to mention that about 30 patients suffering from Duchenne Muscular Dystrophy have been enrolled in this preliminary study.

We are producing the tools that will allow us to study the mechanisms that control stem cell activation and differentiation. To this end, about ten new mouse lines are being set alongside with six new cell lines. First results have already been produced and will lead to promising new developments that will, after regulatory review, help the optimisation of stem cell therapy. For example, we identified a micro RNA as a regulator of stem cell differentiation and we have identified a new bipotent stem cell population that can be purified from any tissue which opens perspectives that go even beyond the scope of the project since they might benefit to the entire regenerative medicine field.

### 1.3 Main results achieved so far

A preliminary study for children affected by Duchenne muscular dystrophy started in June 2009 with 30 DMD patients and 30 individuals acting as controls. HLA-matched donors have been identified for six patients and three of these will receive mesoangioblasts cell therapy likely at the end of this year. In parallel, mesoangioblasts have been produced on large-scale under cGMP conditions for this clinical trial. We have also initiated, ahead of schedule, the preparation of the clinical trials for ocular, urethral and oral epithelial reconstruction.

We have expanded and immortalised human and canine, normal and dystrophic mesoangioblasts carrying a vector for mini-dystrophin. Preliminary results indicate that all immortalised cells, even with active telomerase and Bm-1 did not produce tumors in immune-deficient mice and gave rise to dystrophin positive fibers in dystrophic dogs. This is very important for future clinical translation.

Using preclinical large animal models, we have genetically modified endogenous stem cells, isolated from muscle biopsies of two dystrophic dogs. The modified stem cells produced dystrophin and, when transplanted back into the two dogs, they improved muscle morphology and function.

We have tested the possibility of transplanting genetically corrected mesoangioblats from humans into Mdx-Scid mice. Although homing is good after one week, a macrophage-mediated response limits the number of surviving cells. We are currently running a series of pharmalogical treatments to limit macrophage activity. We have generated a set of double mutant mice and different cell lines that will be used to optimise this approach.

We have identified a new role for a transcription factor, Sox2, in epithelial stem cell mobilisation and have selected anti-inflammatory drugs that could improve stem cell homing.

Using a mouse model, we found that the Notch pathway in implicated in atopic dermatitis and in maintaining corneal integrity.

We have identified PICs (PW1+ interstitial cells) as novel bipotent resident stem cells in skeletal muscle. We have established that PICs are myogenic and recolonise the PIC niche in vivo. We have also generated a PW1-reporter mouse which can be used to purify the PW1+ cells from muscle.

We discovered a transcription factor (Nfix) that is a target of Pax7 and controls the switch from embryonic to fetal muscle formation.

A microarray analysis of the transcriptome of activated satellite cells has led us to the identification of microRNA27 as a regulator of Pax3 mRNA, which triggers its downregulation prior to myogenic differentiation.

Using isolated muscle fibers we are assessing the ability of NO-donors to enhance the number and activation state of satellite cells. Preliminary data suggest that, in differentiating myoblasts, NO is required for the expression of specific myogenic differentation markers. Further ongoing experiments will decipher the mechanisms by which NO controls myogenesis.

We have also initiated work aimed at identifying the targets of the transcription factor FoxN1 in regulating thymus development. We are currently using a combination of transcriptomics and ChIP approaches.

During this period we set up optimal conditions for cell transplantation in the context of increasing skeletal muscle angiogenesis. Promising results regarding the frequency of endothelial and hematopietic cells have been obtained. Preliminary results indicate that mobilisation and homing of the GFP-expressing cells in the skeletal muscle were improved when VEGF-C6 cells were also implanted.

Data in transgenic mouse suggest that the fibrin-depleting drug Ancrod does reduce collagen deposition in dystrophic mice.

Transplanted murine ES cells triggered a milder immune response compared to adult Langerhans islets, highlighting a relative immune privilege of an ES derived tissue upon implantation.

As an indication of the volume and quality of the output, partners of the consortium have published 29 peer-reviewed articles in international journals such as *Cell, Nature* and *PNAS.*

### 1.4 Expected final results & their potential impact and use

The proposed projects capitalize on combined expertise in different areas of regenerative medicine. The proposal involves collaborative interactions that allows us to merge our unique and complementary expertise in the field.

