

**Prof. Steve Wilton Presentation
Monday 26th October 2009
NUI Maynooth**

***Exon Skipping and the Dystrophin Gene:
A Light at the End of the Tunnel?***

On Monday 26th October 2009, MDI was pleased to welcome Prof. Steve Wilton to Ireland to speak to members about his research into exon skipping. Prof. Wilton is the head of the Molecular Genetic Therapy Group at the Australian Neuromuscular Research Institute, University of Western Australia in Perth, Western Australia. He was instrumental in developing exon skipping as a potential therapy for Duchenne muscular dystrophy, an approach which is currently in clinical trials in the UK and the Netherlands.

MDI would like to thank Prof. Wilton for travelling to speak with us and also to thank Prof. Kay Ohlendieck, MDI funded researcher, and his team for hosting the seminar in NUI Maynooth.



*Back: Prof. Steve Wilton, Karen Pickering (MDI Information Officer), Prof. Kay Ohlendieck
Front: Garry Toner (MDI Chairperson), Joe Mooney (MDI CEO)*

The following is a report on Prof. Wilton's talk.

Exon skipping is currently in trials for Duchenne muscular dystrophy, although if it is proved to work for Duchenne, it is hoped that the technique can be applied to some other conditions, such as spinal muscular atrophy and myotonic dystrophy.

The protein that is affected in Duchenne MD is dystrophin. Dystrophin acts as a molecular shock absorber in the muscle, but in Duchenne its absence leads to the gradual breakdown of the muscle. The dystrophin gene is the largest known gene in all forms of life. It is made up of 79 blocks of information called exons. Because the gene is so large, it is an “accident waiting to happen” in that there is an increased risk of a spontaneous mutation occurring in the gene. Spontaneous mutations are thought to lead to approximately one third of the cases of Duchenne, and in these cases there is no family history.

There are various ways in which changes in the gene can result in a diagnosis of Duchenne. There can be deletions of exons, duplications of exons, microinsertions or deletions and splice defects. To illustrate this, we can use a sentence in which every three letter word represents an exon:

THE BAD LAD AND HIS BIG MAD AND OLD DOG DID NOT GAG BUT RAN
AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN
AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN
AND RAN TOO FAR ON A RED MAT AND DUG OUT AND ATE THE SAD
FAT CAT END

Using the example of one kind of mutation, a deletion of the “A” in “AND”, causing a shift in the reading frame, we can see how it corrupts the message:

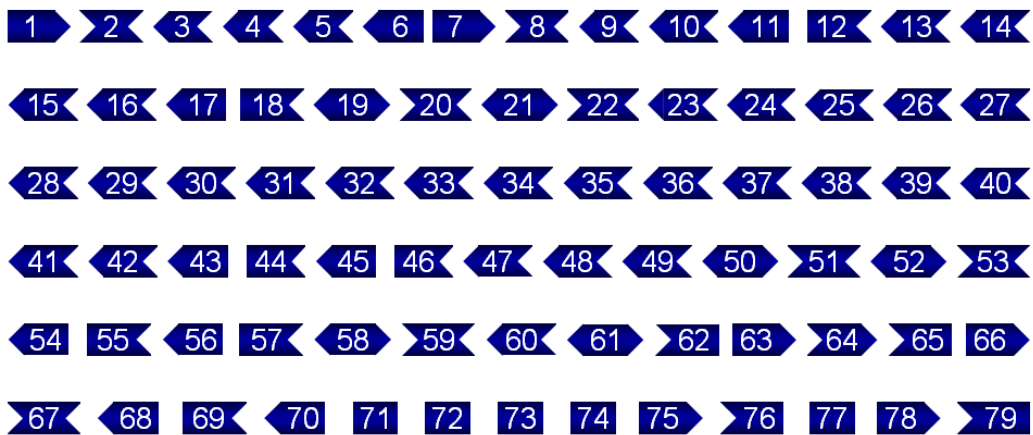
THE BAD LAD AND HIS BIG MAD AND OLD DOG DID NOT GAG BUT RAN
NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA
NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA NDR ANA
NDR ANT OOF ARO NAR EDM ATA NDD UGO UTA NDA TET HES ADF
ATC ATE ND_

The message cannot be read so no protein can be produced. The idea of exon skipping though, would be to skip over the area of the problem. In the example above with the deletion of the “A” in “AND”, if we could also skip over the “ND” in “AND” we would be left with a sentence like this:

THE BAD LAD AND HIS BIG MAD AND OLD DOG DID NOT GAG BUT RAN
RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND
RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND RAN AND
RAN TOO FAR ON A RED MAT AND DUG OUT AND ATE THE SAD FAT
CAT END

It is not perfect, as we have two “RAN”s together, but it is a readable sentence. It would mean that some protein could be produced and the person would have a condition more like Becker. The aim is to get as mild a form of Becker as possible.

