TREAT-NMD Workshop
The Development of Antisense Oligonucleotide Therapies for Duchenne Muscular Dystrophy
25th September 2009
EMEA Headquarters, London, UK
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Aims and Objectives

This initiative is a collaborative venture led by TREAT-NMD as a partnership between members of the academic community and advocacy groups involved in management and research on Duchenne muscular dystrophy (DMD).

The rationale behind our request to meet EMEA derives from the recent preclinical and clinical trial results of antisense oligomers (AOs) as a therapeutic option for boys affected by DMD. These encouraging results, currently with boys who can be helped by AOs to skip exon 51, suggest that this approach could eventually provide a therapeutic option for the majority of boys affected by DMD. Studies that will hopefully lead to the registration of compounds to skip exon 51 will start very soon.

However an issue which is of major concern relating to this novel therapeutic approach is the fact that different AOs will be required to treat DMD boys carrying different mutations. At least 7 antisense will be required to treat 70% of deletion and duplication patients (40% of all patients), but many more antisense will be required to treat less common mutations and eventually all DMD boys who might benefit from this approach (>80% of all DMD boys).

This level of personalised approach is currently without precedent for a genetic disease. It is the concern of advocacy groups that if the common path of taking new drugs into the clinic is followed for each individual AO, this will threaten the viability of this approach. It appears inconceivable to identify resources to bring 30 or more novel antisense AOs in the clinic via the traditional clinical trial pathway. A fresh look at the process is therefore needed to obviate this threat to a novel and promising therapy for DMD boys. We are therefore seeking to identify a pathway that will allow the safe and efficient progress of these drugs, and address the anxiety that there are otherwise significant hurdles to the further development of this potential treatment for DMD, an incurable and fatal disease.

It is with this spirit that the present document had been prepared to address this novel clinical treatment by the members of multidisciplinary steering committee preparing for the EMEA discussions with additional contributions from Prosensa B.V.* and AVI Biopharma* where appropriate.

Chair: Francesco Muntoni*


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A. The parents’ perspective

Duchenne Muscular Dystrophy (DMD) is an unforgiving disease. Parents and family members never forget the day that the consultant gave them the awful news that their son has Duchenne, while their small, smiling son is in the ‘golden age of Duchenne’ and unaware of what lies ahead. Apart from long term use of steroids, with their considerable side effects, little can be done to delay or slow, let alone stop the inexorable and lethal progression of this disease.

The prognosis provides little hope, even with the best supportive care of well equipped modern specialized units, and such basic life achievements such as going to college, getting married, having a job or career or raising his own family are thrown into question. We all love our sons and are very happy when they are able to go to University or find work, and in some countries where care and social services and education are well coordinated young people are now living longer. But even in these best case scenarios, young people are eventually living full time on ventilators sometimes with tracheotomies and require very expensive full time care.

Young men with Duchenne must necessarily rely on others to care for their most personal of needs such as toileting and feeding as well as everyday activities of daily life. Frequent hospital visits, physical therapy, orthopaedic operations to correct joint contractures or scoliosis, dietary supplements, recurrent chest infections requiring hospital admission and antibiotic therapy, cardiomyopathy, ventilatory support, power assisted wheelchair provision – any one of these burdens would be considerable on its own. Duchenne boys and their families have to face many of these and more, simultaneously, and for their rest of their already shortened lives.

The new medicines

The development of medicines using exon skipping with anti-sense oligomers (AOs) has now at last built upon the knowledge of identification of the dystrophin gene in 1986 to bring new drugs into clinical development. We recognise that these drugs will not cure our sons but for the first time we can see the possibility of slowing or even reversing the inexorable progress of the muscle wasting. This is something that can offer our sons a future. A future that may not be always free of the need for a wheelchair but these new drugs could offer a real improvement in the quality and length of life. Preservation of heart and respiratory muscles are life-saving. Preservation of arm and hand function is vital to be able to use a computer, a joystick and eat, all necessary to participate in normal life.

