

## 2009 MDA Research Report

It is again very pleasing to be able to write this year's Research Report for you.

### Duchenne and Becker Muscular Dystrophy

As was the case last year, I am writing rather less on Duchenne and Becker muscular dystrophy (MD) and more on other neuromuscular disorders (NMDs). The reason is that many of you will have been kept up-to-date with developments in relation to Duchenne MD through emails and reports from Duchenne Foundation (formerly Parent Project Australia), from Dr Guenter Scheuerbrandt and from reading the reviews of relevant research that can be found on Dr Scheuerbrandt's and other websites, e.g.:

<http://www.duchenne-information.eu/>

<http://www.mda.org>

Dr Scheuerbrandt's reports list more than thirty different approaches to potential therapies for Duchenne MD, however, dispassionate assessment of therapeutic claims in both humans and animals is essential if everyone's hopes are not to be dashed.

#### *Exon Skipping*

Exon skipping is the most advanced approach to gene therapy of Duchenne MD and phase III clinical trials of exon 51 skipping are about to begin based on the successful outcomes of phase II trials using AVI-Biopharma AVI-4658 in a UK trial (12 boys, intravenous injection, ongoing, no problems) and Prosensa PRO051 in a Dutch trial (12 boys, subcutaneous injection, completed, no problems). Among those responsible for the design of Antisense Oligos (AOs) and the conduct of these trials have been Drs Bushby and Muntoni in the UK, Drs van Ommen and van Deutekom in the Netherlands and Australia's Dr Steve Wilton from Perth. But even if all continues to go well in the phase III trial, exon 51 skipping could only help about 13% of Duchenne boys. Nevertheless, in theory, most boys with Duchenne MD could be treated (not cured) by exon skipping, although other exon or multiple exon skipping would be needed for them. In previous Annual Research Reports, I have explained the principle of exon skipping and a good deal of further information about it is available on Dr. Scheuerbrandt's website. Basically, it involves sticking chemical patches (the AOs), Velcro-like, onto dystrophin genes so that the gene reading machinery in muscle cells will read across the large deletions that are the cause of the MD in most Duchenne and some Becker MD patients. In each case, a shorter, but more or less functional, form of the dystrophin protein would be produced, where little or none was previously present, thus converting the more severe Duchenne form of MD to the milder Becker type.

In a dramatic development, one of the world's largest multinational pharmaceutical companies, GlaxoSmithKline has just announced, on the 13<sup>th</sup> of October, that it will commit to developing PRO051, leaving the way open for the future development of drugs to promote the skipping of other individual and multiple exons.

Multiple exon skipping in laboratory studies and in animals has been attempted and success has been claimed, but with respect to a recent study in dogs with muscular dystrophy, even Dr Scheuerbrandt can be overly optimistic. He writes, "Based on several muscle function tests, the physical state of the dogs was stabilized at the same level as it was before the treatment started while untreated dogs degenerated considerably during this time. Thus, the systemic treatment seemed to have *halted their muscle degeneration.*" My own interpretation of this particular research is that its claims are doubtful and that much more convincing results will be required before human trials could be considered.

### *Other Gene Therapies*

Gene replacement or supplementation continues to hold promise as another form of gene therapy with many published accounts of reasonably successful production of the essential protein “dystrophin” and sometimes functional improvement in dystrophic animals following the injection of appropriate viral vectors containing various forms of the dystrophin gene. Unfortunately, even now, examples of successful gene therapy in other diseases that should be much simpler to treat than Duchenne MD remain few and far between due to the complexities of the immune system and to the problems of packing the relevant genes into suitable and safe carrier viruses.

Additional possible gene therapies have reached the stage of laboratory, animal and clinical trials, but, even then, we need to read the media reports, the drug manufacturer’s reports and those of commentators such as Dr Scheuerbrandt with considerable caution. The most widely publicised of these with some chance of success in the not too distant future is “read-through” of premature stop codons (nonsense mutations) by the drug PTC124, now called “Ataluren”, that is currently in clinical trial. I have commented extensively on this in last year’s Research Report along with the joint statement on it from our Medical Advisory Committee and our Medical and Scientific Advisory Committee. Suffice it to say that this international clinical trial is on-going, that theoretically and at best the drug would help only a minority of boys with Duchenne and that there is no guarantee of a beneficial effect of the drug for any of them. In particular, the majority of Duchenne cases are due to deletions or duplications in the dystrophin gene while only about 10% are due to any of the whole variety of premature stop codons and just a fraction of these might be assisted by Ataluren. This is because the effects of some kinds of premature stop codon are less readily counteracted than those of others. A realistic estimate of Duchenne boys who might be helped by Ataluren, lies somewhere between 0.5% and 5% (i.e. between 1 and 10 in every 200), providing that it proves to be effective, at all, at restoring dystrophin levels in humans.

