‘MOLECULAR PATCHES’ OR EXON SKIPPING AS A THERAPY FOR DUCHENNE MUSCULAR DYSTROPHY:

This question and answer document is aimed at explaining the ‘molecular patch’ technique as a therapy for DMD.

Q: What causes Duchenne muscular dystrophy (DMD) and the milder Becker muscular dystrophy (BMD) form?
A: Both are caused by genetic errors in the dystrophin gene, which affect the production of an essential muscle protein called dystrophin. Without functional dystrophin protein, muscle cells begin to weaken and eventually die.

Q: What is the difference between DMD and BMD?
A: In DMD, there is a total or nearly total absence of functional dystrophin protein. In BMD, a shortened dystrophin protein, which is partially functional, is produced which means the disease is less severe.

Q: How are proteins made?
A: A basic understanding of this is necessary to understand the therapeutic approach discussed later.

The genes we inherit from our parents contain genetic code called deoxyribonucleic acid (DNA). This can be likened to a string made up of 4 different letters (ATGC) arranged in a particular order that is unique for each gene. This genetic code is read to form proteins. These letters can be compared to our alphabet as different combinations of letters can create thousands of different words.

To make a protein there are three essential steps:

i) a copy of the coded DNA is made (called transcription),

ii) this copy is edited to remove any non-essential information (called splicing),

iii) the remaining code is read by the cell’s machinery in groups of three letters (called translation) to form the protein. These groups of three’s make up the reading frame.

These steps can be explained using an example of letters of the alphabet:

THEWTOMMANATIANDPIQHISURDIDOGITRANOVINFORTRIATHEDOOPIBUS

As you can see, this set of letters (representing the DNA) cannot be read because there are extra letters (known as junk DNA or introns, indicated as shadow areas) which need to be removed before the words can make sense. This process of removing the junk is called splicing.
The DNA is copied into RNA and then edited so that only the code essential for making the protein is left:

THE MAN AND HIS DOG RAN FOR THE BUS

If one looks closely and reads this code in groups of three you will see that it reads:

THE MAN AND HIS DOG RAN FOR THE BUS

Q: If both DMD and BMD are caused by errors in the dystrophin gene, why is one form more severe than the other?
A: In DMD the common errors either stop the production of protein because of a fault in the beginning part of the code, or the error changes the code so that it no longer makes sense. In the latter example we say that the error affects the “reading frame”. If we visualise this by imagining the error to be the removal of the letters ND in the example above – (remember the code is read in groups of three): this leaves us with coding which makes no sense and cannot produce any functional protein:

THE MAN AND HIS DOG RAN FOR THE BUS

Q: What happens in BMD?
A: In BMD, the loss of genetic code involves groups of three’s, and the reading frame is not affected. The result is a shorter piece of genetic code, which still makes some sense and produces a shorter but partially functional dystrophin protein:

For example the letters ANDHISDOG are missing. This leaves us with:

THE MAN AND HIS DOG RAN FOR THE BUS

This sentence is not complete but at least it makes sense and a partially functional protein is produced. You can see that the deletion in this example is larger than the example above causing DMD but because it is a multiple of three it does not disrupt the reading frame. There is still some sense, which means that some protein can be produced.
Q: Can we turn a DMD type mutation into a BMD type mutation?
A: Yes, this is the objective of using ‘molecular patches’.

Q: What is the ‘molecular patch’ or ‘exon skipping’ technique?
A: It involves making a very small piece of genetic material (‘molecular patch’), which once inside a muscle cell, will bind to its matching sequence of genetic code. This ‘patch’ is designed so that it binds a region surrounding the genetic error. When editing takes place to remove the non-essential regions of code, the area covered by the patch is not included in the final sequence which goes on to produce the protein. In this way the reading frame is altered so that it becomes readable. This may be clearer using the following example:
Consider the mutation we used above where the letters ND are missing. If a ‘patch’ is made to bind to the letters AHISDOG, and delivered to muscle cells, it will bind to its matching genetic sequence. During editing the AHISDOG will not be included and we are left with:

THE MAN RAN FOR THE BUS

The effect of this is to turn a sentence, which could not be read, into one which can. With the dystrophin gene, this is the difference between no protein being produced and a BMD like protein being produced.

Q: Does this really work?
A: So far scientists have shown this technique to have therapeutic effect in a mouse model of DMD (the mdx mouse) and in human DMD muscle cells grown in the laboratory. It has not yet been tested in living human beings so it is therefore very important that we perform initial safety trials.

Q: Do scientists understand how this works in the body?
A: Not fully. Scientists need to study the editing process further so that we can optimise the use of these ‘patches’ to make them work more efficiently and have maximum therapeutic benefit.

Q: Will it work for everyone with DMD?
A: No, but it is thought that around 60% of the genetic errors associated with DMD could be treated with the use of ‘molecular patches’.

Q: What other options are there if ‘molecular patches’ are not suitable for a particular type of genetic error?
A: This technique is just one of many identified by scientists as having therapeutic potential. Researchers world-wide are investigating many different approaches which may result in other therapies. These include techniques such as transferring a working copy of the dystrophin gene and drug treatments.

Q: How do you determine whether ‘molecular patches’ will be helpful as a treatment for a particular type of mutation?
A: There are specialised tests, which enable scientists to determine the exact nature of your genetic error. In many cases the doctors already know this information, especially if you have been diagnosed in recent years.
Q: Will the same ‘molecular patch’ work for everyone?
A: No, the dystrophin gene is very large and the genetic errors associated with DMD occur in different places along this gene. There are however some common areas for mutations and initially ‘molecular patches’ will be made for these to prove that the technique works. It is thought that several different ‘patches’ will be required to cover the spectrum of genetic errors. Once the technology has been shown to be effective for a particular error it will be possible to design other ‘patches’.

