

**Innovative therapies for rare genetic diseases : the example of muscular dystrophies**

**Olivier Danos, CNRS UMR 8115, Généthon, Evry, France**

## **1. The Human Genome Project : return to the patients**

One of the immediately positive consequences of the Human Genome Project, ever since it started has been to bring hope to hundreds of thousands of patients with genetic diseases. This is far from being anecdotal, because it has taken many of these individuals out of oblivion, and has fundamentally changed their relationship with medicine, science and therefore with the society as a whole. Doctors and scientists, no longer turn away from these diseases in front of which they used to be desperately powerless. Before, they used to save their energy for problems they knew how to solve. Now, they can use the novel elements brought by the Genome databases to identify and design new therapeutic approaches.

The number of human diseases associated with single gene mutations, so-called Mendelian diseases, is over 3000 [1]. They affect less than 1 person in 100 000 for the rarest ones and up to 1 in 1000 for the most frequent. Collectively, they represent a real public health issue.

Twenty five years ago defective genes were identified in less than a hundred of these diseases.

The pace of discovery significantly accelerated in 1994-1995 when maps of the human genome became available. Today, around 1500 genes have been identified

(<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>) and most will certainly be discovered within the next five years.

The consequence of this new-found hope is a great demand from patients who have organized themselves into powerful organizations. These groups have become major players, along with governmental funding agencies and industries in funding and structuring genetic research towards a cure for their diseases. They are the strongest and most dedicated advocates of innovative therapeutic approaches based on the knowledge of genetics. They are important

stakeholders, participating to the ethical debate around these issues. Typically, their strategic choices are only made towards their ultimate goal of finding a cure. This strongly focused attitude can sometimes become in conflict with the choices made by government funding agencies and industries who also respond to other motives [2].

In the USA, several patient organisations including the Cystic Fibrosis Foundation and the Muscular Dystrophy Association, have been actively lobbying and funding research on gene discovery and gene therapy. This is not unusual in this country where the action of disease foundations have lead to spectacular cures in the past, as exemplified by the Polio vaccine which was developed with the support of the national March of Dimes campaign. In France, the action of the Association Française contre les Myopathies (AFM), has often clashed with the established practises and procedures of government funded research. Suddenly, patients collecting large sums of money with their yearly Telethon (over 100 Million Euros in 2004) have found themselves in a position where they could impact significantly on research policy. The concentration of research money on designated programs is often criticized in France as a short-sighted policy that carries the risk of penalizing less visible, but potentially productive, fields of research. A balanced repartition of funding across the various disciplines is undoubtedly an important mission for a governmental agency, but there is no reason why a patient group trying to solve its specific problem should do so. Critics of the charity-based research funding often wonder whether two state-run “public services”, the national television and the research institutions, should be harnessed by a self-promoted group of individuals, eventhough it is for a good cause. Yet, their problem with the way the charity money is allocated is based on a confusion between “public (tax) money” and money from the public.

The patient organisations need to promote two aspects of innovation. The first one is technical, with the goal of using new genome-based knowledge (but more generally each new

breakthroughs in biology, such as stem cells), to identify pharmacological targets and to develop therapies based on gene transfer and gene replacement (gene therapies). The second aspect is legal, regulatory and economic. The classical drug development pathway needs to be adapted to the situation of rare diseases. The Orphan Drug Act, a US law passed in 1983, was the result of intense lobbying by patient groups, and it now allows companies to develop treatments for very small markets and maintain a profit [3].

We focus here on the technical progresses that AFM and the Telethon have made possible. In 1992, scientists from the French public research organisations and AFM leaders created a special laboratory called Généthon. The first mission of this pioneer “genome centre” was to establish the physical and genetic maps of the human genome. It was successfully accomplished within three year and became the stepping stone for the Human Genome Project [4, 5]. The next step was to develop a Gene Therapy Program, still in association with public research, and focusing on gene transfer technologies. I will take examples from the research accomplished at Généthon, to illustrate how, starting from a problem that seemed insurmountable a few year ago, we now see the possibility of bringing treatments (if not yet full cures) to patients. Importantly, this research was undertaken in France and in the USA, only because patient associations have created a favourable environment for it, and not necessarily because it was based on the most timely scientific questions.

