Project number.: **037479**

Project acronym: **MYOAMP**

Project full title: AMPLIFICATION OF HUMAN MYOGENIC STEM CELLS IN CLINICAL CONDITIONS

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

Life Science, Genomics and Biotechnology for Health

FINAL PUBLISHABLE SUMMARY REPORT

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Project coordinator name: **Vincent MOULY**
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DUCHENNE MUSCULAR DYSTROPHY THERAPY: MYO*AMP* A EUROPEAN FUNDED INITIATIVE TO DESIGN NEW CELL THERAPY APPROACHES

Started in December 1st, 2006 MYOAMP gathered cellular biologists, clinicians, and representatives of SME in a European project dedicated to the amplification of human myogenic stem cells in clinical conditions.

The MYOAMP participants together with the Patients Associations at the Monaco Kick-off meeting (January 12th, 2007)

Step by step towards clinical trials

In Europe over 30 000 young people, a number equivalent to the population of Monaco, are affected by Duchenne Muscular Dystrophy (DMD), either from genetic transmission of through new mutations (1/3 of the cases). Among the muscular dystrophies, the DMD has one of the most severe prognosis and finding a treatment to at least improve the quality of life of the affected children is a dramatic issue for the families.

It is a fact that the families are often desperate to see the last results obtained by researchers turned into more clinical trials to at least bring some improvement to their children's health. On the other hand, it is also true that despite this urgent need, families are also aware that designing a therapy is a difficult exercise which involves a lot of assessment and agreements from the regulatory authorities in which safety is the major keyword.

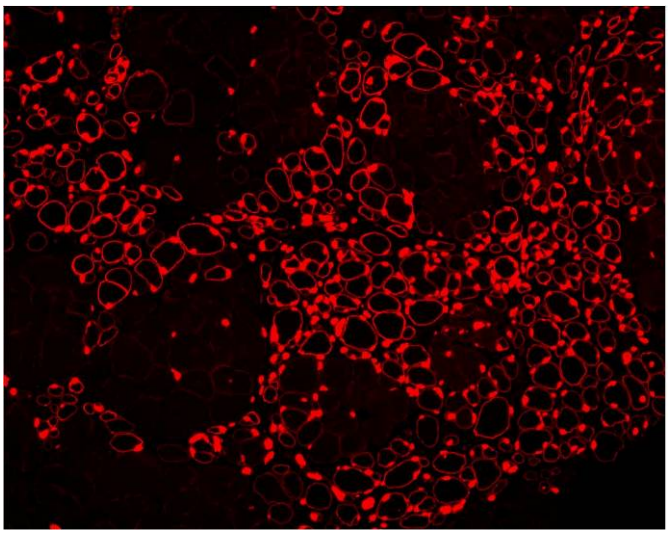
Many clinical trials using muscle progenitor cells have been developed in the past for DMD with very limited success. A solution may be found in combinations of new therapeutic approaches. From this perspective, MYO*AMP* intended to speed up the assessment of the potential of a new cell therapy approach and to set up the conditions for future clinical trials which could not be set-up within its budget and time limits.

By gathering the expertise of the partners, MYOAMP generated guidelines originating from concerted efforts and validated by both clinicians and European SMEs to facilitate the initiation of clinical trials involving stem cell therapy. Furthermore, the participation of clinicians and SMEs ensured that the guidelines generated by MYOAMP are complying with both clinical and economical conditions. The constant exchange with patients' associations allowed a rapid transfer of information to the European population concerned directly by MYOAMP efforts. Finally, the synergy established with other consortia, such as TREAT-NMD, an EU consortium, or ICE, an international consortium, maximized the outcomes of MYOAMP concerted efforts, and ensured that these efforts will be translated into clinical trials, some of them already being set up, and a collaboration between MYOAMP partners and these consortia, initiated within the frame of MYOAMP, is still ongoing.

MYO*AMP* novelty: coupling Exon-skipping to cell therapy

The recent emergence of new therapeutic venues, based upon post-transcriptional genetic corrections called «exon-skipping», or genetic surgery, has raised new hope for the DMD community. Using viral transfer approaches it has given very promising results but unfortunately cannot reach every muscle of the body and several injections would trigger an immune response to the vector. Autologous cell therapy may be used as a complement or alternative if the cell type used fulfilled the criteria of being an efficient vector and bringing a functional benefit to the diseased muscle.

Autologous muscle progenitors cannot be used since these are already defective in dystrophic muscle, while stem cells from other origins are ideal candidates, as long as their myogenic and proliferative potentials are ensured. In this perspective several cell types like mesoangioblasts, which have already been used in mouse and dog models of muscular dystrophy, and AC133 cells have a therapeutic potential for skeletal muscle, as demonstrated in the mouse within MYOAMP. An example of the result of implantation of AC133 cells into regenerating muscles of immuno-deficient mice is shown below to illustrate the myogenic potential of these cells.



