

## Exon Skipping, a therapy for Duchenne muscular dystrophy.

### Interview with Dr. Gerard Platenburg, President of Prosensa B.V. Leiden, the Netherlands.

This interview was recorded in Philadelphia by me, **Guenter Scheuerbrandt** PhD., on 19 July 2008 at the annual meeting of PPM, the American Parent Project Muscular Dystrophy. The following text is an edited and shortened version of the spoken interview. It has been approved by Dr. Platenburg for the information of patients, their families, and care-givers. My questions are written in italics, the answers of Dr. Platenburg in normal print. The chapters about the clinical trial in the Netherlands, as they appeared in my last report "Research approaches for a therapy of Duchenne muscular dystrophy" are shown at the end of this text and should be read as an introduction to this interview.

*In this interview, we should not repeat what I explained about exon skipping in my last Duchenne research report but rather speak about the future, the further development of this technique, which seems to be the farthest ahead of all research approaches.*

**The systemic exon skipping trial.** *Now, you finished the first local clinical trial, and you are starting the systemic trial.*

Yes, we started the systemic trial in May of this year. The boys receive the antisense oligonucleotide, AON, for skipping of exon 51 by subcutaneous injections, under the skin. This trial is an escalating dose study where we will test different dose levels, from one half to ten milligrams per kilogram body weight, injected once a week for five weeks.

*I calculated that for a boy of 30 kg receiving 10 mg/kg that will mean 300 mg every week.*

Yes, 300 mg at the high dose, this would not be more substance than one aspirin tablet has. We start with the lowest dose, and if it is safe to move on to the next dose, we will do so. Every week, another of the 12 little boys will get the first of his five injections. And we have the permission to extend the study with three more boys if we wish to better optimize the dose level. The subcutaneous injection has been chosen, because that would be the most comfortable way of applying the drug, and the family doctor could do it.

*And at the end of the trial, when you are through with all the boys, you have to wait for the skipped dystrophin to appear. Does that mean you will have to do biopsies?*

Yes, we will do biopsies, however not in all the boys before the trial, but in any case afterwards.

*And the boys are all from around the study centers in Sweden, Belgium, and Leiden? I am asking because I am often getting questions from families in Poland, Pakistan, and other far-away countries: "Can't we take part also?"*

The protocol has inclusion criteria. It is the doctors who tell us whether a boy is included or not. So it is the investigator who makes the decision. I do not have any say in that. Our company is just the sponsor. If a boy is enlisted in a local hospital near a study center, then there is a good chance that he will be included. The boys need to be in a facility of the hospital. You cannot fly them in from Afghanistan.

*And anyway, at least in the local trial, no clinical*

*benefit was expected. But here after the systemic injections, there might be functional improvement?*

Yes, it might be, I hope.

*As I understand, you try reaching about 20% to 30% of the normal amount of dystrophin.*

I would be very happy, if we obtain that.

*In the local study, you found only about 3% to 12% of the normal amount in the remaining muscle fibers.*

Yes, but I think, that is very difficult to compare. In the local study, there was only a single injection, it was very localized. When you take a biopsy after the treatment, you get a lot of untreated muscle tissue from the site of the injection. So these results could be an underestimate. And it could have been a lot of other things that had interfered with the analysis. This first study showed that exon skipping works in the muscle of a child. We don't repeat the study, we are happy with the results, and now the real development begins. And we expect a clinical benefit with the systemic trial.

**The next exons to be skipped.** *All the boys who take part in this systemic trial need skipping of exon 51. Which exons will be the next whose skipping you will develop?*

We have two antisense molecules in development for skipping exons 51 and 44. As long as we can financially do it, we will develop them up to the concept stage, that one sees benefit. And then we have also identified AONs for skipping other exons that need to be developed in addition to the two. On this initial list are the exons 43, 45, 46, 50, 52 and 53. We already have made the AONs for each of them.

*How about the other exons, especially those at the beginning of the dystrophin gene?*

I cannot exclude any exon which is not on the initial list. You need to have an initial list to look forward. We have a concept, and it is budgeted, and we will take on further exons if we can do that, that is a question of finances and people.

*But the trial itself is actually being done in the three clinical centers? Are the people there paid by your company?*

No, they are employees of the hospitals, but we contribute to the cost of the trial.

*How much about will the systemic trial cost your company?*

Millions of euros.