The diagram below shows how the exons fit together. If for example there was a deletion of exon 50, you can see that 49 and 51 couldn’t fit together as the shapes at the ends are different. However, if you could skip over exon 51, you can see that 49 and 52 would then join up.



In order to carry out exon skipping, they are using antisense oligomers, which could be described as genetic bandaids. There are different versions. The trial in the Netherlands (the Prosensa trial) is using 2OMeAOs but the UK trial (AVI) is using PMOs. These need help for efficient in vitro delivery but once they are taken up they induce more sustained exon skipping.

Early studies involved intra muscular injections. However, you can't treat a boy with Duchenne by injecting every single one of his muscles, so research started looking at systemic delivery. Prof. Wilton's group demonstrated exon skipping throughout the body in a mouse model (Molecular Therapy, Fletcher et al, 2007). However, in this case it didn't work in the heart. Another group has been trying to improve the efficiency of delivery though, and they have a tag that is added to the PMO and this has resulted in delivery to the heart as well as the other muscles.

In the collaboration between Steve Wilton and Kay Ohlendieck's labs, it was shown that a lot of downstream changes such as levels of proteins including adenylate kinase and aldolase were being normalized after exon skipping in the mouse model. This shows that exon skipping is normalizing the tissue. The aim now is to demonstrate functional and histological improvements.

The steps to develop an exon skipping therapy include:

- Identify the mutation – not all may respond to exon skipping, and it may not be suitable for large deletions or if the mutation involves loss of an important part of the gene.
- Identify the strategy – what exon to target first. For example, if there is a problem between exons 50 and 51, will it work better if you skip 50 and 51 or 51 and 52?
- There is work still to do on clustered mutations. They can take out 2 exons very efficiently and can also do 4. Over this though, it gets messy.
- An individual response will be required – personalized medicine.

Clinical trials are now underway. The Leiden trial with Prosensa have shown that they are getting exon skipping and it looks promising. The MDEX

Consortium trial with AVI Biopharma has also demonstrated exon skipping and dystrophin production after intramuscular delivery, and it is currently in a systemic trial. If the trials are successful and this becomes a therapy, it will need to be a long term, ongoing treatment.

The challenges now for exon skipping are:

- Extending it to all amenable mutations
- Getting regulatory approval for many related compounds – the regulators are aware of this approach and once efficacy has been shown with exon 51 skipping, they will be able to approach the regulators with hard evidence
- Acceptable safety testing – this is a challenge as you cannot test this approach in healthy volunteers
- Patent and licensing issues.

Trials must be undertaken carefully. It is still unclear what dose will need to be given, how often and what the best way to administer it will be, e.g. intravenous, subcutaneous injection. It is evident that as this will be a personalized treatment that it will need to be administered under close clinical supervision, and individual responses will determine the dose regime.

Although much of the work so far has been done on Duchenne muscular dystrophy, the exon skipping approach has potential to be applied to some other neuromuscular conditions. Prof. Wilton has produced and sent a number of these “band aids” to Prof. Arthur Burghes in Columbus, Ohio, to test their potential in spinal muscular atrophy (SMA). SMA is the second most common autosomal recessive genetic disorder and the most common genetic cause of infant death before two years of age (SMA type 1). The gene that is affected, the SMN1 gene, is much shorter than the dystrophin gene, with only eight exons. There is a similar gene, SMN2, which normally doesn't produce normal protein because of a specific sequence in it. The aim would be to skip over this part of the SMN2 gene, and make it produce the protein. It is hoped that this could be a potential therapy for people with SMA type 2 and 3, and although it is in the very early stages, it will be interesting to see how this progresses.

Other conditions that are being looked at to see if exon skipping may benefit them include facioscapulohumeral muscular dystrophy, myotonic dystrophy and Friedreich's ataxia. It is an exciting emerging field of research and although there is a lot of research still to be done, there is optimism for the future.

For further information, please contact Karen Pickering, Information Officer, Muscular Dystrophy Ireland, 71/72 North Brunswick Street, Dublin 7. Tel: 01 8721501. Email: karen@mdi.ie