

## EXON SKIPPING

### NEW PHARMACOLOGICAL STRATEGY FOR DUCHENNE MUSCULAR DYSTROPHY

Duchenne Muscular Dystrophy is a recessive genetic disorder linked to the short arm of the X chromosome (Xp21 loci), which affects about 1 out of 35,000 births. Children with this genetic alteration suffer progressive muscle weakness leading to death in late adolescence or early adulthood.

The genetic basis is a mutation of the gene encoding dystrophin. This gene, one of the longest, known as *dmd* (*Duchenne Muscular Dystrophy*), contains 79 exons. The mutation of exons 47 or 48 causes the synthesis of a shortened version of dystrophin, which retains some of its activity, and is responsible for Becker dystrophy, a less severe type of dystrophy than Duchenne Muscular Dystrophy. However, another mutation, in exon 50 of the *dmd* gene makes dystrophin synthesis unfeasible. The lack of this protein leads to Duchenne Muscular Dystrophy, the most serious version of all muscular dystrophies. The gene associated with Duchenne Muscular Dystrophy <sup>(5)</sup> was identified in 1989.

Dystrophin is found in the sarcolemma, in both skeletal and smooth muscle, and also located in the heart muscle, the central and peripheral nervous system, and the retina. The lack of dystrophin (as in Duchenne Muscular Dystrophy) or the existence of partially functional dystrophin (as in Becker Muscular Dystrophy) leads to a progressive deterioration of muscle function, the most serious, and ultimately fatal consequence of these disorders.

Becker Muscular Dystrophy is milder because a partially functional version of dystrophin is synthesized. The life expectancy of patients with Becker Dystrophy is superior, in terms of decades, to that of patients with Duchenne Dystrophy, in which there is no synthesis of dystrophin at all.

Sadly, there is no treatment for these genetic disorders, except steroid therapy, with which the symptoms become less severe, and the muscle wasting associated with the disease is delayed.

A number of published clinical studies of drugs developed using the cutting edge technology known as "exon skipping"<sup>(5)</sup> have given encouragement to many parents of children affected by dystrophy (in its two forms, Duchenne and Becker). This new technique looks promising, and can be extrapolated for the treatment of other rare diseases, including Huntington's chorea. The idea behind Exon Skipping is that a genetic disease can be alleviated, even cured, not by splicing the defective gene to a correctly

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functioning gene (on which gene therapy is based), but by a partial correction of the defective gene. On Friday, September 20<sup>th</sup>, 2013 the new technique suffered a setback when the results of the largest clinical trial to date with a drug developed using exon skipping technology was reported. This drug, Drisapersen, failed to improve muscle function in children suffering from Duchenne Dystrophy as compared with placebo group. The announcement of the results <sup>(1)</sup> was a slap in the face to families with affected children, raising the question of whether Exon Skipping technology will provide useful drugs in the future.

The results have shown significant efficacy, although many families are reluctant to accept the decision of the Food and Drug Administration (FDA). Debra Miller, head of CureDuchenne, a support group for patients and family members who partially funded the research, believes it is too early to reject this line of research.

Drisapersen has been developed by the British multinational GlaxoSmithKline Pharma, and Prosensa, a small Dutch pharmaceutical company. The market capitalization of Prosensa fell 70% on the announcement of clinical trial failure.

Another drug developed using the Exon Skipping technique, called Eteplirsen, by Sarepta Therapeutics is currently in clinical trial phase <sup>(2), (3)</sup>. The results with Eteplirsen are anxiously awaited.

Gene expression starts with the transcription of one strand of the DNA double helix. The transcript, a chemically reduced nucleic acid, comprises a single-stranded messenger RNA. It contains fragments that are not translated into proteins, called introns, and fragments that do translate into proteins, called exons. The messenger RNA molecule undergoes a sort of "maturing" process, in which the introns are removed, and exons are spliced, to form what is called "mature messenger RNA". Mature messenger RNA is the template for the primary structure (the amino-acid sequence) of the protein.

