



Fourth Round Table conference in Monaco on 15 January 2005: Regulation of muscle growth, a therapeutic issue for Duchenne muscular dystrophy?

Three pharmacological approaches may be faster than gene therapy techniques to develop a drug that can maintain muscles and their function for a limited time.

The fourth Monaco round table conference took place on 15 January 2005. Like the previous round tables, it was organized and financed by the *Association Monégasque Contre les Myopathies* and the *Duchenne Parent Project France*, both belonging to the international *United Parent Projects Muscular Dystrophy*, UPPMD.

Eighteen scientists from six countries were invited to present and to discuss their research results on different approaches to maintain muscle mass and function of Duchenne boys, not by genetic techniques but by more conventional methods like known drugs, approaches which might be able to help the patients for a limited time while they are waiting for a long term cure.

Eleven representatives of parents' associations from eight countries were also present mainly because their

members, the Duchenne families, are those who are most interested in the progress of research that will benefit their children. This report is written for them and for all other Duchenne families in the whole world as also for their pediatricians. This text thus is not a scientific account but tries to summarize what the scientists said in a language which they can easily understand.

This fourth round table conference had three main sessions: On *insulin-like growth factor*, *protease inhibitors*, and *myostatin inhibitors*, each preceded by an introduction. The scientists are mentioned in this report without their titles. Most of them are professors and all have a doctorate either in medicine (MD) or science (PhD).

Insulin-like growth factor, IGF-1 stimulates muscle regeneration.

IGF-1 not yet a Duchenne drug. In her introduction, **Gill Butler-Browne** of the *Hôpital Pitié-Salpêtrière* in Paris warned that the small protein IGF-1, which is offered on the internet as a means to retard aging, should not be used as a therapy for muscular dystrophy before a beneficial effect has been proved in clinical trials. Although it has been shown that this growth factor causes muscle hypertrophy in living mice – an enlargement of muscle cells but not an increase of their number –, it seems only to have a small effect in cultures of human muscle cells, probably because the number of satellite cells, which regenerate damaged muscle fibers, is not increased along with the larger size of the muscle cells. And the enlarged muscles in mice do not seem to have a better function unless they are exercised at the same time.

Thus, before IGF-1 can be recommended for muscular dystrophy children, it must be proved that not only the size of the muscles increases but also their function without the need for excessive physical exercise and without an exhaustion of the limited number of satellite cells, which would mean diminished capability of muscle regeneration.

Mice with more IGF-1 than normal. **Nadia Rosenthal** of the *European Molecular Biology Laboratory* in Monterotondo near Rome described her experiments with mice which had been genetically altered to make larger than

normal amounts of the natural growth factor IGF-1.

That factor, a protein of about 70 amino acids in one chain with three stabilizing bridges, thus with a similar shape to insulin, exists in six forms with slightly different structures. The effects of these different forms are not only positive such as promoting muscle cell growth but also negative such as causing cancer. One of the different forms, called mIGF-1, which is localized in muscle tissue, is of interest for a possible therapeutic use in Duchenne children.

In order to answer the question of what will happen when the amount of mIGF-1 in the muscles is increased above the normal low level, transgenic mice were created which had no dystrophy but which had extra IGF-1 genes in the nuclei of their muscle cells. This is done by genetic techniques which cannot be applied in humans. These *non-dystrophic* mice with high concentrations of mIGF-1 showed a strong muscle hypertrophy after 14 months. Some of the other effects were a decrease of fat, the extended maintenance of muscle mass and strength in aging animals, and an accelerated healing of muscle injury. The additional mIGF-1 did not induce cardiac problems, did not promote cancer, and had no other pathological side effects. *Dystrophic* mdx mice, which also had additional IGF-1 genes, showed decreased inflammation and fibrosis, and much reduced muscle degeneration. Thus mIGF-1

improves the local environment for efficient muscle regeneration.

Before IGF-1 is applied as a therapeutic drug for Duchenne patients, a way has to be found to get its most effective form, mIGF-1, into the muscles without having to transfer its gene, that is, without genetic procedures. Just eating the purified compound or the undefined mixture of the different forms as offered on the internet, or injecting it into the blood stream or directly into the muscles will be ineffective or even dangerous.

Improvement of heart function in hamsters by IGF-1.

