



Association
Monégasque
Contre les
Myopathies

Third round table conference in Monaco on 19 June 2004: Transfer of the dystrophin gene

**There is still a long way until Duchenne muscular dystrophy
can be cured by gene transfer.**

The third Monaco round-table conference in 2004 was organized by the *Association Monégasque Contre les Myopathies* and the *Duchenne Parent Project France*, both of which belong to the international *United Parent Projects Muscular Dystrophy*, UPPMD.

Twenty scientists from six countries met in Monte Carlo on 19 June 2004 and discussed their work on the transfer of the full-sized and shortened versions of the dystrophin gene into the muscles of Duchenne boys.

The conference was opened by *Prince Albert of Monaco* who welcomed the participants and said that "Monaco will always be an open meeting place to all who will give children hope for a better life, especially for those with such a disabling disease".

This report will concentrate on the first clinical trial of a gene transfer technique with Duchenne patients now underway in France and on others planned for the near future. This information is based mainly on two interviews which were conducted immediately after the conference. They are preceded by an introduction to the transfer techniques involved. A conclusion based on comments from Professor *George Karpati* then brings the report to a close.

As the interviews mainly tried to answer the question always asked by the parents "How long do we have to wait, until a therapy will be ready for our boys?", this report was written mainly for the families and their pediatricians and not for scientists who wish to be informed on the details of gene transfer research for muscular dystrophy.

In addition to the presentations and discussions about the clinical trials with the gene transfer technique, there were a number of others which cannot be included here. They dealt mainly with the details of the gene transfer techniques like the advantages and disadvantages of different vector constructions and the problems of immune reactions against the vector and the newly synthesized dystrophin.

A comprehensive research report on the state of Duchenne research as of August 2003 can be seen on the internet at <http://www.duchenne-research.com>. This report will be updated probably in 2005 and will then include information on all approaches toward a cure of Duchenne muscular dystrophy including the work which could not be mentioned here.

Dystrophin gene transfer

Duchenne muscular dystrophy is caused by mutations of the dystrophin gene on the X chromosome. This gene is the largest gene found in the human genome. It is about 2.5 million base pairs (genetic "letters") long which are grouped in 79 exons with a total of only 11.000 base pairs, its active regions, that contain the information for the production of dystrophin. This protein stabilizes the muscle cell membranes. If a mutation of the gene changes the genetic information in such a way that no dystrophin can be made in the muscle cells, Duchenne muscular dystrophy develops. However, some mutations do not interrupt the dystrophin production but only cause the formation of shorter than normal dystrophin. This leads to the milder symptoms of Becker muscular dystrophy.

At the second round table conference in January 2004, exon skipping was discussed as a possible technique to change a "Duchenne mutation" into a "Becker mutation" by instructing the protein synthesis mechanism not to use certain exons with the aim to produce a shorter than normal dystrophin instead of a complete stop of its production. With the techniques discussed at this second conference, one tries to transport the combined exons, the

cDNA, of normal dystrophin into all muscle cells with viruses or plasmids as carriers, called *vectors*.

The most effective viruses for this task are the adeno-associated viruses, AAV. But these small viruses can only transport genetic material that is not longer than about 5,000 base pairs, about one third of all the exons with the information of dystrophin..

Their advantage is that they transfer the gene more effectively than other viruses like the normal adenoviruses. The disadvantage is that the dystrophin cDNA to be transferred has to be shortened considerably to fit into this small vector together with a promoter sequence for activating the gene in muscle cells. Patients with Becker muscular dystrophy have similar shortened dystrophin in their muscles. Therefore, a transfer of one of these *Becker mini-genes* might not completely cure Duchenne muscular dystrophy, but only transform it into the benign Becker form.

The shorter forms of the dystrophin gene are called mini or micro dystrophin genes, depending on their structure which might be as short as one third or less of the natural length.

The first interview was conducted with Dr. *Serge*

Braun, Vice-President Research of the biochemistry company Transgène in Strasbourg, and Dr. Jon Wolff of the University in Madison/Wisconsin and President of Mirus company. Its subject was the first gene transfer trial with

Duchenne patients, in which not viruses, but plasmids were used as vectors to transport not a shortened version of the gene but the entire combined exons, the cDNA of the full-length dystrophin.

Trial in France: Transfer of the dystrophin gene with plasmids

The first phase of this trial was completed at the beginning of 2003. The company *Transgène* and the French muscular dystrophy association *AFM* started their research and development program in 1995. The permission by the regulatory authorities was given in November 1999, and the first injections of the plasmid vectors with the dystrophin gene were performed in September 2000 at the *Hôpital de la Pitié Salpêtrière* in Paris.

The 9 participating boys were all older than 15 years so that they could give their *informed consent*. They did not derive any clinical benefit from this treatment, *it was not yet a therapy*. Its aim was to show that the procedure is safe, i.e., that it does not lead to an immune reaction or an inflammation, and that some new and normal dystrophin appears underneath the membrane of the fibers in the treated muscle tissue.

For this technique, the combined 79 exons without the introns, the cDNA, of the normal dystrophin gene and its controlling structures were part of the genetic material of plasmids. Plasmids are small circular DNA structures without protein inside bacteria to which they mostly confer resistance against antibiotics. As the plasmids do not contain any protein, only genetic material, *naked DNA*, no immune reaction develops against this vector and its charge.