Personalised medicines- our hopes and our concerns

There are now a series of exon skipping clinical trials planned that patient groups have supported by developing new Duchenne patient registries and funding research work. Parents and their families have enthusiastically embraced these trials and have come forward to work with clinical teams to trial the new drugs. The wide range of variations in the gene that results in Duchenne will mean that a series of drugs very closely related in structure (indeed, with the same basic “backbone”) will be needed to promote skipping in Duchenne boys with different type of mutations, which combined comprise up to 85% of patients. These will be compounds closely related in basic chemical structure, but each modified to a very specific genetic type, thus providing truly “personalised medicine” for Duchenne boys.
As parents, we have some concerns

Can the current regulatory requirements for assessing benefit/risk balance for new chemical entities provide a framework to enable the timely assessment of AOs for the majority of patients? Families can see progress with one target exon, for the time being exon 51, but are asking if drugs tailored to meet their own sons needs will be developed, and if so when? We fear that pharmaceutical companies will be unable to fund very expensive and repetitive animal testing over many months for new drugs before they can even begin clinical testing. Then, will the companies be obliged to repeat the same series of clinical trials for every possible gene variation, once the basic chemistry has been studied? Even the most prevalent group of genetic types that can be treated by a single AO, the ones to skip exon 51, only treat about 13% of the Duchenne boys. All other AOs treat even smaller subsets, yet companies now are eager to develop these treatments with the encouragement of patient advocacy groups. Each new AO under the existing regulations might thus have to undergo several years and many millions of Euros cost in animal testing, and then several more years and tens of millions of Euros in clinical testing. For some less common mutations we might even not have enough boys worldwide to fulfill all the regulatory obligations to test this specific AO. What about the boys who need 2 different AO’s because 2 exons should be skipped to be in frame again?

So far, widespread testing of the AOs in both normal and dystrophic animals seems to be well tolerated with little risk of serious adverse consequences detected. Longer term work is ongoing to better define the benefit/risk, but DMD boys and their families are eager to try these drugs now, already believing that the potential life enhancing benefits outweigh the risks of long term exon skipping. It is important to consider carefully and clarify the definition of ‘BENEFIT’ in progressive debilitating conditions such as Duchenne. No further degeneration or steady state should be considered clinically relevant and evidence of benefit in DMD. Also, while we do understand regulatory agencies ask for data to show the drug is effective in ambulant boys, the majority of Duchenne patients are non-ambulant. They should not be the last ones to benefit from new developments as they have less time left.

Without compromising the appropriate level of safety testing for each variation of the same backbone molecules we feel it is necessary to consider fast tracking the development of examples of new sequences using an already tested “platform” for these new exon skipping medicines. In this context, we understand the new European pharmaceutical law contemplates, in Regulation (EC) 726/2004, that special regulatory approval procedures may be reserved for medicinal products of major therapeutic interest in order to meet “the legitimate expectations of patients and to take account of the increasingly rapid progress of science and therapies.” It is gratifying to note that the European Medicines Evaluation Agency and other Regulatory Authorities are currently considering alternative, more flexible approaches to benefit/risk assessment particularly for diseases where there is an unmet medical need, and new, much needed, treatments are in development. We welcome the opportunity to work with the Regulatory Authorities to ensure timely access of the new approach of exon skipping to treating Duchene Muscular Dystrophy patients.

“These are exciting times, full of hope for new medicines to treat our sons but we cannot be left in a position where we have developed drugs for only a minority of patients and the majority is left without access to treatments. The Regulatory authorities can make a huge difference by agreeing new procedures to fast track the trial of medicines with a view to treating up to 85% of our children.”

Filippo Buccella1*, Nick Catlin2*, Pat Furlong1*, Sally Hofmeister1*, Divyesh Popat2 and Elizabeth Vroom1*
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2. Action Duchenne, UK
B. Background

Duchenne muscular dystrophy (DMD) is one of the most common genetic disorders affecting children and young adults. It is a severe muscle wasting condition with onset in early childhood, with the progressive muscle weakness and wasting leading to the patients becoming wheelchair bound by their early teens, which without treatment, leads to death by the early twenties. The social and emotional costs and burden of disease are one of the highest for a childhood onset disease for patients and their families. The economic costs have been estimated in an Australian Access Economics report to be €257 million per year for health care, or €75,000 per affected person, and the value of lost well being (disability and premature death) estimated to cost a further €600 million annually.