Any mutation that causes the deletion of one or more exons, makes protein synthesis impossible, or, in the best of cases, the synthesis of a defective protein which retains only a fraction of its biological activity.

Consider a theoretical example: a set of consecutive exons: CA- UUUU - CAA- GAAG -CC. The code reading codons (set of three nucleotides) in the sequence of exons proposed would be: UUU - CAU - CAA- GAA- GCC. Imagine that a mutation causes the deletion of the second exon (- UUUU -). The reading of the mature messenger RNA codons would be: CAC- AAG -AAG. The amino-acid sequence synthesized would be distinct from the correct

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reading, in addition to lacking two amino-acids.

An ingenious solution to this problem would be to develop a drug that skips the exon adjacent to the mutation removed. This makes it feasible for RNA to continue reading correctly, but the sequence of the synthesized protein lacks several amino-acids. In the case of the sequence initially proposed (CA - UUUU - CAA- GAAG -CC), after the mutation causing the deletion of an exon (UUUU), the drug may skip the reading of the first exon (CA), and continue the correct reading of codons (triplets of nucleotides) (CAA- GAA- GCC). In this example the protein synthesized lacks two amino-acids, but its sequence is very similar to the original, being wholly or partly functional. In any case, the result is better than the synthesis of a non-functional protein or no protein synthesis at all.

In the case of dystrophin (the missing protein in patients with Duchenne Dystrophy, or with only residual activity in patients with Becker Dystrophy), the Exon Skipping technique causes a version of dystrophin that lacks two amino-acids in the central region of the protein, although it is still functional.

Dystrophin, which forms part of the sarcolemma of smooth and skeletal muscle, acts by way of a cushion against impacts and injuries.

In Becker Muscular Dystrophy, the presence of partially functional dystrophin improves the prognosis of dystrophy. In a way, the Exon Skipping technique aims to turn Duchenne Dystrophy into the milder Becker Dystrophy.

However, it is not known how much dystrophin is synthesized in patients taking the drug experimentally, how this modified dystrophin works, and how much protein is really necessary.

Experimental studies suggest that more than half of the dystrophin in muscles is synthesized after an experimental drug developed using the Exon Skipping technique, is administered.

Dystrophin protects muscles from injuries. Probably, the replacement of dystrophin for a modified protein related to the original, will not revert the genetic disorder of Duchenne Dystrophy, but could delay the patient's progressive physical limitations, and, for example, the need for technical aids such as wheelchairs.

According to *Steve Wilton*, professor at Murdoch University, in Perth, Australia, who collaborated in the development of Eteplirsen, treatment should be established as early as possible in order to minimize the progressive deterioration of the muscles.

One common way of assessing the progression of Duchenne Dystrophy is the distance a

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patient is able to walk in 6 minutes. Before the initiation of the clinical study with Drisapersen <sup>(1)</sup>, the 186 patients included in the clinical trial were able to walk an average of 340 metres.

48 weeks after injections of Drisapersen were administered the placebo group had reduced their autonomy by 58 metres, while the average decrease in the study group treated with Drisapersen was 42 metres. The difference is not statistically significant. In other studies with fewer patients, the difference observed between the two groups was just over 30 metres.

The other drug obtained by Exon Skipping technology is Eteplirsen by Sarepta Therapeutics. Eteplirsen has only been studied in a group of 12 boys. Of these, 8 formed part of a study group; and 4 were included in a placebo group. Of the first group, two soon lost their ability to walk after treatment began. The remaining 6 of the study group decreased their ambulation by 20 metres (6 %) over 84 weeks. When the boys from the placebo group were transferred to the study group, the deterioration in ambulation autonomy slowed down significantly.

Dr. Adrian Krainer, researcher at Cold Spring Harbor, is testing a modified Exon Skipping technology, called Inclusion Skipping, to treat Spinal Muscular Atrophy, a disease that is usually fatal during the early years of life. This genetic disorder, characterized by gene mutation SMN1, named loci [5q13.2]<sup>(6)</sup>, inhibits the synthesis of a protein necessary for the functioning of smooth muscles. **[SMN is the acronym for Survival of Motor Neuron]**. A second gene (SMN2) encodes the synthesis of a functional variant of the same protein. Gene SMN1 and SMN2 differ in one exon, in such a way that the encoded protein SMN2 is slightly dysfunctional compared to SMN1.