Q: Will ‘molecular patches’ help the more severe forms of BMD?
A: The effectiveness of ‘molecular patches’ is not dependant on the condition, in fact it has been shown to be therapeutic in other related conditions. The key issue is whether altering the way in which the genetic code is read has a therapeutic effect. There may be certain instances where ‘molecular patches’ might be helpful in severe forms of BMD; however most individuals with BMD will probably not benefit from this approach.

Q: I have heard about several different planned trials, what do these involve?
A: There are three planned safety trials based in Australia, The Netherlands and the United Kingdom. As these have yet to receive regulatory approval, it is not possible to discuss in detail how each of these will differ. The important point is that by having an International Consortium it is possible to ensure research is not unnecessarily duplicated and that the three safety trials will be designed to ensure results can be compared and evaluated. This will move the research forward to the next stage in the shortest possible time.

Q: Who will participate in the initial safety trial?
A: Participants will be selected using specific inclusion criteria. These will include age, the type of genetic error causing DMD, and a number of other clinical parameters. The vast majority of individuals are seen by specialists who keep detailed records, these will be used to select suitable candidates for the trial. It must be stressed that this is only a safety trial and there will be no therapeutic benefit for those participating.

Q: Are ‘molecular patches’ a cure?
A: No, this type of therapy is not a cure because the faulty dystrophin gene is still present. This means that if proven to be effective, this treatment would need to be repeated and how often this would need to be done will become apparent during this project.

Q: How quickly will this research lead to treatments?
A: It is very difficult to predict how long it will take to develop this research into a treatment. The important first step is to demonstrate that this technique works in humans and perform initial safety trials. If these do not highlight any potential problems with the ‘patch’ and/or the carrier used to take it into the muscle, it would then move to further phase II and III trials. These would require considerable sums of funding, as the effectiveness of the patch to restore dystrophin protein will be evaluated in much larger numbers of patients. Only if these trials demonstrated therapeutic effect could it be registered as a treatment. Again it is difficult to put a time scale on this, as it is impossible to know what stumbling blocks need to be resolved. A key point in this respect is the delivery mechanism.
Q: My son is 10. Will it be too late for him?
A: First we need to demonstrate that this technique can be used safely in humans. To reach this stage will take between two and four years. It is impossible to accurately predict how much longer it will take to go through the safety and clinical trials, which would be required in order to develop an effective treatment in humans. A clearer picture will emerge after this initial research and once the delivery issues have been resolved.

Because the potential of the treatment is to slow down the progression of the condition, it is likely that the earlier a boy with Duchenne muscular dystrophy could get the treatment, the more affect it would have on their quality and length of life. It is not possible to say at this stage what the specific benefits of the treatment would be on boys of different ages. It is however unlikely that this treatment would be able to restore significant strength if much of the muscle has wasted.

It’s therefore very important that people with Duchenne muscular dystrophy and their families do not raise their hopes too much at this stage. Whilst we all desperately want a scientific breakthrough, in reality this type of research does take many years to come to fruition. In the meantime it is important that we do all we can to improve the quality of life of those living with Duchenne muscular dystrophy.

Q: What are the hurdles to be overcome if this research is to lead to treatments?
A: Funding is the major hurdles we need to overcome. The International Consortium has received some funding for initial safety trials but much more is required to develop this research further towards the goal of a realistic treatment.

Once the technology has been shown to be effective for a particular error it will be possible to design other ‘patches’. There will have to be a lot of individual ‘patches’ available, to be able to select the appropriate set for any affected child. The safety and efficacy of each of the patches will have to be separately evaluated and this will add significantly to the cost and time scale of the process.

GLOSSARY:

Animal models: Animals having conditions comparable to humans which can be used to study disease processes. Animals represent a simpler system than humans for studying the roles of different proteins and for testing potential therapies. Results can then be applied to humans, the use of a simpler system often allows quicker progression of research.

Becker muscular dystrophy (BMD): A milder variant of Duchenne muscular dystrophy. It is X-linked, slowly progressive, causes muscle weakness and usually only affects boys.

Deletion: The loss of a bit of genetic material from a chromosome or gene.
**DNA:** Deoxyribonucleic acid, the chemical composition of genes. It contains coded information, arranged in a linear sequence. Each cell’s chromosomes contain about two metres of DNA, yet it is so thin that it is barely visible even with the most powerful microscope. If all the DNA in a human body were stretched end to end it would be long enough to reach the moon and back about 10,000 times.

**Duchenne muscular dystrophy (DMD):** A genetic disorder which causes progressive muscle weakness as the muscle cells break down and are eventually lost. Usually it affects only boys and is caused by a lack of dystrophin protein.

**Dystrophin:** The protein which is missing in boys with Duchenne muscular dystrophy and reduced in boys with Becker muscular dystrophy. Dystrophin binds to other proteins in the dystrophin-glycoprotein complex (DGC), absence of these components are implicated in different forms of muscular dystrophy.

**Genes:** The coded instructions that govern the make-up of every human being. Genes are made of DNA. Each gene carries instructions for the production of a specific protein. Genes usually come in pairs, one copy inherited from each parent. They are passed on from one generation to the next, and are the basic units of inheritance. Alterations in genes (mutations) can cause inherited disorders.