DMD is caused by mutations in the DMD gene that leads to a failure to produce a functional muscle protein called dystrophin. Laboratory studies over the last decade have shown that the addition of antisense oligomers (AOs), specific to critical regions of the DMD gene, in cultured patient muscle cells and by injection into muscles of a mouse or dog models for DMD, can induce the expression of a shortened dystrophin protein. More recently, repeated systemic (intramuscular, intravenous or subcutaneous) administration of AOs was shown to be capable of restoring a sustained dystrophin expression in both the mouse and dog models of DMD, and this was followed by a significant functional improvement of the muscle function. Two proof of concept studies using intramuscular administration of exon 51 AOs have been completed successfully in Europe by Prosensa and AVI Biopharma: two follow up studies with the same AOs are assessing the safety and efficacy of repeated intravenous(by AVI Biopharma in the UK) or subcutaneous (by Prosensa in Europe) administration of the AOs in young DMD boys. If these studies prove that this approach is safe and effective in affected DMD boys, the repeated systemic administration of AOs might be the first effective tool to slow down, halt or even reverse the disease progression in this condition.

C. Prevalence of DMD within the community

DMD is one of the most common fatal genetic disorders to affect children on a global basis. Approximately one in every 3,500 boys born worldwide is afflicted with DMD, with a global prevalence of ¼ million. Due to prenatal diagnostic screening the incidence is falling. However, at least one in three cases is due to a de novo mutation, so even if screening operates at maximum efficiency, new patients will still be born with high incidence.

D. Clinical description of the orphan disease

(i) Details of DMD

DMD is a devastating and incurable muscle-wasting disease associated with specific mutations (65% of which are deletions, 15% duplication and 20% nonsense and other small mutations) in the gene that encodes for dystrophin, a protein that plays a key structural role in maintaining muscle fibre stability upon fibre contraction. Most cases are diagnosed by the age of 5. Progressive muscle weakness of the legs muscles eventually spreads to the arms, neck, and other areas. By age 10, braces may be required for walking, and most patients are confined to a wheelchair by age 12. Eventually, the disease progresses to complete paralysis and increasing difficulty in breathing. The condition is terminal and death occurs often in the early twenties. International guidelines for care have recently been generated (Bushby et al, Lancet Neurology submitted). These emphasise that although the underlying disease course cannot currently be modified, attention to the complications of DMD that arise in different systems by an experienced multidisciplinary team does change this natural history. Amongst the interventions which are currently the mainstay of management are corticosteroid use to improve
muscle strength and function, proactive pulmonary surveillance and care including nocturnal ventilation, and early cardiac treatment. The need for these interventions and their intensity varies as the disease progresses. With such interventions it is possible to extend the life of many affected individuals by at least another decade. However interventions such as chronic corticosteroid use come with significant complications which in themselves require proactive prophylaxis and management, and ultimately these patients continue to develop severe muscle weakness and are completely dependent on others for all aspects of daily life activities. Despite the range of interventions that can improve longevity and quality of life in DMD today, none has any effect on the underlying disease process and though they are crucial to the patient and family, in essence most can be regarded as palliative. Novel interventions are therefore eagerly awaited.

**Phases of DMD**

Stage 1: PRESYMPTOMATIC
Toddlers may be diagnosed at this stage if creatine kinase (CK) is found to be elevated by chance or if there is a positive family history. They may show developmental delay including late walking but there is little or no gait disturbance. Speech may also be delayed and both speech and language support may be needed.

Stage 2: EARLY AMBULATORY
Here, Gowers manoeuvre, waddling gait, or even toe walking is observed, but they are still able to climb stairs. At this stage the difference between a boy with DMD and his peers becomes more marked as unaffected children gain motor skills quickly. Physiotherapy interventions help to maintain good and symmetrical posture and use of corticosteroids can lead to a gain in muscle strength which causes also functional gains. Instigation of surveillance of respiratory and cardiac status is important at this point in time. Support with schooling may be called for.
Stage 3: LATE AMBULATORY
Patient’s exhibit an increasingly laboured gait, losing the ability to climb stairs and rise from floor. Physiotherapy input becomes increasingly important at this juncture as the positive effects of steroids begin to be less evident and there are increasing numbers of falls. Orthopaedic input may be prescribed to reduce the impact of joint contractures on muscle function.