Yves Fromes of the *Institut de Myologie* in Paris described his studies to improve the cardiac function of hamsters. Animals with a genetic defect after a spontaneous mutation were used. They had a limb girdle dystrophy with a cardiac involvement clinically similar to the dystrophy of the heart muscles in the late stages of Duchenne and Becker patients.

Two different forms of artificially made human IGF-1 proteins were applied to these hamsters in two ways, either by injecting them under the skin, subcutaneously, or by transferring their gene as naked DNA with a plasmid directly into the heart muscle. After a few weeks, the pathological signs were significantly normalized, especially the

dilation of the heart and its fibrosis were reduced and the contractility of the cardiac muscles was improved.

This IGF-1 therapy seems to be safe without negative side effects. However, there is probably an optimal concentration range of IGF-1 because an excess can induce an undesirable hypertrophy of the cardiac muscles.

IGF-1 treatment, a strategy for muscle growth. In the first part of his presentation, **Lorenzo Puri** of the *Dulbecco Telethon Institute* in Castel Romano near Rome discussed strategies to enhance muscle regeneration. This can be done by activating or inhibiting genes which influence the satellite cells in a positive or negative way. These satellite cells are necessary to repair muscle cells after injury or during muscle degeneration caused by the absence of dystrophin in patients with Duchenne muscular dystrophy.

IGF-1 is one of the many factors that activate myogenic genes which produce new muscle components and organize their correct interplay for creating new muscle cell structures after injury or degradation. The increasingly detailed knowledge of the signaling pathways in which IGF-1 participates, will facilitate discovery of therapeutic interventions with other factors that could positively influence the activity of IGF-1 on dystrophic muscles and thus improve their function.

Protease inhibitors, leupeptin and BBIC, retard muscle degradation.

The role of proteases. **Alfred Stracher** of the *State University of New York* in Brooklyn introduced this session by explaining that the degradation of muscle proteins in Duchenne dystrophy is mainly caused by the enzyme *calpain*, a protein-destroying enzyme, a *protease*, which is activated by calcium. Muscle cells contain an inactive form of this enzyme at their contractile structures. When, as in Duchenne dystrophy, muscle cell membranes are damaged by the absence of dystrophin, calcium ions from outside the cells gain access to calpain and activate it thus leading to widespread destruction of most proteins in the cell. By using calpain inhibitors like the modified tripeptide *leupeptin*, consisting of three amino acids – leucine-leucine-arginine –, the muscle degradation in mdx mice could be almost completely stopped. Leupeptin can be given by mouth and may therefore be ideally suited for a therapy of Duchenne dystrophy.

In a preliminary clinical study in Italy, seven Duchenne boys, 8 to 12 years old, were treated for one year with very low doses of leupeptin. Their creatine kinase (CK) activities decreased significantly from about 10,000 U/L to between 600 and 2,000 U/L. This is an indication of decreased muscle degradation.

Clinical trial of calpain inhibitor C101. The *CepTor Corporation* in Baltimore is developing a calpain inhibitor based on the positive experimental results with leupeptin. **Theresa Michele**, vice president of research for the company, reported on their efforts to target leupeptin to muscles, mainly by combining a modified leupeptin with carnitine for which the muscle cells have a receptor protein in their membranes.

Testing of this new compound, called C101 or *Myodur*,

in mice and dogs is beginning especially to assure that a long-term application in children will be possible.

An investigational new drug application (IND) will be filed with the drug licensing agency FDA at the end of 2005 so that a phase-I/II clinical study to test for safety and pharmacokinetics, the fate of the drug in the body, will begin in the United States in 2006. Because of the orphan drug indication for Duchenne therapeutic compounds, 6 to 13-year old Duchenne children will be allowed to participate in this first clinical study.

Calpain inhibitor BBIC. **Lee Sweeney** of the *University of Pennsylvania* in Philadelphia discussed another protein preparation able to block the activity of proteases called *Bowman-Birk inhibitor concentrate* (BBIC). It is a natural protein composed of 71 amino acids and isolated from soybeans. BBIC is not active against calpain, which is a cysteine protease, but against serine proteases such as the normal digestive enzymes trypsin and chymotrypsin. This protein can be applied orally. It is too big to enter the muscle cells so it acts outside the cells by blocking a signaling pathway. Soy beans contain other proteases also, so BBIC must be purified from them. Eating the beans directly has no effect.

BBIC blocks the dystrophic process in mdx mice by inhibiting the muscle degradation. Muscle mass is increased and muscle force is also increased, so the larger muscle cells also produce increased force. CK activities are reduced considerably and fibrosis also. And from other applications in cancer patients it is known that BBIC is a very safe drug. So, human trials with Duchenne children are already being prepared by the NIH.