**The next clinical trial.** *How about repeat injections later? Will you look into this question too when you are through with the present trial?*

That will be the next phase, a phase IIb trial. But it is not fully planned yet. We did not have any discussions with the regulators yet whom we will have to ask again for approval. That new trial will be the pivotal trial in which we are going to treat many more boys, about 100, for at least six months.

*How long will the present trial and the new IIb trial take?*

The present trial will take about until the end of this year and the IIb trial, I think it will take at least nine months. But after we are through with the injections of the present trial, the results have to be analyzed before we can start with the large IIb trial.

**When will the AONs be ready?** *You remember our first interview about exon skipping in Monaco in 2004 when professor Gertjan van Ommen said, that it will take about 10 years - that will be 2014 - until the first AONs will be ready for the patients. Isn't that more or less realistic?*

I think it is realistic. But I feel that if we have the support of the regulators and the data are good enough that we can really show clear benefit, then it can easily be a little faster.

**The approvals needed.** *Do you need only the approval of the Dutch regulators, or also of others?*

For the trials, yes. But for bringing the product to the market, we have to go through the European EMEA and the American FDA.

*There is still a discussion going on whether the whole approval procedure will be needed for every single AON or not. Is there any hope that one general approval will suffice?*

The present total program will be for two products. If these are safe and efficacious, then we will try to convince the regulators that there should be a possibility to fast-track the other ones, to shorten the procedure for the ones which come after 51 and 44.

*And will that include more than the initial list?*

In principle, yes.

**The production of the AONs.** *Who produces the AONs? I think, you once told me they are being made by your company.*

Part of the production is done by external groups in the United States. And part of it will be done in the future by our company.

*So this will actually be your first product for Duchenne therapy.*

We feel that the technology can also be applied to other diseases. So we are developing molecules and treatment possibilities for myotonic dystrophy, spinal muscular atrophy, and Huntington's disease, which can possibly be treated by interfering with RNA splicing. But those are more in an early stage of research. The AONs against Duchenne will be our first products going into the clinic.

**The price of the AONs.** *Do you know anything about the cost? You will need kilograms of the AONs in pure form.*

*For instance, how much will one injection cost, or a treatment for one month or one year?*

The price depends on many factors: on the way you introduce the products, on how much money we have invested, and on many more things. It would hard for me to give figures. But it will not really be a cheap treatment. You have to be realistic. I mean, we are investing a lot, but if the benefit is there, a high price will be worth it.

*But as far as I understand, our health insurance system in Germany, if a treatment is proven to be effective and has been approved, the insurances have to pay, even if it is very, very expensive.*

It is the same in many other countries, too, also in the Netherlands.

**Marketing the AONs.** *I once calculated that about are half a million Duchenne boys are living in the world. How many of them will need 51 skipping?*

15% of all the boys would need 51 skipping. That would be 75,000, but to be realistic, I would say that the initial market will be about 10,000.

*Do you plan to market the AONs yourself, or will you have distributors?*

That is our ambition, yes. But the possibility to partner with other companies is still there. We will try to bring the product as far as we can. But we never know how these things go.

**The structure of the new dystrophins.** *Now the question about the different exon skipping treatments for different deletions. You will get different skipped proteins in boys with different deletions. Do you know the skipped dystrophins that will be produced?*

We know some of them, especially those that were produced in the boys treated in the first trial. But we don't know all those which will be produced in the other skipplings whether really all of them will lead to Becker symptoms.

**Multi-exon skipping.** *Many patients will need more than one exon skipped. Are you also working on multi-exon skipping? There is a theoretical proposal that skipping the 11 exons 45 to 55 would be a therapy for 63% of all Duchenne patients. And professor Terry Partridge is working with dystrophic dogs who need multi-exon skipping.*

Yes, but a dog is a dog. We are working on multi-exon skipping too, but it is very challenging. Skipping 11 exons at once would be great. If we had a good combination to do that, a cocktail of AONs, we would make it. Up to now, we don't have anything available which would work.

**The two types of AON.** *You are working with the 2'O-methyls. What is the reason for using them when the British and Steve Wilton think that the morpholinos are more effective? But they don't go into the heart, only, maybe, when you irradiate the heart with ultrasound.*

The reason for us was that we had to make a decision on the basis of data we had at the time we started, and the data then was that the 2'O-methyls worked very nicely in our hands. Combined with the fact that they can be produced easily and rather cheaply, we made the decision to move forward with them, because the process of product

development is very complex and very lengthy and costly. You don't change your strategy every day. What the others are doing, is optimization. If there is really a better way to do it, then I would be open for that. But then we would be where we started three years ago. So we are committed to move forward with the 2'O-methyls which, by the way, we are optimizing, too.