Stage 4: EARLY-NON-AMBULATORY
Patient’s may still be able to self-propel for some time and to maintain posture, but are likely to develop scoliosis (abnormal curvature of the spine). More intensive monitoring is essential to check for both this and other orthopaedic complications. There is also an increasing risk of respiratory and cardiac complications, which may also require intervention.

Stage 5: LATE NON-AMBULATORY
By now, upper limb function and postural maintenance is increasingly limited. This is the phase at which respiratory support is frequently required, initially only at night, but later on additionally during the daytime. With time, support for feeding may also be appropriated. However, even with optimal medical management, the lifespan of a DMD patient is severely limited.
(ii) Duchenne versus Becker muscular dystrophy

Mutations in dystrophin leading to DMD disrupt the open reading frame, completely abolishing dystrophin function in muscle fibres. Dystrophin functions to link the skeleton of the fibre to the outer connective tissue layer, providing stability to the fibre upon contraction by buffering the forces generated. Due to out-of-frame mutations, only the first part of the protein can be produced, and the link to the connective tissue is lost. Without functional dystrophin, muscle fibres cannot withstand the continual forces imposed upon them during contraction and become cumulatively damaged during normal exercise. Eventually, this results in the death of muscle fibres and replacement by connective and adipose tissues, resulting in loss of muscle function.

Becker muscular dystrophy (BMD) is also caused by mutations in dystrophin, including deletion of entire exons, but the open reading frame is always maintained. Thus an internally-deleted dystrophin is produced that still contains the domains required to fulfil its structural role within the muscle fibres. These shortened dystrophin molecules are thus partially functional and consequently BMD patients are considerably less severely affected than DMD patients. Generally, diagnosis of BMD is made in adolescence or adulthood, and the overall progression of the disease is slower. The majority of BMD patients become wheelchair dependent 10-15 years after diagnosis, though some remain ambulant till late in life, with essentially normal life expectancy. The consequential functional differences between mutations in dystrophin leading to DMD compare with BMD are illustrated in the below figure.

![Figure 1: Schematic diagram illustrating the central role of dystrophin in muscle. Dystrophin cross-links the extracellular connective tissue to the internal cytoplasmic actin filament network, via a collection of protein complexes at the sarcolemma (plasma membrane). It's repeating spectrin-like structure confers both elastic and rod-like features, which allow it to act as a 'molecular shock-absorber' protecting the muscle cells from forces](image-url)
imposed during continual muscle contraction-relaxation forces. Loss of dystrophin, as seen in DMD, breaks this link and the muscle cell is severely weakened. There is also secondary loss of other sarcolemma proteins. Mutations leading to BMD shortens the dystrophin molecule, but it retains binding ability at its ends and so the cross-link is maintained, albeit it with less strength.

E. Antisense-mediated exon skipping

(i) Exon skipping rationale

Exon skipping represents a novel therapeutic approach for the treatment of DMD, which unlike existing treatments, directly addresses the lack of functional dystrophin protein that causes the disease. As there is currently no such therapy for DMD patients, using exon skipping to convert the severe DMD disease into a condition analogous to the substantially milder BMD is an appealing approach to halt and ultimately reverse disease progression. This can be achieved by modulating pre-mRNA splicing of DMD transcripts in such a way that the out-of-frame mutation is converted into its in-frame counterpart through the skipping of an exon. Specific exon skipping can be induced by antisense oligomers (AOs), which are small pieces of synthetic nucleic acid-based molecules with the complementary sequence targeted to a specific exon. Upon AO binding, the targeted exon is no longer recognized by the splicing machinery and is spliced out (skipped). Thus, specific targeting by AOs can be used to skip most of the 79 exons in the DMD gene, and restore the open reading frame for many different mutations (see Figure 2 for an example). In fact, since a low level of spontaneous exon skipping is found in many DMD patients, resulting in a low percentage of “revertant” dystrophin positive fibres, this protocol mimics a naturally occurring phenomenon.
**Figure 2. Antisense-mediated exon skipping for DMD.** In this example, the open reading frame is disrupted by a deletion of exons 48–50, resulting in a premature stop codon and a shortened dystrophin, which is unable to fulfill all its cellular functions. AOs can be employed to restore the open reading frame (lower panel). Specific AOs bind to exon 51 and hide this exon from the splicing machinery, resulting in the splicing of exon 51 with its flanking intron. This restores the open reading frame, allowing the generation of an internally deleted dystrophin that is partially to largely functional, as it contains both domains for the linker function.