BBIC slows down the degradation process. A future

Duchenne therapy might use both approaches: BBIC or leupeptin for blocking muscle degradation, and IGF-1 for stimulating the regeneration process.

Clinical study of BBIC. Kenneth Fischbeck of the *National Institutes of Health (NIH)* in Bethesda near Washington reported on the first planned clinical study with the natural protease inhibitor BBIC. This compound has been tested as a potential cancer and anti-inflammatory drug in six phase-I clinical studies on about 150 adults without any toxic side effects. But it has not yet been tested in children.

The first phase-I clinical trial with Duchenne children is now being prepared. It will start in the fall of 2005. Fifteen children, 4 to 8 years old, will participate. Different doses of BBIC will be used up to 2,000 units per day for one month. One gram of purified BBIC contains 500 to 600 units. It is a powder which the children can drink dissolved

in fruit juice. The aim of the trial is to detect any side effects and to determine the maximal tolerated dose.

A phase-II trial will follow in 2006, it will be a randomized, double-blind, placebo controlled trial with 24 4-to-6-year old Duchenne children lasting 6 months. The benefits of the treatment are expected to be similar to a prednisone treatment, that is, maintaining of muscle mass and force by slowing muscle degradation. If the results of the Phase-I and -II trials are positive, a phase-III study with a large number of patients and in many clinical centers will follow, unless the orphan drug status of a Duchenne therapy will allow the immediate approval of a BBIC treatment.

It is not known yet in what way BBIC influences muscle growth on the molecular level. But the experimental results with animals and adult humans suggest that it is safe and possibly effective in Duchenne children. Therefore, the immediate start of clinical trials is justified.

Myostatin inhibition releases the limitation of muscle growth.

Innervation of muscle fibers. Jean-Marie Gillis of the *Université de Louvain* in Brussels introduced this session by describing the way the motoneurons, the nerves that transmit movement orders from the brain to the muscle, connect with the muscle fibers.

Each single nerve fiber connects with a group of muscle fibers, a motor unit. Not all motor units are working simultaneously but only as many as are necessary by the demand of force to accomplish a desired movement. During the muscle contraction, the motor units are activated in a rotating pattern. The individual muscle fibers have different diameters, and the larger the diameter, the more force a fiber can produce.

When a muscle fiber undergoes hypertrophy, that is, when it is enlarged by drugs or muscle activity, it can produce more force. Thus, when a hypertrophied fiber has to produce a certain force, it will have to contract less often than a normal fiber. Each individual muscle fiber of a Duchenne boy would then have to work less often when all fibers had been increased in size by a drug treatment. This reduction in individual work load would place less stress on fibers and diminish damage and degradation. However, Duchenne muscle fibers with a large diameter are thought to be more easily damaged than thinner fibers. A treatment involving muscle hypertrophy should then be fine-tuned to produce optimal fiber sizes.

Inhibition of myostatin by an antibody and the propeptide. Tejvir Khurana of the *University of Pennsylvania* in Philadelphia presented the results of experiments to increase muscle tissue by blocking the activity of the protein myostatin.

Myostatin, also called growth-differentiation factor 8, GDF-8, is produced as an inactive protein consisting of 375 amino acids. To become biologically active, the first 266 amino acids, called the *propeptide*, are split off, and two chains of the remaining portion with 109 amino acids combine together to form a double ring. The separate propeptide again attaches loosely to the double ring, still inactivating it. The propeptide is displaced when the complex binds to its receptor molecule on the outside of the

muscle cell membrane. This *active myostatin* then initiates a series of chemical reactions inside the cell, a signaling cascade, that finally interrupts the genetic regulation that would otherwise lead to the biosynthesis of new muscle proteins. How this occurs, is not completely understood yet.

These facts led to the idea that by inactivating myostatin, the regeneration of the muscles of Duchenne boys could be stimulated so that they would not be destroyed as fast or might even increase in size.

In a first approach to block myostatin activity, a monoclonal antibody against the myostatin of mice, JA16, prepared by the company *Wyeth Research*, was used. This is an immune protein that attaches itself very specifically only to myostatin and thus inactivates it. A solution containing these antibodies was injected once a week under the diaphragm of mdx mice. After three months, the treated animals were 12% heavier than control animals without treatment. They had a better muscle function, and their muscle degeneration had decreased along with their serum creatine kinase activities.