In preliminary experiments with muscle cell cultures, dystrophic mice, and dogs, it was shown that this vector construction led to the appearance of new dystrophin at its correct place underneath the muscle cell membrane of the animals, that it restored the dystrophin-glycoprotein complex and that it prolonged the life of the cells. The amount of plasmids injected into the animals was proportionally up to 500 times greater than that used for the Duchenne patients in the trial.

A solution with 0.2 mg plasmids containing 10 trillion (10×10^{12}) copies of the dystrophin gene was injected into one muscle of the forearm of the first three patients. The next three patients received one dose of 0.6 mg and the last three two doses of 0.6 mg two weeks apart. Each patient was treated only when it was certain that the previous one treated did not show any signs of immune intolerance or other problems.

Three weeks after the injections, the treated muscle volume of about 0.5 cubic centimeters was extracted by biopsy and checked for the presence of dystrophin. In three out of six patients in the first two groups and in all three patients in the third group, new dystrophin appeared in less than 1% to more than 25% of the muscle fibers around the injection sites. There were no signs of an immune reaction, neither against the plasmid nor against the newly produced dystrophin. This answered the question of a phase-I study: *Gene transfer with naked DNA is a safe procedure*.

The French scientists are now working with the team of *Jon Wolff* in Madison/Wisconsin, who injected similar plasmid constructions with genes of the marker proteins beta-galactosidase and luciferase into the blood stream of limbs of rats, dogs, and monkeys *under pressure*. The pressure was produced by short-term blocking of the blood circulation in one limb with a blood pressure cuff. Afterwards, up to 20% of the muscle fibers contained the transferred marker protein after one single injection and up to 40% after repeated injections.

The French and American researchers have now joined their forces to take this procedure to Duchenne and Becker patients. Encouraging results have been generated in the Golden retriever muscular dystrophy dog.

Interview with Drs. Serge Braun (B) and Jon Wolff (W) on 19 June 2004

The questions of this and the second interview were asked by the author of this report, Dr. Günter Scheuerbrandt, they are printed in italics.

In this discussion, we should try to find an answer to the question the parents with a Duchenne boy always ask: How long will it take until the gene transfer method will be available to cure our son? In an interview at the last Monaco meeting in January on exon skipping, the answer was: ten years, more or less.

B: It is quite impossible to answer this question. Usually with any new drug, it takes about 15 years to go through phase I, phase II, and phase III of a clinical trial. With a genetic disease, it might be different because we could possibly obtain an orphan drug status and a fast track approval, especially for a disease like Duchenne with no treatment. But even then, it will be a long way. With our plasmid DNA study, we just completed the phase-I trial, which means we went at least one step forward.

How long did this trial last?

B: Two years and a half plus 18 months of discussions with the regulatory authorities, that is four years just for phase I. But it took so much time also because it was the first gene-based trial for Duchenne dystrophy. So, there were more issues to discuss, and more questions and more concerns were raised by the regulatory authorities before any approval was given for clinical trials.

Are these regulations in France different from the ones in the United States?

B: I think, they are similar and very strict in both countries. The French authorities are really very cautious about genetically modified organisms, GMOs, and plasmid DNA is considered as a GMO like modified viruses.

Now, the first phase has been finished for Duchenne dystrophy. Were the regulatory authorities content, did they accept the results?

B: There is still a lot of work to do once the trial is finished. We have to gather all the data and have it prepared by following very strict rules, and to write a report for the

regulatory authorities. So we will go to them this fall, probably in October.

Now, for the first phase, did you actually use the pressure method which was developed in the US?

B: No, because we have to proceed step by step, and the first step was local administration of a low dose. Nothing bad happened. But it was really not a treatment, and brought no benefit to the patients. For the clinical development of a new drug, that is the best way to go. Phase I is to check for toxicity, safety, and tolerance. If the results are o.k, then you can move on to a study in which you want to see some benefit.

That would then be a phase-II trial?

B: Yes, but in our case, it is a phase-I/II trial, because we changed the protocol as we will switch from local administration to local regional administration by intravascular delivery. And this will be done with Jon Wolff's method.

I remember when I visited you, Jon, in 1991 with a father of a Duchenne boy. During the discussion, you stepped out of your office and came back with just a syringe and said "that is all we need".

W: That was the starting point. And even now we basically need only a tourniquet, a needle, and a pump. We tried injections into veins early, but with a big vein, we had a valve problem. With our present technique, that goes back to 1995/96, the administration is done into small veins, it was developed at my company Mirus by *Jim Hagstrom* and *Julie Hegge*. In the phase-I/II trial, we will use this method.

Nine children participated in the first trial who were 15 years old at least because they had to give informed consent. How will this be in the next phase, can you work with younger patients?

B: We wish to work with younger patients, and we will ask both the American drug agency FDA and the French drug agency to allow us to enroll younger patients. But first we have to accumulate more data and then have pre-meetings with them to explore this possibility.

How much time will you need to prepare the next phase?