**(ii) Exon skipping applicability**

Experimental AO treatment in patient-derived cell cultures has resulted in skipping of the targeted exon and new dystrophin expression for patients with different deletions, point mutations and duplications indicating that this approach is widely applicable across a number of different genotypes. Exon skipping could theoretically restore the reading frame in 83% of all DMD patients. There is a number of mutations to which this approach does not apply, including mutations that affect the first or last exon, mutations that affect the domain that binds to the bridging protein dystroglycan, which eventually binds the connective tissue (encoded by exons 64-70, mutations in this region cause DMD regardless of the reading frame) and very large deletions (>35 exons). Fortunately, these mutations are very rare (in total they account for <10% cases of DMD) and the majority (75%) of patients have a deletion that occurs in the mutation hotspot located between exon 43 and 55.

Exon skipping is a mutation-specific approach: different mutations require the skipping of different exons. However, due to the presence of mutation hotspots, the skipping of some exons covers more patients than others (see Table 1 for the “top 10”) Aartsma-Rus et al, *Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations*. Hum Mutat. 2009 Mar;30(3):293-9.

Skipping the exons listed in this table would restore dystrophin expression to >40% of patients. Note that some mutations shown in Table 1 can be restored by skipping of one of two exons (e.g. either exon 51 or exon 53). Therefore, the actual applicability of skipping these exons is not the sum of the applicability but somewhat lower.

**Table 1 Overview of exons applicable to largest groups of patients**

<table>
<thead>
<tr>
<th>Exon</th>
<th>All mutations (%)</th>
<th>Deletions (%)</th>
<th>Duplications (%)</th>
<th>Small mutations</th>
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<tr>
<td>51</td>
<td>13.0</td>
<td>19.1</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>45</td>
<td>8.1</td>
<td>11.8</td>
<td>0.2</td>
<td>2.2</td>
</tr>
<tr>
<td>53</td>
<td>7.7</td>
<td>11.4</td>
<td>0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>44</td>
<td>6.2</td>
<td>8.8</td>
<td>0.4</td>
<td>2.7</td>
</tr>
<tr>
<td>46</td>
<td>4.3</td>
<td>6.2</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>52</td>
<td>4.1</td>
<td>5.7</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>50</td>
<td>4.0</td>
<td>5.6</td>
<td>0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>43</td>
<td>3.8</td>
<td>5.3</td>
<td>0.2</td>
<td>2.6</td>
</tr>
<tr>
<td>687</td>
<td>3.0</td>
<td>3.6</td>
<td>0.1</td>
<td>6.3</td>
</tr>
<tr>
<td>8</td>
<td>2.3</td>
<td>2.3</td>
<td>0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

The fact that different AO drugs will be required to treat different, relatively small, groups of patients presents a challenge for this ultra-orphan approach of exon skipping, especially given that each AO is currently considered a new drug. This makes the development of AOs for different exons expensive and complex. To perform clinical trials, a certain number of patients are needed. For the top 4 exons in Table 1, sufficient numbers of patients (400-700) are registered in the TREAT-NMD global DMD
registry. However, for exon 50, only 235 patients are registered and for exon 8, we anticipate only ~120 patients. These numbers may seem sufficient, but these patients are located in different parts of the world and not all are likely to be eligible due to being unable to cooperate (33% DMD boys have cognitive impairment), or unwilling to participate in clinical trials, or too severely affected to be able to participate to a clinical trial (respiratory and cardiac failure). For patients below the top 10 (which together make up 50% of applicable patients), the numbers are reduced further, making even a very small clinical study impossible to conduct.