In a second approach, an artificially made propeptide was used which, in addition, had to be stabilized by binding it to the Fc fragment of an immunoglobulin normally present in serum. This stabilized propeptide was injected over three months into mdx mice. The results were similar to those of the first approach, except that not only the absolute force of the muscle fibers was greater than expected after the fiber had become larger, but also the specific force had increased, the force per volume, thus the entire fiber was much stronger.

These results support the idea that clinical studies should be performed and, as the following paragraphs show, they have already been planned by the Wyeth company. But it must be understood that this kind of treatment cannot be a complete cure for Duchenne muscular dystrophy because the genetic cause of the disease would not be eliminated. However, compared with other methods, it would have the advantage that no immune or toxicity problems would arise, and no genetic risks caused by viruses. Pharmaceutical companies like Wyeth are al-

ready interested in this technique because increasing muscle mass would also be important for older persons and people with other muscle-degrading diseases.

Clinical study of an antibody against myostatin. **Anthony Celeste** of the *Wyeth Research company* in Collegeville near Philadelphia informed that the antibody blockade of myostatin is already being tested clinically in a phase-I trial with healthy adult volunteers to assess the safety of this approach but not yet efficacy.

The antibody JA16 against the myostatin of mice was used for the preparatory investigations with mdx mice as presented by *Tejvir Khurana*. For the human trial, the specific antibody MYO-029 against human myostatin was prepared and first tested in SCID mice which do not have an intact immune system that would interfere with the human antibody. In these mice, the skeletal muscle mass increased up to 30% after an application of only 1 mg/kg/week of this human antibody for 3 months. The muscle force increased also significantly, and there was no evidence of toxicity. Twenty weeks after the end of the treatment, the muscle mass returned to normal. This result is important because, if in a later human application, some problems should occur, the treatment could be stopped and it may be possible that the patients would return to their original situation. Preclinical studies were also performed with the human antibody in other animals, rats, rabbits and monkeys, with very high doses up to 100 mg/kg. There was again no evidence of toxicity or other side effects.

After these positive results were obtained, "the efforts in the company escalated exponentially". Wyeth is committed to rigorous research to explore the use of this approach as a potential treatment.

The next steps planned are to find a small molecule inhibitor instead of the antibody which is a protein that has to be injected. A small molecular compound could possibly be administered orally as a pill. Work is already in progress to find soluble forms of the binding structures of the myostatin receptor and of the propeptide which would be able to remove the myostatin before it binds to the receptor in the muscle cell membrane. This would leave the receptor free to bind with other substances which also need it to affect other signalling pathways in the cell.

After the meeting, Wyeth has announced the initiation of a phase I/II clinical trial with adult patients with Becker, limb-girdle, and FSH muscular dystrophies, again to assess safety in patients but also to measure any positive effects on the preservation of muscle mass. Results of this study are expected to be available in late 2006. And the American Muscular Dystrophy Association MDA has decided to support these studies with an award of 1.5 million dollars.

Transfer of the myostatin propeptide gene. The research team of **Luis Garcia** at the *G  n  thon Institute* in Evry near Paris investigates the possibility to transfer the gene of the myostatin propeptide into muscles so that they can produce continuously the agent that releases the inhibition of their own growth.

The DNA structure with the genetic information for the 262 amino acids of the propeptide and the 4 amino acids of the cleavage site were packaged into the adeno-associated

virus type 1, AAV1, together with controlling sequences. Billions of these gene transfer vectors were then injected into leg muscles of normal mice. After two weeks, the injected muscles had increased by 25%, and this increase of their mass remained stable.

A similar vector construction was used for injections into the arteries of the legs of normal and dystrophic mdx/REX mice while the venous outflow was blocked by a blood pressure cuff. Six weeks after this partly systemic injection under pressure, all the leg muscles of the *normal* mice had increased by about 35%.

As mdx mice/REX start to lose their muscles when they are about 5 months old, the intra arterial injections were started at this time. Two months later, their muscles had not increased significantly, possibly because the number of their satellite cells, which are responsible for regeneration, were diminished by the dystrophic process. But the muscle tissue looked more healthy than untreated control animals with less necrosis and with very little fibrosis.

When the genetic structure for the propeptide has a mutation causing the replacement of the amino acid aspartic acid in position 99 by the amino acid alanine, the mutated propeptide is much more stable than the natural one. Experiments with this mutated structure are being prepared as well as trials with dystrophic dogs.