## **2. Gene therapy**

Gene therapy is a technology by which genes or small DNA molecules are delivered to human cells, tissues or organs to correct a genetic defect, or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases. Drug-based treatments typically modify components of the cell or its environment in order to induce a therapeutic response. In contrast, the purpose of gene therapy is to compensate a deficiency or avoid its pathologic

consequences by genetic reprogramming. This view, which has been proposed since the late sixties, is universal and simple. It can be applied to all fields of medicine, from sport injuries to neurodegenerative diseases and it initially generated a lot of optimism. High hopes were based on the development of efficient gene transfer technologies, on the promise of an abundant harvest from the Human Genome Project and on several spectacular proofs of principle obtained by researchers over the past twenty years. Not surprisingly however, numerous difficulties still exist to bridge the gap between those experiments and clinically relevant treatments for patients. Fifteen years after the first gene therapy clinical trials, only a handful of positive biological responses in human patients are documented, essentially in very rare genetic immuno-deficiencies, but also in hemophilia, vascular disease and cancer. A first set of limitations for the development of gene therapy is technological: efficacy and potency of gene transfer vectors, accessibility of the relevant cellular targets in the patient, vector manufacturing and toxicology. A second category of limitations stems from our incomplete knowledge of vector biology, of the consequences of gene transfer procedures on the target tissue homeostasis and of the immune response associated with the treatments. Yet, over the past five years, important progresses have been made in developing efficient vector systems for clinical use. Vectors derived from Lentiviruses and Parvoviruses are the most adapted for permanent genetic modification in the context of genetic diseases. Besides simple gene complementation, alternative strategies such as gene or mRNA repair have proven successful *in vivo*.

### **3. Gene Therapy for Muscular Dystrophies: a worst case scenario?**

Skeletal muscles are formed during embryogenesis through the migration of progenitor cells present in the somites. A sequential myogenic program drives the differentiation of these cells into multinucleated myotubes which then develop into myofibres. These giant cells

contain hundreds of nuclei, and are grouped into bundles, with their extremities attached to the skeleton by tendons. Each myofibre is connected to a nerve terminal and irrigated by a vascular capillary. It contains the contractile apparatus (the myofibrils) and a highly specialized cytoskeleton that allows contraction in response to motoneuron-induced membrane depolarisation. The dense vascularisation provides high levels of nutrient and metabolite exchange and frequent contacts with blood-borne cells, including those of the immune system.

Mononucleated muscle progenitors called satellite cells persist in the adult tissue. They are located between the external side of the myofibre membrane (sarcolemma) and the layer of extracellular matrix called basal lamina. Upon fibre injury, they are induced to multiply, fuse and repair the broken syncytia.

Muscular disorders can affect many different components in the skeletal muscle system. Over 80 different monogenic diseases are known to affect muscle function including 25 different progressive muscular dystrophies (i.e Duchenne Muscular Dystrophies, Limb Girdle Muscular Dystrophies), Congenital Myopathies (ie myotubular myopathy, protein surplus myopathies), myotonic syndromes, periodic paralysis and metabolic myopathies (Pompe disease) [6]. In addition, the muscle can be affected in the course of diseases linked to the immune system (inflammatory myositis, chronic fatigue syndrome) or as a result of cancer or aging (cachexia).

Gene therapy for muscular disorders is certainly a tall order. The skeletal muscle represent around 40% of the body mass, and many deep muscles are difficult to access. The diaphragm and the heart are affected in many muscular disorders and reaching them efficiently would be paramount to the treatment of patients. Thus, a widespread delivery of an active principle will be required to fully compensate the deficiencies. This can only be envisioned by systemic

delivery of gene transfer vectors or of genetically corrected cells that would efficiently contribute to muscle reconstruction. Unfortunately, the dose of gene transfer vector that would have to be used is often beyond the current production possibilities. Besides, it is likely to provoke a catastrophic toxic shock response in patients.

In addition, many muscle disorder deteriorate the tissue down to a point where rescue becomes hopeless, with fat or fibrous tissue progressively invading most of the parenchyma. Any intervention would then have to take place as early as possible during the course of the disease, before severe symptoms appear. This grim state of affairs has often discouraged gene therapists and many of them have chosen more appropriate “model diseases” to achieve their “proofs of principle”.

#### **4. Gene Therapies for Muscular Dystrophies: solving some of the difficulties**

Yet, the indefectible support of patient associations has convinced scientists and clinicians to keep tackling the apparently overwhelming difficulties of applying gene therapy to muscle diseases. As a result, significant progresses have been made over the past few years, up to a point where true benefit for these heavily handicapped patients can now be foreseen.

Efficient vectors for gene transfer into the muscle have now been identified. The best ones are based on non-pathogenic human parvoviruses , the Adeno-Associated Viruses (AAV), among which specific subtypes display a significant tropism for skeletal and cardiac muscle [7]. These vectors can be applied locally by intra-muscular or intra-myocardial injections, or regionally using high pressure vascular perfusion. The later approach is minimally invasive and allows reaching every muscle in a limb at once [8]. Successful whole body gene transfer has even been documented in mice, using a bolus administration of high concentrations of

vectors [9]. Non virus based vector technologies have also been developed. DNA injected alone in the muscle, often with electric field-induced permeation of cell membranes, remain in the tissue and efficiently reprogram muscle cells [10].