Question: Once one AO has been approved, do all subsequent AOs need to go through large scale clinical trials?

(iii) Double exon skipping

Close to 20% of patients require the combined skipping of two exons (double-exon skipping) to restore the reading frame. Double exon skipping using AO technology has been achieved in patient-derived cells, and in mouse models and dog models. In each case dystrophin was restored, which was accompanied by functional improvement. This implies that double-exon skipping is feasible. Notably, exon 51 and 45 double-exon skipping applies to 1.1% of all patients. As the single exon skipping of these exons is in the 1st and 2nd position, respectively (Table 1), it is anticipated that AOs to skip these exons will be among the first to become registered (exon 51 AOs are already in clinical trials).

Question: When AOs to skip exon 51 and AOs to skip exon 45 are both registered, will patients who require both of these be allowed to use them? Or are additional trials needed first to test this combination of AOs? If the latter, this will be difficult to test as this applies to an estimated 60 patients in the TREAT-NMD registry.

F. Issues relating to the development of treatments for DMD

(i) Non-clinical development Issues

AO therapeutics, while relatively unknown in the market place have been used in clinical trials for more than 15 years and it is estimated that more than 3,000 patients variously suffering from cancer, metabolic diseases, cardiovascular complaints, asthma and DMD as well as many healthy volunteers have been exposed to them. Duration of exposures exceeds a year in some trials. There are two oligomer drugs that have been approved for use in man. Both are drugs directly injected into the eye and both have been approved in the US and the EU. There are approximately 60 or more oligomer drugs currently in clinical trials. While these drugs are modified nucleic acids, this kind of therapy is NOT gene therapy. Why antisense drugs should not be considered gene therapy can be summarized as follows:

- AOs contain fewer than 100 nucleotides in total (single stranded, double stranded, or partially double stranded); AND
- AOs are unable to integrate into the genome (i.e. do not contain known viral vector, transposable element or other known sequence designed to promote integration of the molecule into the genome) AND
- AOs cannot be autonomously replicated in cells (i.e. do not contain elements known to interact with DNA or RNA polymerase); AND
- AOs do not comprise a gene (i.e. do not contain promoter/enhancer elements, transcription initiation elements or polyadenylation sequences designed to enable the molecule to be transcribed into mRNA); AND
• AOs cannot be reverse transcribed into DNA (i.e. cannot be recognized by reverse transcriptase); AND
• AOS cannot be translated into protein (i.e. do not contain elements that interact with ribosomal subunits); AND
• AOS have a transient effect (reversible in time, not permanent - must be re-administered to sustain effect).
• AOs are designed to interact with mRNAs, not the nuclear DNA that comprises the genome.

(ii) Biochemistry of antisense oligomers

Two classes of candidate AOs are currently being studied in patients with DMD, the phosphorodiamidate morpholino oligonucleotides (PMOs) such as AVI-4658 and phosphorothioate oligonucleotides like PRO051 (Figure 3). To provide safety information on the AOs in clinical trials, a broad range of toxicity studies in animals have been performed as summarised below.

(a) Phosphorothioate Oligonucleotides (PS oligomers)