B: It is difficult to say, because it takes several months to get an answer from the regulatory authorities, usually about three months. In our case with the first phase, it took 18 months.

Is there a reason why it takes so long? There are important people in these committees. Are they not interested? This is a disease that is just awful, it is terrible. And the children are dying away, the family's time is running out. Is there just red tape, bureaucracy, that they cannot work faster?

W: They are understaffed, at least in the FDA. **B:** And in France it is not different.

But in general, these people are interested that trials are being done?

B: They just don't have the experience and they are not alone, because this is a completely new field of research. They have to learn, we have to learn. We have to do the trials in a very safe way, and their role is to assure we are doing it very safely. That is why they take so much time and precautions and raise so many questions. And this is for the patients' sake.

At the meeting in January, Nick Catlin who represents

the British Parent Project, said quite emotionally what the parents really want: Even if there is risk, the scientists should go ahead.

W: That is not the right way to do things. **B:** If anything happens during such a trial, it would do a lot of harm to the whole field, it would stop everything. So we have a huge responsibility. And that was also what we felt with the phase-I trial: Had anything negative happened, that would have been detrimental to the whole field of gene therapy. **W:** Clinical trials are very expensive and time consuming, so you want to do it right, you need all the information you can think of.

We know that you have spent already 25 million dollars for the first trial. Has this being paid completely by the AFM? And your company, Transgène, must have provided something, too.

B: This amount was necessary for the whole trial but also for research, and for the salaries of the people working on this project. The AFM paid this completely and continues to pay for these trials. Our company Transgène provided know how, manpower, equipment, expertise, and scientific development. All this is highly valuable. We have three-year contracts with the AFM, the present contract ends at the end of June of this year. And as usual, every year, we provide them with complete reports and have meetings with the scientific committee. On a regular basis, they verify all the expenses. Transgène is a public company. So these financial matters are open to everybody.

If it costs so much money for the development, then how much will the treatment about cost to the parents? Will it be expensive?

B: Probably. But there is one example: Gaucher's disease. It is a rare disease, and the treatment with the missing enzyme is very very expensive, 300,000 dollars per year. It is covered by health care. We do not now how much the Duchenne treatment will cost, but it should not be as high,

How big are your companies, and how many people are working on this project?

B: Transgène has 165 people, and about 30 of them are working on Duchenne gene therapy, some of them part-time. **W:** And at Mirus and at the university in Madison there are about 6 people working with me.

To come back to the next phase of the trial: When will you be able to start and how long will it take?

B: It will take about a year for the preparation, and when the next phase is approved, we will probably need as much time as for the phase-I trial. That lasted two years and a half, maybe we can do it quicker this time. But it all depends on the regulatory authorities. If they ask again to enroll patients on a sequential basis, then it will take two and a half years. But again, that would be for safety reasons. And there is nothing we can do about.

And how many patients will take part in the phase-I/II trial?

B: It is still a matter of discussion. Maybe 15 patients.

Our families will say: Oh, we take our child and go to Strasbourg as others went to Memphis to Peter Law.

B: All the patients will probably be American and French boys. Nevertheless, this remains still open. But we are not responsible for deciding whom to enroll in the trial. Only clinicians have the right to make those kinds of deci-

sions. The principal clinical investigator in France will probably be again, as for the phase-I trial, Professor *Michel Fardeau*. The new trial will also take place in the US, but we do not yet know who will be the principal investigator there.

Will you start in the two countries at the same time? Will you use the same method, will you just have more patients?

B: Probably. We will ask the FDA and the French drug agency and we hope that the trial will be carried out in both countries with more patients than in France alone.

So you will need four more years, including one year to prepare, before starting with phase III?

B: If we see some clinical benefit in phase I/II, we might be eligible for fast track approval, because Duchenne is an "orphan disease", and because there is no treatment at all. But again, it is only "if". For a normal phase-III trial, we expect to need one hundred patients or more and will need another four years.

So can you calculate about how long it will take?

B: Well, we can answer in terms of best and worst scenarios. Best scenario would be a fast track approval by 2008.

And worst scenario would mean if something goes wrong with this or another trial for another disease, this would be a complete stop. That is possible, too?

B: It is possible, too. You cannot rule it out, no.

If dystrophin transfer works, at what age should the children be treated?

W: The younger the easier it will be. Maybe at 5 years of age, or 3 to 5 years

Will the medication be the same for all Duchenne boys and not have to be individually designed as is expected for exon skipping? And how often will one have to repeat the treatment?

B: Yes, the plasmids with the cDNA of the dystrophin will be the same for all patients. **W:** Perhaps we will need a booster every 6 months. So there might be an initiation dose to get to a certain level of dystrophin and thereafter only maintenance doses.

The cDNA of the gene, that is transported, is not integrated into a chromosome?

B: It should not. The probability is very low. It has never been demonstrated using plasmids or viruses in intramuscular delivery. **W:** And we should get long-term expression.

How will the treatment look? How will you produce the pressure?

W: With a blood pressure cuff. We will have to see how easy the procedure is. Maybe it could be done in the doctor's office. It will look like an infusion. The pressure is produced with a pump, about 500 mm mercury, less than an atmosphere.

So we can have some final words, addressed to the parents.