MYO*AMP*, coordinated by Inserm and managed by Inserm-Transfert, synergized expertises from European leaders in their respective field to set up conditions for autologous transfer of human myogenic stem cells in GMP conditions for the treatment of DMD by exon-skipping. MYOAMP working plan included efforts so that these conditions and guidelines are transferred to SME and clinicians, defining efficient integration through dedicated partners within the 3-years duration of this project.

Paving the way to clinical trials

Very little was known about how the conditions required to amplify these stem cells isolated from humans can be set up in Good Manufacturing Practices (GMP) conditions, which is an essential step required before any clinical trial. Many groups have used animal models to investigate the possibilities of using autologous cell therapy for muscular dystrophies, but these data are dispersed, not always comparable and information regarding human cells is sparse. Moreover, little attention has been focused on the transfer of this knowledge towards applications for therapeutic trials.

The feasibility of autologous myoblast transfer therapy has already been demonstrated for cardiac repair, even if in cardiac therapy, injected cells were mainly used to counteract the development of fibrosis in patients devoid of any defect in skeletal muscle. The fact that pre-clinical trials developed in mouse models of muscular dystrophies have been successful as compared to clinical trials, which used mostly allogenic cells and resulted in very limited clinical benefit for the patients, illustrated the urgent need for pre-clinical studies using human cells.

MYO*AMP* addressed the question of the amplification of myogenic stem cells used in autologous cell therapy and their safety. The cells transduced with a lentiviral construct allowing exon-skipping have been selected through functionnal tests and further amplified. The safety and ethics have been defined through two specific workpackages at all steps of the amplification of the transduced cells. *In vitro* and *in vivo* approaches aimed at improving our understanding of the regulation of proliferation, while the culture conditions and effect of cytokines have been assessed, and telomere length measurements (reflecting the mitotic clock status of the cell) have been defined and are being adapted for SMEs.
The number of cells to be injected at each implantation, which is purely empirical in many clinical trials, has been tested and optimized in a model of implantation of human cells in immuno-deficient mice, in order to define the maximum number of cells to be finally amplified in GMP conditions.
The stability of the parameters initially examined in non-GMP conditions, have been checked through amplification in various conditions, to allow the definition of both guidelines for GMP production and key-parameters to be followed during the GMP amplification.
Therefore, MYOAMP succeded in defining conditions to isolate and amplify human myogenic stem cells to be used in future clinical trials in GMP conditions. Fuethermore, the constant collaboration between academic, R&D and SME partners ensured that these conditions comply with SME requirements, and an extensive market and patent analysis has been carried out within MYOAMP.

European added value

The main outcome of MYOAMP is the definition of protocols to obtain amplified human stem cells with an optimized efficiency for clinical trials.

In addition to basic knowledge on the amplification mechanisms, MYO*AMP* provided guidelines to obtain these cells in a reproducible and safe manner that can be directly transferred to clinical applications, and adressed technical, ethical and safety issues in a GMP environment.

To ensure a maximum success, MYO*AMP* partners have worked from the beginning in close contact with several patient associations including the Duchenne Parent Projects in France and United-Kingdom, the Association Monégasque Contre les Myopathies, and the Association Française contre les Myopathies. The end-users had thus the possibility to be the first to know about the progress made by the participants of MYO*AMP*. This coordination between patients association, scientists and clinicians was a key-effort to bring the expected results to the next step, convince the national health institutions of the necessity to support the clinical trials, and define the conditions for these trials. An international concerted effort, named ICE, was set up as a result of MYOAMP concerted efforts, and one of MYOAMP partners is setting up a clinical trial in Italy using the results generated by the MYOAMP consortium.

Key words: Human stem cells, Duchenne Muscular Dystrophy, neuro-muscular diseases, cell proliferation, cell therapy, exon-skipping

Coordinator :

Vincent MOULY
INSERM-UPMC
UMR S 787 Myologie
Institut de Myologie
105 bd de l'Hôpital
75634 - Paris Cedex 13 - FRANCE
Tel: 33 (0)1 40779635
Fax: 33 (0)1 53600802
Email: mouly@ext.jussieu.fr

Partners :

Luis GARCIA
INSERM- Institut de Myologie
Paris, FRANCE

Yvan TORRENTE
University of Milan
Milano, ITALY

Giulio COSSU
Fondazione Centro San Raffaele del Monte Tabor
Milano, ITALY

Jenny MORGAN, Francesco MUNTONI
University College, London
London, UK

Otto MERTEN
Généthon,
Evry, FRANCE

Mallen HUANG
3H Biomedical
Uppsala, SWEDEN

Stephan THOMA
Cellgenix Gmbh,
Freiburg, GERMANY

Séverine POUILLOT
Genosafe
Evry, FRANCE

Anton OTTAVI
INSERM-TRANSFERT
Paris, FRANCE