There is now a drug in the early stages of clinical trial that can prevent Exon Skipping while reading the SMN2 gene, achieving the synthesis of a fully functional protein.

One problem with the strategy of Exon Skipping is that different exons should be skipped to counter the various mutations that can trigger Duchenne Muscular Dystrophy. Thus, the skipping of exon 51, achieved by Drisapersen, could be helpful to about 13% of all children suffering from Duchenne Muscular Dystrophy.

Drisapersen was developed at Leiden University Medical Center, in collaboration with GlaxoSmithKline Pharma. According Annemieke Aartsma-Rus, associated with the investigation of Drisapersen, Exon Skipping technology could be useful to 83% of all patients with Duchenne Muscular Dystrophy. However, to achieve a very high response

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rate, the skipping of 100 different exons would be necessary. Instead of developing a drug for Duchenne Muscular Dystrophy, a variety of drugs would have to be synthesized, some of which would be useful to a small number of patients. This new Exon Skipping technique would therefore be the first approximation to "personalized medicine". But today, it seems unlikely that such a project could be carried out.

To date (January 2014) no drug developed using the Exon Skipping technique has been authorized.

GlaxoSmithKline Pharma and Prosensa are committed to the re-evaluation of the results of the failed clinical trial using Drisapersen.

Moreover, Sarepta Therapeutics plans to apply for approval of its drug Eteplirsen, from the results of the clinical study in 12 patients. In ideal conditions, Eteplirsen could be marketed late this year (2014).

Associations of the parents of children with Duchenne Muscular Dystrophy are trying to make the FDA change its position of not authorizing Drisapersen; as well as fighting so that the approval of Eteplirsen is not hampered as well. The parents' associations feel it is paramount that the day when the children affected by the disease need to be confined to a wheelchair be delayed as long as possible.

However, the FDA does not seem likely to facilitate the approval of Eteplirsen, considering that the preliminary results of trials using Drisapersen also showed favourable results.

We must assess the results of these potential medications from the standpoint of solidarity, compassion, and a deep respect for the victims and their families.

For many people with this genetic disorder, just to be able to walk a few paces independently is an enormous stimulus for enduring this terrible disease.

### Bibliography

1. GSK and Prosensa announce a primary endpoint not met in Phase III study of drisapersen in patients with Duchenne Muscular Dystrophy.  
<http://www.gsk.com/media/press-releases/2013/gsk-and-prosensa-announce-primary-endpoint-not-met-in-phase-iii-.html>. Consult.: December 2013.
2. Efficacy Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy Patients.  
<http://clinicaltrials.gov/ct2/show/NCT01396239?term=%22Eteplirsen%22+AND+%22AVI-4658%22>

- [22Sarepta+Therapeutics%22&rank=1](#). [www.ClinicalTrials.gov]. Consult.: December 2013.
3. Dose-Ranging Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy (DMD) Patients.  
<http://clinicaltrials.gov/ct2/show/NCT00844597?term=%22Eteplirsen%22+AND+%22Sarepta+Therapeutics%22&rank=3>. [www.ClinicalTrials.gov]. Consul.: December 2013.
  4. Aartsma-Rus A, den Dunnen JT, and van Ommen GJB. New insights in gene-derived therapies: the exemple of Duchenne Muscular Dystrophy. Annals of NY Acad Sci 2010; **1214**: 199-212.
  5. Aartsma-Rus A, Bremmer-Bout M, Janson A, den Dunnen J, van Ommen G, and van Deutekom J. Targeted exón skipping as a potential gene correction therapy for Duchenne muscular dystrophy. Neuromuscul Disord. 2002; **12**: 571-577.
  6. SMN1 gene.<http://ghr.nlm.nih.gov/gene/SMN1>. Consult.: november 2013.

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