Follistatin, an inhibitor of myostatin. In the second part of his presentation, **Lorenzo Puri** reported that it was possible to up-regulate substantially the activity of the gene for the protein *follistatin* with the drug *tricostatin A* (TSA) which is a deacetylase inhibitor that acts specifically on chromosomes in the nuclei of muscle cells. And follistatin in turn blocks the activity of myostatin.

Solutions of TSA applied under the diaphragm of mdx mice for 3 months increased their muscle mass and reduced the activity of creatine kinase significantly and had other beneficial effects. But TSA has undesirable side effects such as hair loss. The anti-epileptic drug *valproic acid* has a similar effect to TSA, and as this is an approved drug for long-term treatment of children, it would not need new toxicity studies. However, before clinical trials are started, the complex relationship of all the many factors involved has to be investigated.

Function of myostatin. In his presentation, **Ketan Patel** of the *Royal Veterinary College* in London discussed his work to find out where and in what way myostatin inhibits the growth of muscle tissue.

Chickens were used as experimental animals because it is easy to follow the embryonic development of their muscles at different stages times during the incubation. Among the *somites*, which are the earliest precursors of all skeletal muscles, only some cells contain active myostatin and thus do not proliferate. Other somites contain follistatin which inactivates myostatin, and thus assures that these somites can divide to form the next structure in muscle development, the myotome. This means that cell division only occurs where there is no active myostatin.

In order to see the local effect of myostatin, very small glass beads were coated with myostatin and then injected into the proliferating myotome structures of the early chick embryos. The beads themselves did not interfere with the

growth of the embryo, but the additional myostatin down-regulates, inactivates, the genes of many, but not of all, protein factors necessary for further muscle development. The myostatin beads thus stop muscle growth where they are located, and this is a long-term effect. The beads can also be taken out, then the growth factors appear again and muscle growth resumes.

In other experiments with mice that have their myostatin gene inactivated, it was found that the muscle mass increased significantly, but that the muscle force did not increase in line with the larger size of the muscle fibers, that is, their specific force decreased. And the muscle cells of the male mice contained cytoplasmic inclusions, tubular aggregates of unknown structure, which seem to be the cause for the decreased specific muscle force.

Long-term effect of myostatin. Kathryn Wagner of the John Hopkins University in Baltimore investigated the long-term effect of myostatin inhibition. By examining the muscle structure of non-dystrophic mice which were between 3 months and 2 years old and which had their myostatin gene knocked out, inactivated by genetic methods, it could be shown that even in the absence of myostatin for periods of up to their entire normal life time, all mice, including the older ones, had normal muscles with increased mass. Mdx mice whose myostatin gene was also inactivated maintained their increased muscle mass and strength into old age.

To test whether muscle regeneration is impaired in old normal and mdx mice without myostatin, muscles were injured by toxic drugs. In both cases, the regenerative capacity of the muscles was not altered, but the degeneration of the mdx muscles caused by the absence of dystrophin continued as before.

These results show that the prolonged absence of myostatin does not have negative effects in mice. But whether this means that the inactivation of myostatin for a long time in Duchenne patients would also not produce any side effects has to be checked in long-term clinical studies.

A young boy without myostatin. Markus Schülke of the Charité Hospital of Humboldt University in Berlin described the first known human child, a now 5-year old boy, whose myostatin genes on both of his chromosomes 2 are inactivated by a mutation. The adenine in the 5th nucleotide of the first intron of his myostatin gene is replaced by a guanine. Thus, this mutation has changed a non-coding part of the gene, a region highly conserved during evolution that affects the splicing of its three exons. This caused an alternate splicing of 70% of the boy's myostatin pre-mRNAs leading to the addition of 109 coding nucleotides after the first exon with the result that a premature stop codon appeared which interrupts the biosynthesis of this small protein. These molecular facts explain the very low myostatin concentrations in the muscles and its complete absence in the serum of the boy.

Because of the reduced myostatin activity, the boy's skeletal muscles are about twice as large as in a normal child, and he is physically very strong. His mother, a professional short distance sprinter, is a heterozygote, she has

the same mutation on only one of her chromosomes 2. Several male relatives of the mother were or are also unusually strong. However, the father of the boy could not be investigated,

The Belgian "blue-white" cattle have no myostatin. Therefore, they have an increased muscle mass of about 20%. However they do not tolerate physical stress very well. They have another mutation, a deletion of 11 nucleotides, in their myostatin gene. So myostatin inhibition in Duchenne boys might lead to still unknown health problems. Possibly, a treatment should not be started too early. But the German boy does not show any medical side effects at the age of five years.

