

## Exon skipping for the therapy of Duchenne muscular dystrophy.

### Interview with Dr. Annemieke Aartsma-Rus. Leiden University Medical Center, the Netherlands.

On the 1<sup>st</sup> of April, at the 20<sup>th</sup> Congress of the Scientific Council of the “Deutsche Gesellschaft für Muskelkranke” (German association of patients with muscle diseases) in Ulm in Germany, I, Günter Scheuerbrandt, PhD, recorded the following interview with Dr. Annemieke Aartsma-Rus (associate professor at the Leiden University Medical Center) on the present state of the exon skipping technique for the therapy of Duchenne muscular dystrophy, which is currently being tested in clinical trials on patients, to prove if this technique is safe and effective. This technique, in which Dr. Aartsma-Rus’s team is very actively involved, is the farthest developed of all research approaches and could potentially lead to an effective treatment for many Duchenne patients in the not too distant future.

The following text is an edited version of the spoken interview. It has been approved by Dr. Aartsma-Rus and released for publication. My questions are printed in italics, Dr. Aartsma-Rus’s answers in normal print. The scientific name of the potential exon-skipping drugs is *antisense oligoribonucleotide*, which is often abbreviated to *antisense oligo* or *AO*, also in this text.

#### **Introduction.**

*We will talk about exon skipping in this interview which will mainly be read by the families of Duchenne boys and young men. I have explained the details of this technique in my research reports which can be seen on the internet at [www.duchenne-information.eu](http://www.duchenne-information.eu). So many of the readers will know what exon skipping is, you do not have to explain it here. Let’s concentrate, therefore, on the clinical trials with Duchenne boys of this very promising technique and its results that will lead, hopefully soon, to an effective therapy for which we are all waiting for. But, please, Annemieke, start with introducing yourself.*

Since the year 2000, I have been working at the Leiden University Medical Center in the Department of Human Genetics, where I currently lead the group of about 10 persons that works on exon skipping. We focus on the development of exon skipping for Duchenne muscular dystrophy.

#### **Clinical trials for skipping exon 51.**

*Professor Gertjan van Ommen, the director of your Human Genetics Department, said in 2004 in an interview I recorded with him in Monaco, that it will take about 10 years until the first exon-skipping drugs will become ready for the Duchenne boys.*

I think that was a good estimate, because we are close to something now. But it will only be the skipping of exon-51 that may make this estimation. The phase-III exon-51 trial with 180 Duchenne boys is now enrolling patients. It is placebo-controlled and will hopefully show that exon skipping is effective and safe. For registration and marketing approval, the results will have to be presented to the regulatory agencies, the EMA in Europe and the FDA in the USA, who will assess these. This discussion will focus on many things, but mainly on the efficacy and the safety. Generally, in trials, there are side effects. The question is whether they are drug related or not and this can be assessed by comparing the side effects in the placebo group and the treatment group.

*The analysis of the results and registration application will take some time after the trial is completed. And then you will possibly be able to buy it in the pharmacy for those who need skipping of exon 51.*

It will only become available for the boys who need exon-51 skipping.

#### **Clinical trials for skipping the next exons.**

*And then the next skipping to be developed will be for exon 44.*

Yes, skipping of exon 44 is now in a phase-I/II trial with a small group of boys, but the results are not yet out. Thus, we don’t yet know whether the drug for skipping exon-44, the antisense oligo or 44-AO, leads to dystrophin restoration. Also, we don’t know whether a phase-III trial will be required.

*Does this mean that we may perhaps not need children to be on placebo for exon-44 skipping?*

Maybe, because if we know that exon-44 skipping leads to dystrophin restoration and if we know that dystrophin restoration leads to functional improvement based on the results of the 51-skipping, one could question whether a placebo-controlled trial is needed for exon 44 skipping. If placebo-controlled trials are not needed, then exon-44 skipping may become available sooner.

*Is this trial also a cooperation between GlaxoSmith-Kline, GSK, and Prosensa?*

Prosensa does it alone at the moment. For 51-skipping, GSK and Prosensa work together. GSK has an option for 44-skipping.

*And the next exons will be the others on the priority list?*

Prosensa is preparing the pre-clinical trials for skipping exons 45, 53, and also for exons 52 and 55.