B: Because we are getting e-mails from everywhere, asking the same question about the time we need, we always give the same answer: I know it is frustrating for the parents that it takes so much time for the clinical development of any drug, but especially for Duchenne dystrophy. It is also frustrating for us, but we try to do it as quickly as possible. On the other hand, we also have to follow very strict rules, because this is for the sake of the patients, to make sure that nothing wrong happens. So this is why it takes so long.

W: Another thing the parents should know is that this gene transfer will not be a cure. The objective in all three phases of the trial is to preserve hand function. We are only treating a limb, the forearm. And this is an important first step that will improve the quality of life. When we see that this first step works, the next step will be treatment of the legs and then possibly the respiratory muscles and the heart. But right now the technique does not work well with heart muscles. Maybe we can make it working better somehow at a later time. The injections are regional, not systemic. In these first trials, we inject the plasmids into a vein at the wrist so that they get into all the muscles from the cuff down to the end of the hand. Again, it is not a cure but it is something to start with.

That was quite important to say. Thus this treatment will not affect the life expectancy of the children. To achieve this, will it take 20 to 30 years more?

W: Oh no, it will be sooner. **B:** Because if you show clinical benefit with the forearm, then you can move on to other limb muscles and even to the respiratory muscles.

W: And as more people work on the regional technology, it will become better and better. So, please understand, that these are important advances and that things are moving into the right direction.

On behalf of the organizers of this conference and certainly also in the name of the families with Duchenne boys, I wish you all the necessary success with your research work and thank you and your colleagues for your dedication and efforts to find an effective treatment for Duchenne muscular dystrophy.

Transfer of the micro dystrophin gene

In the second interview, the planned clinical trials using the transfer of very shortened cDNAs of the dystrophin gene were discussed. As an introduction, this technique is summarized first.

In the laboratory of Professor *Jeffrey Chamberlain* in Seattle, the scientists have performed considerable engineering of the shortened dystrophin cDNAs, the combined exons of the gene. They have identified a certain short version that is very functional and highly effective at combating dystrophy in the dystrophic mdx-mouse. This new micro dystrophin gene lacks most of the central portion of the dystrophin protein and the very end, the C terminal end of

the normal protein. This means that the last 17 base pairs of exon 17 and all following exons up to and including exon 59 were removed and at the end the exons 70 to 79, too. The resulting protein that normally has 3,685 amino acids was then 2,539 amino acids shorter, meaning that it was only 31.1% as long as the normal protein.

With experiments published in the August 2004 issue of the journal *Nature Medicine* (10, 828-834, 2004), the American researchers found a new method by which the type 6 of the adeno-associated virus (AAV6) with the micro dystrophin gene was injected *systemically* into the bloodstream of adult mdx mice. The vectors were injected

together with the protein VEGF (vascular endothelial growth factor) which makes the blood capillaries permeable for a limited time. It could then be shown that essentially all the voluntary muscles of the mouse were now making new dystrophin at very close to normal levels. The muscles showed an improvement in their function, and testing the muscle breakdown by measuring serum levels of creatine kinase showed a whole body improvement of the dystrophy.

This work shows for the first time that it is possible to deliver new dystrophin genes to all the muscles of an adult mouse. The focus of research now will be to determine if the method is safe and whether it can be scaled-up for larger animals and eventually applied in the clinic.

Dr. *Olivier Danos* is the Scientific Director of G n thon at Evry near Paris. He presented data on several

different serotypes of adeno-associated viruses used for therapy studies, viruses with different surface structures. Serotypes 1 and 6 are much better than types 2 and 5 for skeletal muscle gene transfer in mice. Preliminary studies showed that an arterial injection of AAV containing microdystrophin into the hind limb of a GRMD dog with muscular dystrophy led to production of low levels of new dystrophin in muscles of the leg. His group is also studying different gene regulatory elements to put into AAV vectors to control the dystrophin production, and showed very encouraging data using a promoter of the desmin gene.

With the support of AFM, G n thon is currently gathering pre-clinical data on the use of AAV vectors for microdystrophin gene transfer into the muscles of Duchenne patients. This work should lead to a clinical trial within the next three years.

Interview with Drs. Jeffrey Chamberlain (C) and Olivier Danos (D) on 20 June 2004

At the end of the first interview it was said, the aim of the study in France is to improve hand function only, it will not be a complete cure for Duchenne dystrophy. And if that works, one can probably extend this method to other limbs and to the lungs. If you are using microdystrophin for a therapy, what you will really get will be a Becker-type disease, isn't it?

C: We hope that, at the minimum, it would convert the characteristics of the disease into the milder Becker form of muscular dystrophy. It is possible that it may work better than that. From the experiments with animals it is difficult to predict the clinical effect in children. That is one of the reasons why we hope to get the technology into the clinic to find out how effective it is.

D: We still do not know the mechanism of correction with micro dystrophin. We can only make educated guesses based on our many years of experiments with transgenic mice. So, we have data that tell us that it will lead to some correction. However, whether it is going to be Becker-like, or whether it is going to be better, is impossible to say until we have done the trial with humans. That is one reason to do localized injections in the first trial, and then we can look at the muscle function in a human patient and be able to understand how well this micro dystrophin works.