General discussion. At the end of the meeting, Terence Partridge of the Hammersmith Hospital in London again raised the questions as to the nature of the hypertrophies caused by over expression of growth factors or inhibition of myostatin that had been discussed briefly in the course of the presentations. The point was made that no stringent stereological studies had been made to ascertain whether the enlargement of muscle that resulted from these treatments was accompanied by an increase in muscle fibre nuclei or of satellite cells. Such an investigation would be relatively simple to perform and would give valuable information on the type of mechanisms involved in the observed muscle enlargement.

A brief survey was also given of methods for examining the effects of these reagents on the numbers and the proliferative capacity of satellite cells removed from muscles of animals treated with growth factors or inhibitors. The evidence presented, argued strongly against any direct effect of myostatin on the behaviour of satellite cells in vivo or in vitro, a conclusion that was in accord with the findings presented earlier by Luis Garcia. Further discussion of these points addressed the matter of reconciling the increase in muscle size induced by IGFs, or by blocking of myostatin, with the apparent benefits observed in both cases on the dystrophic phenotype. Theoretically, larger muscle fibres, should be more susceptible to damage arising from transmission of force across their surface, and there are a number of observations in support of this idea. So, the findings of reduced disease activity in mdx mice whose muscles had been increased in size has been hard to explain. It may be that the activation of satellite cells and increase in size of muscles are not the only effects of these muscle growth promoting strategies and that robustness of muscle fibres is enhanced by some other, as yet un-described, mechanism. As a general conclusion, it seemed that a number of different methods of increasing muscle size had a beneficial effect on muscular dystrophy in mice, which was surprising in that it seemed to protect muscle fibre integrity. Several of these approaches are being, or are about to be, tried in human subjects, but it was generally agreed that great caution must be exercised, particularly in view of the incomplete understanding of the modes of action of these various reagents.

Interview with Nadia Rosenthal: Parents should not loose hope while waiting for a genetic therapy for Duchenne dystrophy.

Nadia Rosenthal is head of the *European Molecular Biology Laboratory (EMBL) Outstation at Monterotondo* near Rome. After the round table meeting, she answered questions (in *italics*) by Guenter Scheuerbrandt about possible interventions with muscle-growth promoting drugs, while more permanent genetic therapies are still being developed and the hope the parents should not lose.

Parents with Duchenne boys need hope for a cure of this terrible disease. They are collecting money, often in rather small amounts, which they donate to the parent projects hoping to see their sick children cured. When the dystrophin gene was found in 1986, everybody thought that there would be a cure the following year. At the two last Monaco Round Tables, it was realized that it will still take several more years until exon skipping will be ready as a therapy, and that it does not seem that the gene transfer techniques will become a therapeutic option any faster.

If we look at the history of gene therapy, we see that promises were made as early as in the 1970s, even by scientists, about the prospects of gene therapy, and here we are 30 years later, and we are still not there. Of course, gene therapy could break open any day, new discoveries could be made, a new viral vector could be found that suddenly would make the promises a reality. If it were that simple!

But even then, a gene therapy would have to go through clinical trials. And that is what I tell parents, since they all want their child to be the first guinea pig. The problem is that a child cannot make an informed decision whether to take part in a dangerous trial. Of course it is a personal tragedy to have a child with a terminal disease that may kill him before a cure is found. But more harm would be done by rushing into these trials. If a treatment were so fantastic that it had no side effects and would cure the child, we would have found it by now. The dreams of researchers that they would stumble suddenly on something that would work perfectly, are not realistic.

So obviously, the researchers have had many hypotheses to check, and as a result it takes a long time to get all the basic information. I am now working on a promising therapeutic approach, but the ignorance in this field is just profound. I have spoken many times to different audiences and tried to explain very rationally the seemingly miraculous potential of IGF-1 to regenerate tissue, but we have to know much more about which is the right IGF-1 protein to use.