#### **The 6-minute walk test to measure muscle function.**

*Concerning the 51-trial: if we look at the publication of the details of the trial on the NIH internet pages, they say the 6-minute walk test is the primary outcome measure, they do not mention the determination of dystrophin. What is the reason for this? Is it too difficult to test reliably for dystrophin?*

A drug cannot be approved just because there is dystrophin restoration. A drug can only be approved if there is functional improvement. Functional improvement can either mean that the boys get better, or it can mean that the progression of the disease slows down. For the functional

outcome measures, there are only some techniques that are validated and allowed by the regulatory agencies. The 6-minute walk test is a validated test which they are willing to accept for registering a drug. If you would like to use another test, then you have to validate that one and that may take years. So, it is easier and quicker to use the 6-minute walk test.

*Was this test being developed for Duchenne dystrophy?*

The 6-minute walk test was developed for patients with heart disease, not for neuromuscular disorders. The company Genzyme used it to find the medicine for Pompe's disease. They were the first to show that you can use it as an outcome measure for a neuromuscular disorder. And then the PTC Company in New Jersey validated it with Duchenne boys.

*After the PTC study for their drug Ataluren to read through premature stop codons, which didn't really work, they have published the 6-minute walk test done during their trial, and you could really see by the declining line on a chart that the lower dose of Ataluren had a better effect than the higher one.*

What we could see in the publication of the Ataluren trial that the boys in the placebo group got worse during the 48 weeks of the trial. That is the natural history of the disease, but there may be a placebo effect and the real natural history might have been even worse. For the boys treated in the present phase-III 51-trial, we hope that the declining line is less steep, or that some boys even maintain their function, and in the best case, even become better.

We have some more data from Prosensa's phase-I/II trial: all the boys participate now in the extension study where they get the highest dose of the trial, 6 mg/kg once a week. They have been treated for more than 18 months now and this study will continue. And we know that after 3 to 6 months, most of them did better. But, of course, in an extension trial, the placebo effect may be large, because all the boys know they are being treated and they also know that they would only be treated if there was dystrophin restoration in the initial trial. Although we cannot rule out this placebo effect, the data look very encouraging. Nathalie Goemans from the University Hospital in Leuven, Belgium, recently presented 6-minute walk data from patients after 48 weeks treatment showing that the effect on functional outcome improvement seems to persist.

*Will biopsies be made during the extension study?*

There was a biopsy after 6 months after the trial itself. I don't know whether there will be other biopsies. A muscle biopsy is quite invasive and painful, so it is not something you can do every month, every 6 months or even every year. After all, the boys don't have many muscles left at the beginning of the study. We want to limit the biopsies as much as possible.

And a muscle biopsy would be necessary if one wishes to find out whether the skipping drug leads to the production of new dystrophin and its messenger RNA during the treatment. But both, the messenger RNA and the new dystrophin, are difficult to test for. There are many variations of testing procedures, each laboratory has its own variety. As I said, every outcome measure has to be validated for the registration process, and it would take many years to develop standardized methods for dystrophin and messenger

RNA analyses. Researchers working on exon skipping are trying to standardize the assessment of dystrophin levels, but this will be challenging.

So, to get around this problem, we are now also working on a project to find biomarkers in the serum. This is an EU project with other scientists in Europe. It is called BIO-NMD and led by Alessandra Ferlini at the University of Ferrara, Italy, [www.bio-nmd.eu](http://www.bio-nmd.eu). If we know that the levels of certain proteins are higher in serum of Duchenne patients and these levels go down after dystrophin is made, these levels may act as a surrogate marker. It is much easier to take some blood every six months, than to make a muscle biopsy.

### **Clinical trial for skipping exon 50.**

*I know that AVI BioPharma in the US was concentrating on skipping exon 50.*

I have to base my information on press releases. So I know that for skipping exon 50, they developed a peptide-conjugated morpholino antisense oligo. Aurélie Goyenvalle in Kay Davies's laboratory in Oxford used such a modified morpholino oligo for skipping exon 23 in a very severe Duchenne mouse model that neither contains dystrophin nor utrophin. It worked really well in these mice which tolerated the modified drug very well. The problem is that we are not mice. When AVI tested it in monkeys, there were some toxicity issues. So I don't know whether they are now trying to optimize the drug by changing the peptide for making it less toxic. I don't know whether they stopped their work on clinical development of peptide-conjugated morpholinos entirely or whether they only delayed it.