### *Cell Therapies*

Some further approaches to potential therapies for Duchenne MD deserve brief comment. Many of these are a long way from being generally accepted and seem unlikely to succeed. “Embryonic stem cell” therapies are still at a very early stage of being trialled in animals with MD and success in humans will, probably, be very difficult to achieve in the foreseeable future because of ethical considerations, problems associated with extracting the correct stem cells from human embryos, amplifying them to sufficiently high numbers in cell culture and avoiding their tendency to develop into tumours. Despite reputable physicians and scientists being well aware of these ethical, technical and practical difficulties, some unscrupulous practitioners in other countries continue to offer injections of “embryonic stem cells” as a therapy for MD (at a high cost). No one knows what if anything, other than a salt solution, is contained in these injections or whether they might contain something that could be dangerous to the patient.

“Adult stem cell” therapies so far tested in animals have included the use of primitive muscle cells or cells similar to muscle stem cells from the walls of blood vessels, as well as stem cells from menstrual blood, bone marrow, fatty tissue and “fibroblasts” (from skin and tendons) that have been converted into primitive muscle cells in cell culture. These have been used in attempts to repopulate dystrophic muscle with functional muscle cells. There is no accepted consensus on the success or otherwise of these cell transplantation studies but most researchers concede that it is worth persevering with them even though, at best, they are likely to be imperfect in boys and young men whose muscles are severely wasted and replaced by fibrous and fatty tissue.

### **New Developments in NMDs - of worms, flies, mice, dogs and humans:**

*Caenorhabditis elegans* is a microscopic “elegant” worm. Its genome is completely known and so is every aspect of its development from egg to adult and every cell of its body. A dystrophin-deficient form of *Caenorhabditis* exists that has obviously ineffective wriggling. This is allowing the screening

thousands of potential drugs that could potentially counteract the lack of dystrophin. In this way, two carbonic anhydrase inhibitors, methazolamide and dichlorphenamide, have been found to be quite effective and they are already in use in humans for other reasons. Dr Segalat and colleagues at the University of Lyon in France have shown that these compounds not only enhance function in the worms but also in MD mice.

Fruit flies, like the worms, have very short life spans but are much more complex and have many naturally-occurring mutations that can be related to human diseases. One of these was discovered many years ago that affected survival and the muscles and eyes of the flies and so was called the “muscle blind” mutation in the relevant gene and protein of the fly. It has since turned out that myotonic dystrophy in humans is an equivalent disease and that there is an equivalent gene and protein called “muscle blind like” (MBL) that is involved. Already, more than a year ago, Dr Artero and colleagues from the University of Valencia in Spain showed that survival of the muscle blind flies could be improved by certain simple chemical compounds creating hope that something similar might be useful for humans.

As in the flies, human myotonic dystrophy is a multi-system disease affecting not only the muscles but also the eyes, the gastro-intestinal tract, the nervous system and glucose-tolerance. In humans with myotonic dystrophy, MBL gets stuck in the cell nucleus by binding to the abnormal CTG repeats in the DNA and CUG repeats in the RNA. I first heard an account of the potential for treating human myotonic dystrophy presented by Dr Ami Mankodi at the Mumbai meeting of the Asian and Oceanian Myology Center in May this year. Dr Mankodi and Dr Charles Thornton at the University of Rochester in the USA had been at the forefront of this new understanding of myotonic dystrophy. In very recent research performed at the University of Illinois in the USA by Drs Baranger, Zimmerman and their colleagues it has been found that MBL can be displaced from the DNA and RNA by certain simple chemical compounds that bind to the DNA and RNA in preference. This releases the MBL to perform its normal essential functions. These compounds have already been tested successfully in mice with myotonic dystrophy and there is great hope that this apparently complex disease in humans, will succumb to relatively simple treatment.

New drugs and methods for exon skipping continue to be developed. Among these are peptide-conjugated AOs that enter muscle cells much more readily than the bare AOs being trialled at present and even enter heart cells effectively, the “Vivo-Morpholinos” – octa-guanidino AOs - being produced by Gene Tools, Oregon, USA, that have similar advanced properties, peptide backbone AOs that are more stable than the others and allow increased flexibility in their design along with virally delivered U7 snRNA AOs being tested by Dr Kaye Davies at Oxford University in the UK that have promise not only for Duchenne MD but also for many other disease applications.