But if it works, will it be a systemic method?

D: Today we cannot tell you how the treatment is going to be done, with what vector and by which delivery. We are just beginning. We are discussing how we can administer the vector systemically and get a correction in the entire musculature of a mouse. This is quite different from the situation a year ago. I think this is progress, but it is just the beginning of something new, and we are not there yet. The outcome of our experiments is just totally open and there is still much more work to do.

C: There are several stages to find out, how we can apply this technique to the patients. The first must answer the question of how well the micro dystrophins work in a human muscle compared to the mini dystrophins and the entire full-sized dystrophin. And that will likely start with injections into small muscles.

Will this be done in human studies in what you call phase I?

C: Yes, that's right. And if that is successful, we can

scale it up like it is being done in the other trial with plasmid DNA, to try to improve the hand function. At the same time, we will have to develop methods with animals to get a distribution of the gene to a much larger region of the body, perhaps into an entire leg, an entire arm and then, eventually into the whole body. But it's going to take a lot of work to see if that will really be possible to do first in animals and then in patients.

D: There are a number of things that have to be tested in clinical trials: The function of micro dystrophin after it is newly made, the best route of delivery, and also the vector itself. If adeno-associated viruses are used, we must know which particular type would be best. In clinical trials for a normal drug, you check for toxicity in phase I, then you increase the dose, then you do that in more patients, that is quite straightforward. Here, we plan local injections to look at micro dystrophin function and safety, and then we may try another mode of distribution. So, regulatory agencies will say: this is not a phase I and then phase II, this is a phase I and another phase I, because the conditions are different. It just means, we are going to a complex system with our clinical trials.

Will you work together in the future on these trials?

D: This is not decided. So far we have been working in parallel with the same kind of ideas and tools. This workshop, this round table, is so useful because we started to talk and are convinced it would be much better if we would work together. Otherwise, Jeffrey in Seattle and we in France would have completely different clinical trials.

C: It is important to have the different groups talking to each other and to meet on a regular basis and to see where we can help each other out. But at the same time, one does not want to completely merge all the research efforts in the world and have only a single program because that tends to stifle innovation and creativity. If you don't have independence and competition, then you may not make the next breakthrough.

D: In such a new and complex program, it is always difficult to make decisions. Should we work with this or that micro-dystrophin? Should we use this or that AAV serotype? Now we have several centers going in the same direction. Each is going to make different choices and that is good because you can never know what the results will

be before you do the experiment. If you have only one big program that goes in one direction, the chances that something goes wrong are much higher.

And then you have to start from scratch again.

D: Yes, and I don't think that it would save money in the long run. Such a large program would be much less motivating for the people doing the work. The new way is to work in parallel, to exchange information and to try not to duplicate efforts. Eventually this will reduce the costs of a clinical trial.

To get at the important question the parents always ask for instance: Will my son, who is 10 years old, still profit from this, when he is 15 or 20? Will it come fast enough to save my son? Therefore this question: When will you be able to start a trial, how long will it take for each phase?

D: There are other trials, but I do not see that any of these trials are going to save patients. They are likely to bring us important information, but they will not save patients. Of course, anything can happen, something we do not know today can be found and have positive impact. We are doing the best we can with the information we have today. It is a long process, development of a gene therapy is full of hurdles and problems. We solve one problem, then we go on to the next. We would love to be able to speed up the process, but it is just humanly impossible.

C: Research has to go in phases, from one area to another. We have worked for a long time mostly with the mouse model for muscular dystrophy, trying to understand whether it will even be possible to have an impact on this disease. And to take that from the mouse into the patients is a very slow and difficult process. Together with several groups over the next two or three years, we will probably begin studies with the microdystrophins to see if they are going to be safe and the methods of delivery will also be safe. Then we will look at the safety of more large-scale experiments.

But these experiments with micro dystrophins are only one type of approach to cure the disease. There are also advances being made in other areas of research. Known drugs are being studied, better steroid hormones are being looked at, and things like this. And hopefully, they will have a very positive impact on the disease also. All this will help the patients do better and live longer. And hopefully, an improvement in the disease will come a little bit faster. However, it is quite difficult to know how long it is going to take to really have an effective treatment.

D: It is always a problem giving dates. Anything can happen. We decided at Généthon to design an optimal pathway. This is just to put on paper, the time lines, how long each step will take. And if we do that, we can say, o.k., we will finish the animal studies at this time, then move on to toxicology, and then to the clinical trial. If everything goes perfectly well, and if there are absolutely no scientific problems arising – but that is not going to be true – we would start a trial in two years, in October 2006. That is about the time it takes to start one initial clinical study with microdystrophin. We can say that, but we know that we will have to revise these predictions all the time. So, it is by no means a firm date, and we will not say we have to meet a deadline. That is all I can tell you about times and how long it will take.

It was said at the meeting that the development of a normal drug takes 15 and more years.

D: Yes, and we are still at the first stage of the 15 years. We are really at the beginning. Obviously, we could say: from now on it will take 15 years. But, on the other hand, we do not want to lose the hope that something else will happen that is not gene therapy. There could be a new drug that will totally change everything, and we have to be ready for that.