**The company Prosensa.** *Can you tell us something about your company Prosensa? Is it independent, how is it financed, can one buy stocks and, if yes, at what price?*

We are a private independent company, so you cannot buy any stocks, not yet. Concerning financing, we have many parent organizations helping us. And without the help of these organizations, we wouldn't be here. We have also some venture capital firms that are backing us. At the moment, we are well financed for the current trial, and I am trying to raise more money for the next, the pivotal trial.

*How many people are working in your company?*

As an introduction to the interview, the chapters about exon skipping in general and about the clinical studies in the Netherlands are reproduced. They are part of my last report "Research Approaches for a Therapy of Duchenne Muscular Dystrophy" published in May 2008. The entire report in English, German, and Spanish together with the two previous ones can be seen at the internet under [www.duchenne-information.eu](http://www.duchenne-information.eu). Those who wish to receive my future reports and interviews as soon as they are ready should send me their e-mail address to [gscheuerbrandt@t-online.de](mailto:gscheuerbrandt@t-online.de).

**Exon skipping is not a cure.** The *exon skipping* technique tries to slow down the fast Duchenne dystrophy into the much milder Becker dystrophy. It *does not alter the gene itself with its mutation*, but affects how the defective gene is read and processed. Exon skipping will *not be a complete cure for Duchenne muscular dystrophy*, it should only reduce the severity of its symptoms, it will only be *a therapy*.

If a mutation, a deletion, duplication or point mutation, disturbs the reading frame of the messenger RNA, mRNA, and thus causes Duchenne dystrophy, the frame can be *restored* by preventing the inclusion in the mRNA of one or more exons with *antisense oligoribonucleotides*, AONs. They are short pieces of chemically synthesized RNA whose sequences are designed in such a way that they attach themselves precisely to the complementary sequence of the pre-mRNA inside the exon to be removed or at its border regions, *and nowhere else*. These AONs thus interfere with the splicing machinery so that the targeted exon or exons are no longer included in the mRNA, they are *skipped*.

As this skipped mRNA is shorter than normal, the dystrophin protein is also shorter, it contains fewer amino acids. If the missing amino acids are part of non-essential regions, like the rod domain, the shorter protein can often still perform its stabilizing role for the muscle cell membrane. The result would be the change of the severe Duchenne symptoms into the much milder symptoms of Becker muscular dystrophy.

For the first exon skipping trials, two kinds of chemically protected AONs are used. They have to be protected because then they are not or only slowly destroyed in the muscle cells by nucleic acid destroying enzymes. The two types of AONs are the *2'O-methyl-*

We have now about 30, so we are quite young yet, but we will be growing rapidly, if this goes well.

**Some final words.** *At the end of this interview, would you please say some final words, not to me alone, but to the patients and their families?*

Yes, I am very positive. The data of the first clinical trial shows that we get the AONs into the muscle tissue and that exon skipping works in boys. It will be very exciting times now, in the coming months, to see whether the investment of years of intense work and of substantial capital has paid off. I think we have been able to move forward successfully step by step, and I am optimistic. In about half a year we will know more, much more. And again, I am really happy with the support of the parent organizations, because without this help from the families, I would not be here.

*Thank you very much, also on behalf of the families and their sons and all those who will read this interview.*

*phosphorothioates*, also called *2'O-methyls* and the *morpholinos*.

**Exon skipping trial in the Netherlands.** The *first in-human trial with the exon skipping technique* was performed in the Netherlands between January 2006 and March 2007. It was designed to provide a *proof of principle* only and not a therapeutic benefit to the treated boys. It was a *local study* on a small area of a single muscle, the *tibialis anterior* muscle of the shin, which was treated with a 2'O-methyl antisense oligoribonucleotide against exon 51 called *PRO051*. With this type of a chemically protected AON, the Dutch researchers had worked in pre-clinical experiments for many years and were able to successfully skip dystrophin exons in muscle fibers not only in cell cultures but also in living mice and dogs after local and systemic (into the blood circulation) injections.