PRO051 has the structure of chemically modified RNA. The substitution of a sulphur for an oxygen in the phosphodiester linkages between nucleotide units and the addition of a methyl group on the 2’ position of each ribose, produces 2’O-methyl RNA with a negatively charged phosphorothioate backbone (2’OMePS). These modifications increase the half-life and the distribution to tissues. PS oligomers like PRO051 are highly water soluble and have distinct chemical characteristics that drive the biology of members of this class. After injection (either subcutaneous or intravenous) PS oligomers are cleared rapidly from plasma and taken up by cells. In the period immediately after injection, little of the drug is excreted in urine and faeces because they are protein bound in plasma and thus spared from glomerular filtration in the kidney. The addition of the sulphur atom results in distinct patterns of binding to plasma and cellular proteins. It has also been implicated in the activation of the innate immunity pathway when injected at high doses in both rats and monkeys. Thus, both the pharmacokinetics and toxicity of these compounds are largely driven by the polyanionic nature and the water solubility of the oligonucleotides. These properties are largely independent of sequence and as such, PS oligomers as a class have more pharmacokinetic and toxicologic similarities than differences from sequence to sequence. PS oligomers have been studied in laboratory animals in acute, subchronic and chronic assays. With these data it is possible to predict the pharmacokinetic and
toxicologic profiles based on these similarities (reviewed in Henry et al 2007, Levin et al 2007, Kwoh et al 2007). This concept has potential regulatory consequences.

The vast majority of oligonucleotides in clinical development belong to this class and therefore the most has been published about the safety of these molecules. After 15 years there is considerable insight into the effects of high doses of PS oligomers and the toxicities observed in animal studies and the relationship between the effects observed at high doses in animal studies and their relevance or lack of relevance to responses in man.

(b) Phosphorodiamidate Morpholino Oligonucleotides (PMOs)
The morpholino-based oligonucleotide AVI-4658 is one of but a handful of this class of PMOs studied in clinical trials. The chemistry used to synthesize this class of AO utilizes the typical DNA bases but the backbone that connects those bases is completely synthetic (known as phosphorodiamidate) and unlike that in endogenous oligonucleic acids. In contrast to PS oligomers, the morpholinos are uncharged and as a result do not bind to plasma proteins and are thus rapidly cleared from plasma by renal filtration and excreted in urine. Thus the majority of the administered morpholino oligonucleotide is lost, but that fraction taken up by cells the absence of protein binding inside cells is an advantage because protein binding sequesters active drug. Reduced protein binding intracellularly allows a greater fraction to be free and available for binding to the target mRNA.

Because such a high percent of the PMO is excreted rapidly on a mg/kg body weight basis, PMOs are presumed to not be toxic, but little has been published on this issue. However, it may be necessary to treat at higher doses because of the rapid urinary clearance. How this effect will translate into clinical dose regimens is currently being investigated. Ongoing studies conducted by AVI Biopharma, where over 400 human subjects were safely dosed with PMOs at total exposures of up to 600 mg in clinical trials for treating either West Nile Virus, HCV, polycystic kidney disease or restenosis will be presented in a seminar at the workshop. In all trials, no treatment-related adverse effects were detected. Safety data is also emerging for AVI-4658 in the current systemic study of the drug in DMD patients, where cumulative exposure has already exceeded 2000mg without obvious adverse effects to date. In toxicity evaluations of PMO performed in mdx mice by Dr. Qi-Long Lu of Carolinas Medical Center, USA (unpublished observations) doses of up to 1500 mg/kg were administered for up to 3 months, with no adverse findings seen in the treated animals. Similarly, in a study in the CXMD dog where three different sequences were evaluated, doses of up to 200 mg/kg were well tolerated (Takeda 2009).

The general features of the morpholino oligonucleotide have important implications for their regulation and registration. Because morpholino oligonucleotides are uncharged there may be more significant differences between the behaviour of different sequences. How this affects toxicity will have to await toxicity studies on multiple sequences under similar conditions as has been performed for PS oligomers.

(iii) Safety and toxicology issues using AO’s as clinical drugs

Questions:

1. Once approved, therapeutics for DMD patients are going to be used chronically. What are the consequences of long term administration of phosphorothioates or morpholino-based AOs? And what models are the best for studying those effects?

Why these questions are more important for AOs to treat DMD patients relates to the fact that effective treatment of DMD will require multiple new AOs to be developed for different groups of genotypes in whom skipping of an individual exon will restore the reading frame. In a paradigm that presages personalized medicine, the issue of how to test AOs for very small numbers of patients makes a thorough understanding of drug safety for the individual classes critical. How can we regulate
these compounds safely and yet allow for the registration of therapeutic agents for patients with extremely rare mutations?