But what can one say to the parents about the time? That it may take 10 years or 20? Their sons will be dead by that time. What can they do? Treat them as well as possible to keep them in good shape if something comes along, that they can benefit from?

C: That is really the best they can do, to take advantage of the best possible medical care and management of the disease that we currently have available. And even without a miracle drug or gene therapy break-through, the quality of care for the patients has improved tremendously compared to what it was 10 years ago. So this better quality of life of the patients is also slowly increasing their life span.

One must just take advantage of even small improvements in medical care and of new drugs that come along. Similar things are happening with cancer. There is no magic bullet that will cure cancer, but there are drugs that slow down some types of cancer. And there are many different types of treatment that can come together and have a very major impact in improving the life of the patients. My guess is that we will see things like that with muscular dystrophy.

Gene therapy may not be a complete cure, but it could strengthen the muscles enough so that patients can benefit from other approaches which will take them to an even better state of health. It is difficult to predict how these things might work, and the best hope is to keep as many research approaches under way as possible.

Another area is to try to avoid the birth of some Duchenne children by better carrier detection and genetic advice, for instance after a Duchenne boy was found by screening soon after birth. But not only the immediate family should be counseled, but also the families that are related to the mother. If the mother of a Duchenne boy is a carrier, her sister can be a carrier, too. This way, one can even avoid first cases in the related families. Carrier detection becomes more and more precise, the latest development is the new MLPA method of the MRC company in Amsterdam.

D: That will reduce the number of patients, sure, but will not eliminate the disease.

Because there are too many new mutations. But if there is a new mutation, it can start a new series of Duchenne boys in the following generations of families.

C: Screening at birth and being able to offer genetic counseling in time is a very important approach. And these new carrier assays are another advancement that we have seen over the last 10 to 15 years. But even with the information available, it seems to be difficult to get that out to the families and their relatives and let them know what it means.

The family doctors know the families, they should tell the immediate family what their responsibility actually is: to warn their relatives.

C: One of the problems of this disease is, that the gene that is defective, is such a large and very complex gene. For a long time, it has been difficult to screen it effectively

for mutations. But the advances of DNA technology have really simplified that enormously. It is going to be important to get the advances out of just a few research laboratories and make it more widely available throughout the world, so that we can have an effect on the frequency of the disease.

One of the reasons that Duchenne dystrophy is a common genetic disease, is that it arises spontaneously and at a higher rate than in any other known inherited disease. So it is always going to be with us. And that is why we need to continue working as hard as we can to develop a treatment.

Now, one other question: What actually is the relationship between Généthon and the French association AFM?

D: Généthon is a creation of AFM. It is a non-profit research institute whose main goal, 15 years ago, was to establish the physical map of the genome and to identify genes associated with genetic diseases. In 1997, I was invited to join Généthon in order to start a research activity centered around gene therapy. At present, most of Généthon works on gene therapy for genetic diseases. And 85% of the budget of Généthon comes from AFM. The rest comes from government grants and other sources.

At Généthon, we live together, literally in the same building, with the AFM. Every day, we meet, talk and have lunch with parents and the patients. This is a very important and constant motivation for us

The AFM is very successful in getting money for research, that is unbelievable. --- How many people are actually working on this gene transfer project at Généthon?

D: We have a team of about ten people who are responsible for moving this project forward using the common resources of Généthon. *Luis Garcia* and *Jean Davoust* who participated in this conference are important players in this project. All together over 50 scientists and technicians are working on projects related to muscular dystrophies at Généthon.

And how many are working in Seattle?

C: There is core of 6 or 7 people in my laboratory who are working on the micro-dystrophin project, but we have about three times as many who are working on other approaches towards muscular dystrophy.

There is another research laboratory in Seattle, headed by Rainer Storb, in the Hutchinson research laboratories.

C: *Rainer Storb* is one of the pioneers on bone marrow transplantation, working mostly with dogs. He has now developed a colony of Duchenne dogs. We are collaborating with him to test our microdystrophins in dogs. But his own research group is looking more at stem-cell based approaches using the dog to develop a treatment.

Over the last year, several researchers have moved to Seattle and begun working on muscular dystrophy. Among them are *Marie-Terese Little*, *Rainer Storb*, and *Stanley Froehner* who came from the University of North Carolina. We have now informal collaboration between many different laboratories.

We have time for a final word to say to the parents..

D: The parents are in a very difficult situation. Because

we are asking them to wait, this is the answer to your initial question. They will have to wait for a long time. The first trial will start in a couple of years. We researchers should be very humble, because we cannot make big predictions. We are only certain about the data we have and we know what we are going to do next. But, the parents should not lose hope, and that is very important. We are asking for faith, and that is very difficult. Of course, we can think about the future and carry some hope for the future. I wish I could do more than that, but nobody can.

C: I agree with that entirely. The important thing is to have hope, and to know that targets have been set. It is a slow progress and it never moves as fast as one would like it to, including us in the laboratory. But it is coming along, and, looking back 15 or 20 years, when I first started working on muscular dystrophy, it was very difficult to imagine that there could be an effective treatment for this disease. And now, we can imagine that there will be a treatment for this disease. We do not know exactly what that treatment is going to be or when it is going to come. But what we have seen with the animals, it is possible to have a major impact on this disease. Now we just have to struggle to find a way to make that a reality. And we can only bring things along as fast as we can.