One of the major problems that occur in the various forms of cell transplantation, such as “myoblast transfer” and the stem cell therapies, is the relatively poor rate of engraftment of the transplanted cells onto the existing dystrophic muscle cells. Although still inadequate, as is growth of the transplanted cells into new muscle cells, considerable advances are also being made in these areas.

Another serious problem for all attempts at therapy of NMDs is the general replacement of degenerating muscles with fibrous and sometimes fatty connective tissue. Imatinib (anti-TGF-beta – transforming growth factor beta) and some other promising compounds inhibit inflammatory infiltrates and fibrosis (scar formation in the dystrophic muscle) in mdx mice. But, a delicate balance is required between the beneficial actions of TGF-beta (in the repair of wounds) and its harmful actions in degenerating muscle.

Naturally, worms, flies and mice cannot give us all of the answers to therapies. They are too small, their muscles do not suffer the stresses and strains of those in larger animals and humans and drugs that might work in them might be toxic to us. For these reasons, most potential therapies first require testing in large animal models such as dogs with muscular dystrophy and only when this proves positive, is it reasonable to establish clinical trials in humans. Even then, clinical trials in humans

must go through several phases, first to establish safety, then to establish effectiveness and finally to establish an effective dose and safety range.

## **Other NMDs**

### *Myotonic Dystrophy*

The amazing recent advances towards therapy of myotonic dystrophy have been discussed, above, under “New Developments....”

### *Congenital Muscular Dystrophies*

With respect to Congenital MDs laminin-111 therapy and a drug named Omigapil show considerable promise in testing in mouse models of these diseases.

### *Limb Girdle MDs*

Limb girdle MDs are typically caused by an absence of muscle proteins called sarcoglycans. There are several sarcoglycans each labelled with a Greek letter (e.g.  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) with the absence of each giving rise to a slightly different kind of limb girdle MD. As in the case of Duchenne and Becker MD, these could theoretically be treated by cell therapy, by appropriate gene therapy or perhaps by drug therapy. Mice with the relevant forms of limb girdle MD are available and are being thoroughly studied in the search for potential treatments. In one case, gene therapy has been so successful in the mouse model that volunteers are being recruited for a clinical trial in the USA. Another group of Limb girdle MDs results from an absence or inadequacy of the protein, dysferlin. In the USA, the Jain Foundation has been established to fund research into this disease. Just this year it has been found that three muscle proteins, dysferlin, caveolin-3 and mitsugumin 53 work together as a membrane repair complex. It was suggested some 30 years ago that MDs could result from excessive tearing of muscle cell membranes and inadequate repair. It is at last certain that excessive membrane damage is responsible for Duchenne and Becker MD as well as for the sarcoglycanopathies and some congenital muscular dystrophies while now it is apparent why a failure of membrane repair after damage has similar consequences. Mutations in the dysferlin gene and the caveolin-3 gene both cause types of limb girdle MD.

### *Facio-scapulo-humeral MD*

There is currently a resurgence of interest in FSHD with Mr Bill Moss, one of Australia's richest men and with FSHD himself, having established FSHD Global Ltd dedicated to “making up for lost time” in FSHD research. Along with the mouse model of FSHD available to study, many researchers are coming to a similar understanding of how the genetic defect on chromosome 4, in combination with certain environmental factors (now being called the “Exposome”), causes muscle cell death and failure of muscle cell regeneration in this disease. Hints to possible future treatments can be anticipated as a result.

### *Spinal Muscular Atrophy (SMA)*

As reported at the 4<sup>th</sup> Conference on Birth Defects and Disabilities in the Developing World in New Delhi by Dr Andoni Urtizberea just a few weeks ago, three new drugs are in clinical trial funded by the French Muscular Dystrophy Association (AFM). Also, a pilot trial of the anti-epileptic drug, valproate, was recently completed in 42 subjects aged 2 to 31 with SMA type I, II or III. Importantly, there was an increased mean score on the modified Hammersmith Functional Motor Scale (MHFMS) in 27 patients but there were some problems with weight gain and carnitine depletion in many subjects.

Interestingly, PTC Therapeutics, the company that is trialling Ataluren as a potential therapy for Duchenne and Becker MD, has discovered candidate substances for increasing levels of the protein,

“survival of motor neuron” (SMN) that is essential, as its name implies, for the survival of the movement control pathway from the spinal cord to muscles. We await further developments here.

### *Motor Neurone Disease*

In the case of motor neurone disease (MND), also known as amyotrophic lateral sclerosis (ALS), numerous substances are either being readied for testing and trials or are entering clinical trial phases. One of these seems to have great potential for treating one form of familial MND.