AVI together with the British researchers around Francesco Muntoni and Kate Bushby performed a year ago another systemic phase-Ib/II study for skipping exon 51 with their morpholino antisense oligo which is now called Eteplirsen. Preliminary results have been reported at the meeting of the American Academy of Neurology in Honolulu in April.

### **Different types of antisense oligos.**

*Let us discuss now the two different types of antisense oligos mainly used in the present clinical trials.*

They have different chemistries and the chemistries have different characteristics. Their names are: 2'O-methyl phosphorothioates AOs also called 2'O-methyls which are used by Prosensa/GSK and morpholino AOs used by AVI. If you inject them intravenously or subcutaneously, they go into the blood stream and then will be eliminated by the kidneys. They are small molecules, so small that they are just filtered out. This is true for all AOs. The advantage of the 2'O-methyls is that their phosphorothioate backbone binds to serum proteins. Then the serum proteins act like a carrier. The unbound AO is cleared by the kidneys, but the AO bound to the serum is protected and therefore the serum half-life of 2'O-methyl AOs is much higher, several weeks. By contrast, the morpholinos are unable to bind to serum proteins and are thus filtered out much quicker. The serum half-life of the morpholinos is between only 2 and 4 hours. That is a big difference between the morpholinos and the 2'O-methyls.

If you compare the results of the morpholino trial and

the 2'O-methyl trial, you see that the morpholinos act very locally while the 2'O-methyls lead to a more homogeneous dystrophin restoration. The reason probably is that the 2'O-methyls have a greater chance to go to the muscle as they are present longer in the serum. The morpholinos have a very brief window of opportunity, they either go to the muscle and are very effective there, or they are filtered out by the kidney before they have a chance to do anything. We don't know for sure whether this is the main reason for the difference.

*The morpholinos are more expensive, yes?*

They are more expensive to make than the 2'O-methyls and higher doses are needed, therefore they are more expensive, but they are off patent now, so they should become less expensive than they used to be.

*How about Luis García's work of exon skipping with gene transfer?*

Of course the advantage of gene transfer is that you have to treat only once, and then this genetic medication is there forever. The problem at the moment is that it is not very efficient. Although you can treat all the muscles in an entire mouse, but a mouse weighs only 20 grams. At the moment it is quite difficult to treat an entire limb in a human because you have to inject the virus vectors into an artery under pressure. That is the first problem: you cannot treat the whole body of a child at once at the moment. There are some risks with gene therapy, because if it is toxic, it is there, you cannot stop it. With skipping drugs, you can stop the treatment, and the AOs will be cleared from the body and you can use another one, perhaps further optimized. However, if gene therapy is shown to be effective and safe, then of course it would be more attractive: just one single treatment. But we are not there yet.

### **Exon skipping for different mutations.**

*Now, would you please say something about exon skipping for the different dystrophin mutations?*

What is now being developed is single-exon skipping. Most deletions of the dystrophin gene need single-exon skipping. But there are also deletions that need double-exon skipping. Point mutations are more often in out-of-frame exons than in in-frame exons, because there are more out-of-frame than in-frame exons in the dystrophin gene. If it is in an in-frame exon, you need to skip only that one exon. But more often you need to skip two when the mutation is in an out-of-frame exon.

We know from experiments in cultured cells and animal models that skipping two exons is possible. But we don't know yet how to do clinical trials for two exons because the regulatory agencies might ask for more toxicity studies or maybe they will allow us to make cocktails of antisense drugs which are already registered. Or, maybe, we could just do separate injections of two AOs. That would be easier to get through the approval process than to use cocktails.

But we don't know yet what the regulatory agencies will say for a boy who has a deletion of, for instance, exons 46 to 50 and thus needs skipping of exons 51 and 45, which is quite common. So, skipping of exon 51 is in development, skipping of exon 45 is high on the priority list. When both are available, both can be prescribed and taken. We don't know yet how to deal with double-exon skipping

from a regulatory perspective.

*And how to repair duplications?*

Duplications are very difficult. The problem is that the AOs recognize both exons that are exactly the same. So if you want to skip one of those two, you will skip both of them. For single-exon duplications, there might be a way out, because you can skip a third exon before or after the duplicated ones, and thus restore the reading frame. But for larger duplications, it becomes very complex and thus very challenging, but we are working on it. So single- and double-exon skipping for deletions and point mutations may become available in 10 years, or maybe in 15 years, but for duplications it will take in all likelihood much more time or it may turn out not to be feasible to do exon skipping for duplications.