The very last question: Is there enough money for this type of research? The parents themselves, when they have a young Duchenne boy, believe, they have to collect money, so that research is being performed to help their son. The amount of money will not be large, but it is important, too, isn't it?

C: Money is always important. There is a lot more money going to muscular dystrophy research now than it was 10 years ago, but I would never say, it is enough. There are always more things that we can imagine doing if we had more money. There are things you could bring on faster, but unlimited amounts of money would not bring an instant cure for this disease. There are things that cannot be made faster.

D: We need money for all kinds of things and we need it for the long term. But we need money also today to train young people, and they will be the ones to make progress in 10 years from now. I think the action of parents in collecting money is very very important, because they are a group of people who have said, "we together are all going to get money and give it to this project", then it is money that is earmarked for a certain activity and must be concentrated on this activity. So it is very different from paying taxes, and then waiting for the government to perhaps allocate some to Duchenne research. That is why it can be so efficient, and this is why parent associations are so helpful. Without them, our work could hardly exist.

Thank you very much for this interview. And certainly also on behalf of the families, I thank you for your dedication and wish that through your efforts a treatment for Duchenne muscular dystrophy will be found rather in the near than in the far future.

Proposed gene therapy trial at Ohio State University

Professor *Jerry Mendell* at the Children's Research Institute of Ohio State University in Columbus/Ohio is preparing a gene transfer trial with the aim to convert the symptoms of Duchenne into the much milder symptoms of Becker dystrophy.

The vector used will be a modified adeno-associated virus of serotype 2, called AAV 2.5. It will contain a mini dystrophin gene construction, $\Delta 3990$, that lacks the protein regions R3 to R21 and the C terminal end of the normal cDNA. This corresponds to the exons 14 to 54 and part of exon 55 as well as to the exons 70 to 79. Instead of 3,685 amino acids of the normal dystrophin, the protein made by this minigene will only have 1,230 amino acids, meaning that it would be exactly one third as long. This minigene has been developed by Dr. *Xiao Xiao* of the University of Pennsylvania in Pittsburgh and then used for successful gene transfer studies in mdx mice. Dr. *Jude Samulski*, director of the Gene Therapy Center at the University of North Carolina in Chapel Hill and of the bio-

technology company *Asklepios* will be responsible for the large-scale preparation of the vectors. This work is being supported by the Muscular Dystrophy Association of America with an award of 1.6 million US\$.

After the toxicology and biodistribution studies with animals are completed and the permission of the regulating agencies obtained, the trial will begin in the second half of 2005. It will be a phase-I/II trial with 6 Duchenne patients whose mutations of the dystrophin gene are known. They will be at least 10 years old so that they can give their informed consent.

The injections will be done double-blind into the biceps muscles using vector on one side and salt solution on the other, guided by magnetic resonance imaging. Two different doses will be used for each group of 3 patients. After 3 and 6 weeks, muscle strength will be measured quantitatively. Also after 6 weeks, a muscle biopsy will be performed to check for the presence of new but shortened dystrophin and of possible side effects.

Conclusion

To finish this report, a conclusion is attempted which is partly based on comments made by Professor *George Karpati* in Montréal.

At this meeting, two research gene transfer approaches were thoroughly discussed: First, the planned administration of a micro dystrophin gene construction with adeno-associated viruses into the blood circulation of dystrophic mice and, second, the regional administration under pressure of the combined exons of the full-length human dystrophin gene with plasmids into the muscles of Duchenne patients in the first clinical Duchenne gene therapy trial.

Microdystrophin transfer with viruses. This approach has shown impressive results. A single tail-vein injection of a relatively small amount of adeno-associated virus carrying only about one third of the dystrophin exons with a muscle specific promoter resulted in a generous production of shortened dystrophin in all skeletal muscles and in no other organs of the experimental animal used, the dystrophic mouse. There was no appreciable toxicity.

These impressive results may be explained by (1) an extraordinarily specificity of this type of viruses for skeletal muscle fibers, (2) the use of a promoter which activated the gene construct only in muscle cells, and (3), the simultaneous administration of VEGF which makes the capillary blood vessels permeable for a short time.

Of course, it is not guaranteed that all these favorable circumstances would also materialize in children, and the therapeutic effect of the very short micro dystrophin may also produce only a mild mitigation of the severity of the Duchenne symptoms or none at all. However, in view of the possible relative safety of the procedure, cautious human trials appear to be justified. And major technical and financial problems might arise when the large-scale production is attempted of the viruses with their genetic charge in the required quality necessary for this novel type of drug to be used in children.

The positive aspects of this approach are in summary: (1) The preclinical studies in mice gave favorable results,

(2) the method seems to be safe, and (3), no immune reactions were caused against the vector material or the new dystrophin.

However, there are also uncertainties and possible negative aspects: (1) The microdystrophin might produce only an insufficient clinical improvement, (2) the time course of any therapeutic effect has not yet been studied, (3) studies with larger animals than a mouse, for instance with the dystrophic dog, should be undertaken before children are treated, (4) the large-scale manufacturing of the vector may be prohibitively expensive, and (5), the maximal amount of the adeno-associated viruses injected into the blood circulation of children without negative side effects is not known.