These gene transfer technologies have permitted to study the effect of gene complementation in animal models of muscular dystrophies. The stable correction of disease associated lesions has been documented in a number of cases, sometimes accompanied by a recovery of muscle function [11-14]. These approaches however are fraught with imperfections, due to the difficulty of controlling the expression of the therapeutic gene in intensity and to restrict it to the relevant cell only. For this reason, it has been important to develop methods that would directly target and modify either the gene itself, or perhaps more interestingly its message which is in most cases correctly produced by the cell but contain mutations that cause the disease.

In the particular case of Duchenne Muscular Dystrophy (DMD), our research group has tested the possibility of repairing the mRNA transcript of the dystrophin gene [15]. Dystrophin is a modular protein with a spectrin-like central region whose size can be reduced without much effect on function. Mutations in the dystrophin gene which are associated with severe DMD are nucleotide changes or deletions that disrupt the translational reading frame, leading to a non-coding mRNA. Our approach has been to modify the dystrophin mRNA in the region bearing the mutations, in order to re-establish the translational frame. We have removed selected exons among the 79 that compose the final dystrophin mRNA, by locally preventing the formation of a functional spliceosome. This operation results in slightly shorter proteins that remain functional (quasi-dystrophin).

Therapeutic exon-skipping was obtained in the muscle of mice or dogs carrying mutations in the dystrophin gene, by administrating short anti-sense RNA sequences that were designed to



mask key determinants of spliceosome formation. The anti-sense sequences can be continuously produced in the treated muscle when an AAV vector is used to deliver them. As a consequence, normal levels of dystrophin were permanently restored in these muscles and they recovered their mechanical and functional properties.

These experiments have laid a foundation for organizing clinical trials in DMD patients. It can be seen, from the genetic analysis of patient populations that up to 80% of Duchenne individuals are eligible for such a therapy. Treating most these patients would involve the design and manufacture of only eight different vectors. The first trial will address the questions of feasibility of exon-skipping on a limited group of patients receiving local vector injections. In a second trial, a larger group of patients will receive a limb perfusion, with the hope that muscle function will be restored. Today, this step is technologically within reach and could bring a sizeable benefit to the patients. Further progresses are needed to reach a fully curative therapy: repeated or full-body vector administration, gene transfer to the heart, and possibly to the brain, etc. Yet the promise of even a modest improvement in the patient's quality of life is enough to justify embarking into the tortuous process of developing gene-based therapies for a rare genetic disease.

1. Austin, C.P., *The impact of the completed human genome sequence on the development of novel therapeutics for human disease*. *Annu Rev Med*, 2004. **55**: p. 1-13.
2. Rabeharisoa, V. and M. Callon, *Patients and scientists French muscular dystrophy research*. *States of knowledge*, ed. S. Jasanoff. 2004, London: Routledge.
3. Rinaldi, A., *Adopting an orphan*. *EMBO Rep*, 2005. **6**(6): p. 507-10.

4. Cohen, D., I. Chumakov, and J. Weissenbach, *A first-generation physical map of the human genome*. Nature, 1993. **366**(6456): p. 698-701.
5. Gyapay, G., et al., *The 1993-94 Genethon human genetic linkage map*. Nat Genet, 1994. **7**(2 Spec No): p. 246-339.
6. Wagner, K.R., *Genetic diseases of muscle*. Neurol Clin, 2002. **20**(3): p. 645-78.
7. Louboutin, J.P., L. Wang, and J.M. Wilson, *Gene transfer into skeletal muscle using novel AAV serotypes*. J Gene Med, 2005. **7**(4): p. 442-51.
8. Gonin, P., et al., *Femoral intra-arterial injection: a tool to deliver and assess recombinant AAV constructs in rodents whole hind limb*. J Gene Med, 2005. **7**(6): p. 782-91.
9. Gregorevic, P., et al., *Systemic delivery of genes to striated muscles using adeno-associated viral vectors*. Nat Med, 2004. **10**(8): p. 828-34.
10. Hagstrom, J.E., et al., *A facile nonviral method for delivering genes and siRNAs to skeletal muscle of mammalian limbs*. Mol Ther, 2004. **10**(2): p. 386-98.
11. Bartoli, M., et al., *Noninvasive monitoring of therapeutic gene transfer in animal models of muscular dystrophies*. Gene Ther, 2005.
12. Cordier, L., et al., *Rescue of skeletal muscles of gamma-sarcoglycan-deficient mice with adeno-associated virus-mediated gene transfer*. Mol Ther, 2000. **1**(2): p. 119-29.
13. Harper, S.Q., et al., *Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy*. Nat Med, 2002. **8**(3): p. 253-61.
14. Sun, B., et al., *Correction of glycogen storage disease type II by an adeno-associated virus vector containing a muscle-specific promoter*. Mol Ther, 2005. **11**(6): p. 889-98.
15. Goyenvall, A., et al., *Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping*. Science, 2004. **306**(5702): p. 1796-9.