Still I think there is a lot of room for hope. I started working in muscular dystrophy in 1984, and at that time the same problems existed as now but we knew so much less. Nobody knew about myostatin, nobody knew even what the disease was caused by. Since that time, we have made extraordinary progress, and yet, for parents, none of that means anything because there is no cure yet. And so we have the problem how to tell the parents that in spite of the progress we are not yet at the cure. I think the fact that

potential therapeutics are already being assessed for safety in phase-I clinical trials is enormously exciting.

And also the growing number of laboratories working in this field.

Yes, and the number of approaches. And I think the suggestion to combine approaches is very important. And what these workshops in Monaco do, is to encourage discussion among us researchers. So, for instance, I have already offered my help and to send reagents to my colleague Kathryn Wagner in Baltimore who works on the IGF-1-myostatin combination, because we need to know whether those two things could be put together. I am also very interested in protease inhibition, because we know that IGF-1 actually does that anyway. So, the question is how many different attacks do you need, and the answer is: as many as possible because the cell is an extremely complex little machine. And we do not know which approach will arrive at a cure.

Although the lesion in Duchenne muscular dystrophy might be quite simple, just one protein is missing, it cannot be repaired like a broken-down car which can be fixed by exchanging one broken piece. Because a cell has many different ways of working around problems. And so there may be ways for us to induce the cell to make up for its fragility and maintain the muscle at least for the time we need until we can actually replace the gene, which is the ultimate goal, or repair it, so that it makes functional dystrophin again.

The other thing to say to the parents is, that the research that they are supporting is increasingly enhanced by the recognition that there are many other kinds of muscle weakness occurring in aging, cancer, chronic heart failure, infectious diseases, and even AIDS, which also have to be addressed. So there is an enormous need for intervention, because many of the same processes of degradation that we see in these maladies are potentially treatable by the same therapeutic avenues that we try to develop for muscular dystrophy.

And that would then also be interesting for pharmaceutical companies.

That's right, I have talked to several pharmaceutical companies and realized that they see muscular dystrophy as a very important and extremely attractive first path for treating these other diseases with muscles deteriorating, because the level of knowledge is much higher for muscular dystrophy than it is for, let's say, the usual muscle atrophy in aging people. One knows very little about that, and that has mostly come from the research we have done on muscular dystrophy. So, the Duchenne parents are not the only ones in their search for a cure for their disease. More and more pharmaceutical companies are becoming aware of muscle as a key target for therapeutics. And what that means is not necessarily that we will get the Duchenne cure faster, but that when something looks good, then there would be money for trials and for the development of a therapy.

And also to get it to the patients everywhere, because marketing will also be important.

Yes, and the research can only come up with the proof of principle. And the time then to develop a drug and to bring it to the market is a fairly predictable number of years. And that does not always happen fast enough for the individual. All we can say today is, that the clinical trials we saw proposed today, some of which rely on substances that are already in approval for use, are the best possible fast track to ameliorate the disease, maybe not to cure but at least to stave off the severe problems.

Then can we say, that with the methods we have discussed today, one could perhaps buy time until something better and more permanent comes along?

Yes, absolutely. And obviously that is the key advantage of using pharmaceuticals that are already present in the drug books for something else. That is why I brought up AIDS and muscular dystrophy. If people who are suffering from both are using some of the anti-inflammatory protein inhibitors, we might be able to discover promising new facts. That is why clinical data banks are so important,

sometimes providing us with unexpected information.

So, there is hope that something is going to happen?

There is always hope! Especially because there is real movement now in a way that I have not seen before. I now think that it is easy to imagine how certain processes in the cell can be modulated by existing ordinary small molecules. This could lead to a real breakthrough, as happened with AIDS when protease inhibitors were developed. So, diseases as devastating as AIDS, when it first came to be an epidemic, looked seemingly incurable and rivalled muscular dystrophy. And now people are managing it. So that is what we hope, managing the disease, and later curing it.

When researchers like you speak directly to the parents in interviews like this one about the consequences of their work, their words are especially important for them and give them real hope. I thank you and all your colleagues for your efforts and dedication and wish you – also on behalf of the families with Duchenne boys –

the success we all need.

Some scientific facts

To understand better the different approaches towards a pharmacological intervention in the dystrophic process of Duchenne children, some basic facts of muscle development and its most severe disease, Duchenne muscular dystrophy, are briefly summarized here.