Before the start of this first clinical exon-skipping trial, clinical and molecular genetic tests were performed on each boy to make sure that the exon skipping procedure in the living boys would produce the shortened Becker-type dystrophin with the expected structure. Four boys, who already were using wheelchairs, participated in this open study. They were between 10 and 13 years old and had proven deletions of the dystrophin exons 50, 52, 48-50, and 49-50. They were treated sequentially, meaning that only after the results for one boy were positive and did not show any serious side effects, the next boy was treated. Each boy received four injections of 0.2 mg PRO051 dissolved in 0.2 ml saline (0.9% NaCl) under local anesthesia into a small region of 1.5 cm length of the shin muscle *tibialis anterior*.

After four weeks, muscle tissue was obtained after a

biopsy from the injection site and tested for the expected skipped mRNA and shortened dystrophin. These tests showed that 64%, 85%, 97%, and 73% of the muscle fibers still present in the dystrophic fibers contained new dystrophin at their membranes after this 4-week treatment. Relative to laminin  $\alpha 2$ , a protein not affected by the dystrophic process, the dystrophin content was 33%, 35%, 17%, and 25%. This comparison takes into account the extent of the muscle degeneration. Without this adjustment, the 13-year old boy with much connective tissue and fat in his muscles had only 3% of the normal amount of dystrophin, whereas the boy with the least affected muscles had 12%. Molecular sequencing methods then proved that the new dystrophin had exactly the expected structure with a restored reading frame. It was impossible to determine whether the amount of the new dystrophin would have been able to slow down the progression of the disease in the entire muscle because the treated muscle tissue volume was too small.

These results signify that an exon skipping treatment, when it becomes available, should be started when most of the muscles are still intact, that is, immediately after the precise dystrophin mutation is known which causes a shift of the reading frame.

The Dutch researchers have now started a phase-I/II clinical study to explore the effect, safety, tolerability and possible side effects of *systemic* injections of the PRO051 AON into the blood circulation so that it can reach all muscles including those of the lung and the heart. This trial is performed in collaboration with the University of Leuven in Belgium, the Leiden University Medical Center and the Queen Silvia Children's Hospital in Sweden. Patients are now being enrolled.

The injections will be done subcutaneously (under the skin) because it had been shown with mice and monkeys

that this type of application caused exon skipping without serious side effects in all tested muscles, also in the heart and the diaphragm, and because it would not require frequent visits to doctor's offices and hospitals if repeated treatments will become necessary.

This exon skipping technique with the 2'O-methyl AONs was developed at the University of Leiden by Prof. *Gertjan van Ommen*, Prof. *Judith van Deutekom* and their team, but for the organization and performance of the clinical trials, the company Prosensa B.V. in Leiden with its president Dr. *Gerard Platenburg* is now responsible. Prof. van Deutekom is now head of research of Prosensa.

The systemic study will be done on twelve 5-15-year old Duchenne boys and will last five weeks with one subcutaneous injection every week. Because it has not been proven that the AON doses used in the animal studies will be safe to use in children, too, one will begin the systemic trial with a very low dose which will be increased slowly to approach an optimal level from initially 0.5 mg/kg/ injection to a maximum of 10 mg/kg.

Prosensa has already produced gram quantities of the AON against exon 51 in clinical grade quality for the coming trial. Also, AONs with optimized structures against exons 43, 44, 45, 46, 50, 52 and 53 have been prepared. These AONs together, including the anti-51 AON, would allow the treatment of over 65% of all patients with deletions. But, for financial reasons, Prosensa is developing at present only the AONs against exons 44 and 51 towards full clinical application and marketing.

The company needs more investment capital for the development of other AONs and appreciates the substantial funding received from parents' organizations like the Dutch and German Parent Projects, the French muscular dystrophy association AFM and others.

The details of the local clinical trial were published by Dr. Judith van Deutekom as the main author with a commentary by professor Eric Hoffman on 27 December 2007 in the New England Journal of Medicine: Van Deutekom JC, Janson AA, Ginjaar IB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007; 357; 2677-86. Hoffman, EP. Skipping toward personalized molecular medicine. N Engl J Med 2007; 357; 2719-22. Those who wish to obtain the pdf files of these, in my opinion, most important publications of Duchenne therapeutic research, should send me their e-mail address.

The financial support from the TREAT-NMD Neuromuscular Network for writing and editing this interview as well as my Duchenne research reports is gratefully acknowledged.

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