### *Ataxias*

As I wrote last year, polyglutamine (polyQ) expansions, gene variations that give rise to increased lengths of polyQ in proteins (i.e. longer than normal runs of QQQQQQQQQQQQQQQQQetc) are the cause of a number of diseases including several varieties of spinocerebellar ataxia and Huntington’s disease. Using a mouse model of spinocerebellar ataxia due to a polyQ expansion, a Japanese research group showed a dramatic disease reversal after gene therapy to introduce an enzyme into brain cells to destroy accumulated poly Q.

Many other varieties of spinocerebellar ataxia involve ion channels. Given the understanding and success that has been achieved with respect to the treatment of epilepsies, many of which are also ion channel diseases, significant developments can be expected here in the future.

The antioxidant, idebenone, is now well accepted as being an effective treatment for the cardiomyopathy in Friedreich’s ataxia although any beneficial effect on neurological function is less certain. To test for neurological effectiveness, two new high dose phase III clinical trials are being conducted, one in young ambulant patients in the US and one in Europe that is unrestricted as to age or disease severity. As well, additional drugs with considerable potential are undergoing laboratory testing or are already in clinical trials.

### **International conferences, co-operation and coordination**

Last November and December, I attended and presented scientific research communications on the work from my students and myself at two conferences in Melbourne: Firstly, at the 7<sup>th</sup> Annual Meeting of the Asian and Oceanian Myology Center (AOMC) and then at the Annual meeting of the Australian Physiological Society. Since then, I have attended two conferences in India, the 8<sup>th</sup> Annual Meeting of the AOMC in Mumbai in May and the 4<sup>th</sup> International Conference on Birth Defects and Disabilities in the Developing World (ICBDD-DW) in New Delhi in October. I gave two presentations at the Workshop on Neuromuscular Disorders preceding the ICBDD-DW conference and attended a Board Meeting of the World Alliance of Neuromuscular Disorders Associations (WANDA), as its president, as well as a Board Meeting of the International Genetics Alliance, as an observer.

WANDA is an organisation with a mission to facilitate the development of therapies from the laboratory to the bedside: the Roadmap to Treatment. If we are to reach our destination with respect to Care, Treatment and eventual Prevention of NMDs through many potholes, washaways, construction sites, detours, road blocks and gridlocks, we must demonstrate the skills and ingenuity of the Indian drivers that I saw on their roads and highways as they tried novel approaches, daring, bravery and utter persistence. And then again, we could follow the example of the Indian pedestrians who were prepared to risk their very lives, weaving between oncoming bicycles, motorcycles, auto-rickshaws, cars, buses and trucks in their determination and ultimate success in crossing to the other side of the road.

Perhaps the most encouraging developments in the whole area of neuromuscular disorders are the recent amalgamations and agreements between Industry, University and Patient and Parent Groups in the USA, UK, Europe and elsewhere in the world to facilitate research in exactly this way. We can expect a quite amazing increase in the rate at which basic discoveries can be translated to effective therapies as a result.

## **Research in South Australia**

With respect to my own contribution to neuromuscular disease research over the last year, I have edited several further Commentaries published under the heading, “What the Journals Say” in the journal *Neuromuscular Disorders*. I have also had scientific research papers published from my own research group in the *International Journal of Biochemistry and Cell Biology* and submitted for publication in other journals.

Recent work from my laboratory in the Sansom Institute in the Division of Health Sciences at the University of South Australia, by my PhD student, Linlin Ma, in collaboration with our colleague Dr Grigori Rychkov at the University of Adelaide and Dr Jie Zheng and his student, Ekaterina Bykova, at the University of California, Davis, involves fluorescent proteins as markers in cells. Our Adelaide and Davis research collaboration has involved the use of fluorescent proteins, namely, Cyan (blue-green) Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP) to help us work out the molecular defect in the muscle disease, myotonia congenita (Thomsen’s Disease). Because the fluorescence of one such fluorescent protein can influence the fluorescence of a neighbouring fluorescent protein, provided it is close by, it is possible to use two of these to work out distances between any two positions inside cells and to determine if and when this distance changes. Dr Zheng visited our laboratories at the Sansom Institute and the University of Adelaide in May this year for discussions regarding further research collaboration and publication of our results so far.