Full-length dystrophin gene transfer with plasmids. The intravenous administration of a very large number of plasmids containing all the combined exons of the dystrophin gene, its cDNA, with its own control elements into a leg of dystrophic mice under pressure resulted in a variable but often appreciable production of normal dystrophin in all the leg muscles with relatively little side effects. In the completed human phase-I-trial, the local administration of similar "naked" genetic material proved to be safe, but the percentage of dystrophin-producing muscle cells was never larger than 25%.

In the second phase of the trial with Duchenne patients, it is planned to inject a rather large volume of similar naked DNA into a vein of the forearm shortly blocked with a tourniquet. It is expected that a high percentage of muscle cells will produce new dystrophin and thus improve the hand function of the patients.

The positive features of the proposed study include: (1) The feasibility of this procedure was proven in monkeys, (2) a large amount of naked DNA can be safely introduced into human muscles, and (3), naked DNA does not cause immune problems.

Uncertainties or even negative aspects include: (1) For practical reasons, one tries to treat forearm muscles first

although the hand function of Duchenne patients deteriorates rather late, (2) the large volume injected and the tourniquet application may cause significant collateral damage, (3) the longevity of the therapeutic effect is not known, and (4), the large-scale production of the plasmids in the required quality will be expensive.

The future: Since the discovery of the dystrophin gene in 1986, research for a therapy of Duchenne muscular dystrophy has progressed considerably. In addition to the two techniques discussed at the meeting and now in development for clinical trials, there are other promising approaches as, for example: exon skipping, stop codon read-through, upregulation of utrophin, and treatments with

drugs like prednisone or other substances found active in animal studies. As explained in the interviews, it will take still many years until an effective and safe therapy, based on the two discussed methods, will be available for the patients. Therefore, research on these and on all other approaches must go on without any delay and as fast as possible. But practically all these other techniques, as soon as they are sufficiently tested in animals, will have to go through the different phases of clinical trials, too. Thus, it is unlikely that any one of them will be able to produce an effective and safe therapy for Duchenne boys faster than the transfer of the gene with plasmids or viruses.

Participants of the round table conference

Scientists:

The scientists are listed with their abbreviated addresses and without any titles. Most of them are professors and all have an MD and/or a PhD.

Serge **Braun**, Transgène, Strasbourg, France
Barry **Byrne**, University of Florida, USA
Elisabeth **Barton**, University of Pennsylvania, Philadelphia PA, USA
Jeffrey **Chamberlain**, University of Washington, Seattle WA, USA
Jamel **Chelly**, Institut Cochin, Paris, France
Giulio **Cossu**, Università la Sapienza, Rome, Italy
Oliver **Danos**, Génomax-CNRS, Evry, France
Jean **Davoust**, Génomax-CNRS, Evry, France
George **Dickson**, Royal Holloway University, London, UK
Luis **Garcia**, Génomax-CNRS, Evry, France
George **Karpati**, McGill University, Montréal, Canada
Robert **Kotin**, National Institutes of Health, Bethesda MD, USA
Jerry **Mendell**, Ohio State University, Columbus OH, USA
Terence **Partridge**, Hammersmith Hospital, London, UK
Thomas **Rando**, Stanford University, Stanford CA, USA
Lee **Sweeney**, University of Pennsylvania, Philadelphia PA, USA
Sin'ichi **Takeda**, National Institute of Neurosciences, Tokyo, Japan
Jon **Wolff**, University of Wisconsin, Madison WI, USA
Dominic **Wells**, Imperial College, London, UK
Xiao **Xiao**, University of Pennsylvania, Pittsburgh PA, USA

Parents' representatives:

Filippo **Buccella**, Duchenne Parent Project, Italy
Nick **Catlin**, Duchenne Parent Project, UK
Christine **Dattola**, Duchenne Parent Project, France
Brian **Denger**, Duchenne Parent Project, USA
Sally **Hofmeister**, Aktion Benny & Co, Germany
Rod **Howell**, Muscular Dystrophy Association, USA
Peter **McPartland**, United Parent Project Muscular Dystrophy, UK
Luc **Pettavino**, Association Monégasque contre les Myopathies, Monaco
Christine **Cryne**, Muscular Dystrophy Campaign, UK
Jenny **Versnel**, Muscular Dystrophy Campaign, UK
Michel **Villaz**, Association Française contre les Myopathies, France
Elizabeth **Vroom**, Duchenne Parent Project, Netherlands

This report was written by:

Guenter **Scheuerbrandt**, PhD
Im Talgrund 2,
D-79874 Breitenau, Germany.
gscheuerbrandt@t-online.de

A report on all research approaches with results up to August 2003 can be seen in English, German, French, and Spanish at <http://www.duchenne-research.com>. Those who wish to receive the report on the second Monaco Round Table of January 2004 and future updates should send their e-mail address to Dr. Guenter Scheuerbrandt. All Monaco round table reports are available at <http://www.duchennefr.org> or <http://www.uppmd.org>