### **Exon skipping for rare mutations.**

*But is this time estimate meant also for single exon skipping in boys with rare mutations?*

Yes, but this is another problem, because there are only very few patients worldwide for each of the more than 100 groups of boys with rare mutations. Then it is very difficult to develop something, even if you want to do clinical trials, there are so few patients that it becomes statistically very problematic.

But the problem is that most of the boys with rare mutations who have a proper diagnosis are in the western world, and the majority of the patients live in China and India. There are some parts of China and India where diagnosis is very well done and care is also provided. However, for the majority of patients care is probably less than in the western world, there is no diagnosis, no-one knows their mutation, and they probably don't even recognize the disease. I think that is the first problem. Theoretically there may be more than thousand patients in some groups of rare-mutation boys, but we don't know them.

*I am trying to interest the Rotary organization to which I belong, to help me to get information to the pediatricians all over the world. And through them, we might get in touch with these "unknown" Duchenne families in the developing countries.*

I think that is a good initiative, and it is also surprising that in many countries where you wouldn't expect it, there are one or two labs that do very nice DNA diagnostics. I was in Rumania two weeks ago, and saw that there is a genetic lab that does mutation analysis with the modern MLPA technology. In India there are also laboratories where MLPA tests are being done. As Duchenne is a rare disease, generally diagnosis for Duchenne is done only at one location for the entire country like in the Netherlands or for a region.

*Does a list exist with these laboratories in the different countries? I am getting many e-mails with questions where these laboratories are.*

There is a list on the internet with the locations where you could have DNA diagnoses being done not just for Duchenne alone, but for other diseases too, for Europe the address is [www.eurogentest.org](http://www.eurogentest.org).

### **Other research approaches.**

*Now let's talk about the other methods which are also being developed and which might help the patients for*

*whom exon-skipping cannot be a therapy.*

Yes, for instance Idebenone, which may take care of the quality of respiratory and heart muscles, and perhaps also of skeletal muscle. Biomarin was developing a drug to upregulate utrophin, but stopped the development as in healthy volunteers the blood levels of the drug were too low. The problems which the stopped utrophin upregulation shows again that if something looks very promising in mice, then, the reality is often different in humans. It is very easy to get high enough concentrations in mice, but in humans it will be different. Why? We are not mice. That means, if things look very promising in mice, you have to be aware that we are much larger than mice and also different, and that the majority of techniques that work in mice does not work in humans.

For exon skipping, GSK is in a phase-III trial, and these studies will reveal whether this technique will work. For the other approaches, which are in earlier phases, it is very difficult to make any prediction whether or not it will work. Because again, if something looks very promising in mice or other animals, it might not work in humans. We are working on exon skipping for over ten years now, and it is still not there. So that shows that it takes a long time, until something is found that works, so it is very difficult to make any prediction about when a certain technique becomes an effective and safe medication against Duchenne muscular dystrophy.

#### **Living with Duchenne.**

*To finish our interview, I have a difficult question: is it really worthwhile to prolong the life of older and very handicapped Duchenne patients with all the modern management methods?*

I think it is a good question. And there is only one per-

son whom you can ask it: the patient himself. The patient has to decide whether or not he wants treatment and care. From our perspective, only to be able to move your finger or raising your finger and move your eyes, may seem like a horrible situation. But I know patients who are in that situation, and they say: “my life is very valuable to me, I like my life, and I have a computer, I can go to the theater, I can go to the movies, I can go to the soccer matches, and I am happy”.

I think if a patient is happy and wants to live, then it is his decision. But if he says, “I don’t want to live, I don’t want this kind of life”, then it is also his decision. And once he is an adult, he can make this decision, not to continue care. The positive attitude of Duchenne patients makes you quite ashamed about the complaining you do, while you are able to walk and to do everything, and this patient has only so little, and in spite of this, he is very content and happy.

And if you look back 20, 30 years ago, Duchenne patients didn’t have assisted ventilation then. Now they have it and the quality of their life improves a lot, because having enough air is very nice. Not only the duration of their life improved, but also its quality, and that is what counts even more. So, if you want an honest and convincing answer to your question, please ask the boy or young man with Duchenne dystrophy himself.

*With your so understanding answer to my last and not so easy question, we conclude our discussion about exon skipping, the most advanced Duchenne research, and – also on behalf of all the families and patients, who will read what you were saying to them here – I am thanking you for your explanations and everything you and your colleagues are doing to find a therapy for “our boys”.*

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