Muscle development. In the early embryo, the first cells to become muscle tissue are the **somites** which proliferate to form the **myotome**. The myotome cells give rise to **myoblasts** and to **satellite cells**. The myoblasts fuse with each other to form **myotubes**, whose many cell nuclei are centrally located, and then develop to mature **muscle fibers** whose nuclei are located at the cell membrane. The satellite cells, which are a type of local stem cells, attach themselves to the outside of the muscle cell membrane. When the fibers are damaged by injury or by a disease like Duchenne dystrophy, the satellite cells repair and regenerate the muscle cells in a way similar to the biosynthesis of new fibers by myoblasts.

Many different proteins influence the development and structure of the muscles. Some of them are mentioned in this report such as IGF-1, myostatin, and follistatin.

Muscle structure and protein synthesis. The force generated by the actin-myosin structure inside the muscle fiber is transferred to the entire fiber by a protein complex located in and at the cell membrane, the protein **dystrophin** being one of its most important components.

The gene which carries the information for this protein is the **dystrophin gene**. It is located on the X chromosome. With a sequence of 2,220,223 **nucleotides**, “genetic letters”, it is by far the longest human gene. It has 79 active regions, the **exons**, with a total of only 11,058 nucleotides, which specify the sequence of the 3,685 **amino acids**, the building blocks, of the dystrophin protein. The large remainder of the gene, 99.5%, is formed by the **introns**, which do not contain the information for the amino acid sequence of the protein.

The nucleotide sequence of the gene is copied in the cell nucleus to the pre-messenger ribonucleic acid, **pre-mRNA**. The intron sequences are then eliminated and the exons spliced together to the messenger RNA, **mRNA**.

This messenger moves out of the cell nucleus to the **ribosomes** in the cytoplasm where the protein is assembled according to the genetic information preserved in the structure of the mRNA.

Duchenne muscular dystrophy. This hereditary disease is caused by a **mutation** in the dystrophin gene, a change that disturbs the genetic information so that the protein dystrophin cannot be produced any more by the muscle cells. Without dystrophin, the muscle fibers degenerate, mainly because their membranes become permeable. Then large quantities of calcium ions enter the cells and activate enzymes like the protease **calpain** that destroy all the other proteins of the muscles.

To counteract this degeneration, the satellite cells are activated to initiate and to maintain repair procedures. The two processes, **degeneration** and **regeneration**, are in equilibrium for some time until the number of satellite cells becomes so small that regeneration slows down and finally stops. This leads to the appearance and worsening of the clinical symptoms of the disease.

Approaches towards a therapy by gene techniques. The repair of the mutation by **exon skipping** and the replacement of the gene by the **transfer** of its entire normal active structure or of shortened versions are research techniques that have progressed considerably. Their encouraging results, mainly obtained with dystrophic **mdx mice**, are now being tried in clinical studies with Duchenne patients. At the present time, these genetic techniques do not seem to provide a complete and long-term cure of the disease, but will only be able to slow down muscle degeneration, that is, they could change the fast Duchenne into the much slower **Becker muscular dystrophy**. It will, however, still be many years before a therapy, based on these techniques, will be ready for Duchenne patients.

A note: Scientists like to talk about the things they are working with as if they have only one of each: *one* protein, *the* dystrophin, *the* gene, *the* muscle fiber. In reality, they are producing and are working with millions or billions of them. This way of using the singular instead of the plural is followed also in this report.

Buying time with a “normal” drug.

A “normal” drug already used against other diseases, and being able to slow down muscle degeneration or to speed up muscle regeneration, would need much less time to be approved as a Duchenne therapy than a completely new technique like gene therapy. This would be of immense benefit for the half a million families with Duchenne children worldwide. Some of the potential drugs which were discussed at this meeting, IGF-1, BBIC, leupeptin, follistatin and other myostatin inhibitors, would be such “normal” drugs. Although it is not yet well known how they work at the molecular level, they are so promising that they are being clinically tested anyway. And they would probably have far fewer side effects than the steroids prednisone and deflazacort, which are still the only approved drugs that have a proven retarding effect on

degrading muscle, but whose precise mechanism of action is not known either.

As has been suggested at the meeting, some of the drugs under consideration could be taken simultaneously, for instance IGF-1 to stimulate regeneration and calpain inhibitors to stop degradation. They could even later be used in combination with a genetic technique like exon skipping. And they should be applied as soon after birth as possible, when most of the muscles still have a normal structure. This would “buy time” for the children while they are waiting for the possibly more effective genetic therapies that try to correct the gene mutation itself.

The further development of the potential “normal” drugs discussed here might be a short-cut, a way to treat many of the sick children in time.

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