Within my institution, the Sansom Institute at the University of South Australia, other relevant research is also occurring:

1. New agents that might promote read-through of premature stop codons (nonsense mutations) continue to be assessed by Prof Doug Brooks and his colleagues. As above, these might be useful in treating a minority of boys and young men with Duchenne or Becker MD or children with spinal muscular atrophy or cystic fibrosis.
2. Prof Brooks is also studying a variety of different diseases known as “liposomal storage diseases” of which Pompe Disease is one. Pompe Disease fits classically into the category of MD-type diseases and is one of those that are typically catered for by MD organisations internationally. It also remains one of the few MD-type diseases for which therapeutic success has been achieved (with “Myozyme” produced by the pharmaceutical company, Genzyme), as I have reported in previous Research Reports.
3. Dr Corey Xiao and his students are conducting research on bone growth, which might initially seem to have little to do with muscle disease. On the other hand, osteoporosis is an adverse side effect of the corticosteroid treatment of Duchenne MD and the study of bone growth and strengthening is directly relevant. Also, even closer to my own research, the muscle cell membrane protein that is mutated in the muscle disease, myotonia congenita, has a close relative that occurs in osteoclasts, cells that normally dissolve bone. When bones are healthy, the activity of these cells is closely balanced by the activity of other cells, called osteoblasts, which lay down new bone. Excessive osteoclast activity causes osteoporosis, but if the function of the relevant protein could be blocked this could constitute a treatment for osteoporosis and counteract the adverse effects of corticosteroids.

## **Impossible Things**

You will remember that in past years I have asked you to practice believing in impossible things as suggested by both Lewis Carol, the author of *Alice in Wonderland*. It seems that you have been doing this increasingly because more of the things that, even last year, seemed impossible, are now at the point of becoming possible with regard to treatment of neuromuscular diseases.

The US government has recently committed US\$117million to NMD research – about A\$130million. This will have required intensive lobbying by patients, parents and researchers.

In France, patients and parents run the AFM and its telethon raised 105 million Euros in one day last year = AU\$170 million. On a population basis (we have 22 million people and France has 60 million)

Australia should raise AU\$60 million in a one day telethon. There is every possibility that this could happen as David Jack, CEO of Muscular Dystrophy Foundation Australia, is negotiating just this kind of telethon for Australia with recent agreement from Jerry Lewis to host it in 2011. Many of you will know that Jerry Lewis has hosted the MDA telethon in the USA since 1966 and has raised several billion dollars for NMD services and research in the process.

### *Impossible Equipment*

Genuine brain to brain (B2B) thought transmission has just been achieved by researchers at Southampton University in the UK. One person with EEG electrodes has only had to think about moving their left arm or right arm and their brainwaves have been de-coded by a PC into a string of zeros (left arm) or ones (right arm). These were then sent over the internet to a second PC that controlled flashing LED lamps which were set to flash at a slightly different frequency depending on whether zeros or ones had been received. A second person observed the lights but the different frequencies were so subtle that they could not be registered consciously. When a set of EEG electrodes was used on this second person and their brainwaves decoded by a third PC, it could be determined whether zeroes or ones had been unconsciously received by the second person. It is suggested that B2B communication could be useful for people with no muscle function and for people with neurological conditions such as “locked in” syndrome.

Obviously, thought controlled wheelchairs and other equipment are possible by these means and some examples have already been tested.

Petaflop computing (peta means one million billion – a one with 15 zeroes after it – and one flop in computing terms means a single computation in one second) is also now a reality. In the US, a Cray XT5 super computer, Kraken, contains 16,000 AMD six core 2.6 GHz Istanbul processors and 129 terabytes (one thousand billion bytes) of memory and has a peak performance of 1.03 petaflops. It is stated that this enormous computing power will enable increased understanding of everything from the physical makeup of the universe to the causes of global warming to the roles of proteins in disease. It bodes well for our understanding of NMDs, almost all of which result from absent or mutated and non-functional proteins.

*So, don't give up. Remember with respect to developments of helpful equipment, advances in research and increases in fundraising, as Mark Twain proposed on his visit to Adelaide in 1895:*

**TRUTH IS STRANGER THAN FICTION. IMPOSSIBLE THINGS CAN TURN OUT TO BE TRUE.**

And continue to dream – dreams can also come true – but they often need a lot of hard work. You - MDA's members, Patients, Parents, Carers, Researchers, Clinicians, Allied Health Professionals, Staff and Volunteers - you are the ones who have the reason and the commitment to put in the hard yards. No one else will do it for you. And from what I have seen at the two conferences that I have attended in India this year, NMD organisations in developing countries are advancing rapidly because their members have more reason, more commitment and more determination to succeed than we do.

So if you can, please help by making a donation or by assisting, however you can, to raise funds for our People who have NMDs, our Association and for our Research Foundation.

Professor Allan Bretag