## Impact on science

We shall translate our expertise in stem cell biology into realistic strategies that will increase the efficacy of stem cell therapies. This project aims to develop and implement clinical trials for genetic and acquired diseases of epithelia and skeletal muscle, by transplantation of donor or genetically corrected, autologous adult stem cells. While transplantation of epithelial stem cells for skin and cornea are now routine clinical procedures, none of them is yet optimal as skin appendages are not restored and bilateral corneal damages cannot be treated. At the same time, a clinical trial with autologoous genetically modified stem cells has produced excellent results for a severe genetic disease (epidermolysis bullosa). In the case of muscle diseases, donor cell transplantation has produced encouraging results in a large animal, pre-clinical model of Duchenne muscular dystrophy (DMD); based on this result a first clinical trial is planned. The proponents of this proposal have pioneered this work and are world leaders in the field.

It is widely recognized that the efficacy of these trials relies on continuous optimization, based on in depth understanding of the biology of the stem cells used, of the pathology of the host tissue as well as the immune response against donor cells, viral vectors and transgenes. This project addresses coordinately all these issues, ranging from clinical experimentation, pre-clinical tests in large animals and in immune deficient mouse models, to cell biology of stem cells and targeted use of pharmacological tools. The project also addresses the biology of epithelial and skeletal muscle regeneration, as well as the control of angiogenesis, inflammation and immune response in the host. The expertise of the participant groups, the documented long lasting collaboration among most of them and their prominent position in the field guarantees high probability of success for this project.

## Economic benefits

By the end of next year the musculo-skeletal market globally is anticipated to reach € 262 million, while soft tissue and wound repair, for which epithelial stem cells are used is anticipated to reach greater than € 912 million by 2010.

In 1995, only 5 percent of companies involved in regenerative medicine research were based outside the U.S. By 2002, this percentage of non-U.S. regenerative medicine companies had increased to 46 percent. There are a total of 436 tissue-engineering related companies currently exist in the EU, indicating a strong commercial base, however due to disparities amongst the partner nations, national support programmes are limited.

OptiStem and its European partners will demonstrate a proof of principle through the interaction and integration of the fundamental, pre-clinical and clinical partners which will boost the European biotechnology industry, especially the SME sector, and contribute to developing standards and regulations in the area. Industry, particularly SMEs, will be kept routinely informed of progress and therefore play a significant part: this is specifically pertinent in paving the way for the application of the diversity if cellular based products in development in the diverse pathologies that can be targeted via cell therapy.

## Impact on society

Degenerative diseases create a life-altering experience for the person with injury, for their partner, parents, siblings, and children. The impact on and subsequent dimishment of body functions associated with the diseases can cause depression and loss of self-esteem. It has been considered essential, based on European policy consistent with human rights principles that people with disabilities should be treated with dignity, encouraged to have independence,

be given equality of opportunity, encouraged to have an active participation, a full citizenship and a high quality of life.

Given the diversity of degenerative diseases indicated above, pathological manifestation can occur at any age: either as a child, during an individual's most productive years, or as an aged person. In most cases, patients require continuous physical and medical care depending on the disease, severity of manifesation, degree of disability, and location of injury. The burden of caregiving most frequently falls on the partner. Care giving partners are often severely stressed, particularly due to health issues that arise after tissue degeneration initiates and suffer emotional stress that is comparable to or greater than those of the injured partner. Caregivers have a higher incidence of physical stress, emotional stress, burnout, fatigue, anger, and resentment.

Hope is considered an important coping strategy for both the person and family with degenerative diseases. Goal-directed hope based on realistic perceptions of life, focusing on progress, positive interpretation of events, are important in helping people and families cope with the disease. Hope is also focused towards the society at large, that new therapeutics are developed.

In addition to imposing direct medical costs on society, degenerative diseases also result in indirect costs, primarily related to reduced productivity due to disability with a further loss of self-esteem of the sufferer and diminished integration into society.

OptiStem aims to provide more than hope: we aim to provide validated cell therapies.

## 1.5 Project Contact Details and Logo

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