In some respects oligonucleotide drugs are like all other drugs in development. Until there is widespread use and long term safety data in clinical practice, the possible long term toxicities associated with their use will not be known with certainty. In some respects however, oligonucleotide drugs are different from other drugs, in that there are many properties that are shared by the oligonucleotide classes that allow us to predict the safety profile before full characterization is performed. There is not the level of predictability that would allow one to avoid doing toxicity testing, but there are many more similarities in the toxicity and pharmacokinetic profiles than there are differences. This should permit more efficient testing with fewer different doses, on smaller number of animals, in fewer species predicted to be sensitive from previous testing with different sequences on the same backbone.

(a) PS oligomers

For PS oligomers the target organs and the toxicities associated with chronic administration are well characterized and toxicities that would be predicted are highly dependent on the species. From years of experience with the class, we know that rodents exposed to PS oligomers have profound pro-inflammatory effects with cellular infiltrates observed in multiple organs. These are thought to be related to stimulation of innate immunity. The distributions of receptors that stimulate innate immunity and the differences in the stimuli for innate immunity have always led researchers to conclude that rodents over-predict for humans.

For many this conclusion supports the concept that immune responses in rodents are not predictive of the response of humans. This conclusion in turn suggests that toxicities in rodent models administered PS oligomers acutely or chronically are not as relevant as the responses of other species, for example primates. In moving from short-term to chronic dosing the pro-inflammatory effects can potentially become even more exaggerated and thus some regulators have openly speculated that chronic rodent data may not be useful for predicting the responses of humans. Cynomolgus monkeys have been traditionally used in toxicity studies with compounds of this class and this species is generally predictive for man. However monkeys are more sensitive than human beings to complement activation and therefore they do not necessarily faithfully allow predicting what toxicity will be found in the human (Levin et al, 1999). It is on this background that we need to make recommendations as to how to define toxicity profiles with chronic administration.

Stimulation of innate immunity in primates is weaker than in rodents but NOT absent. In primates, low levels of cytokine release that tend to reverse over time after dosing was observed. However, even low levels of chronic immune stimulation over time can add up to significant adverse responses. Whether these effects observed in monkeys, often at high doses, are predictive of humans is an important question for the PS oligomers.

Based on both experience and an understanding of pharmacokinetics the primary target organs are known. Liver and kidney accumulate the majority of the oligomers and are subject to accumulation-based effects. In general these accumulation-based effects are proportional to dose and for many sequences these effects are not severe. In addition, first order kinetics would suggest that accumulation will only continue until steady state is achieved. Thus toxicities that are dependent on concentration alone would plateau with steady state. Many of the accumulation based effects are simply evidence of drug being collected or sequestered in cell types known to phagocytose or endocytose oligomers which accumulate to such an extent that they can be visualized microscopically. Long term consequences of those accumulations will depend on the extent of the accumulation and, to some extent, the nature of the sequence. In monkeys and man, liver has not proven to be a major target organ in either acute or chronic administration with either the PMOs or the PS AOs.
In monkey there are morphologic changes associated with accumulation of AOs in the proximal tubular cells. Renal proximal tubular cells accumulate the greatest concentration, but appear to be relatively resistant to any adverse effects of this accumulation. The tying up of cellular membranes by the vesicles that encapsulate the oligonucleotide alone can lead to morphologic effects, such as reduced cell and brush border height and compensatory changes that result in reduced cell height. Occasionally these membrane-bound vesicles are associated with the formation of vacuoles because in fixation of tissues for histopathology analysis, when the oligonucleotide is washed out of the membrane-bound vesicles, a resultant space appears as a vacuole.

Some chronic effects may or may not be related to accumulation. Haematologic effects have generally been limited to mild anaemia and in some cases PS oligomers produce a reduction in platelet number. From previous experience, when reductions in platelets were observed, they were independent of synthesis because megakaryocyte morphology and numbers have been normal or even increased, suggesting that the reductions are secondary to platelet destruction or sequestration. How these data predict for humans is unclear, although there are examples in the public domain that suggest that the primate response could predict for reductions in platelets in humans. Only closely monitored clinical trials will provide that answer.

(b) PMOs

Large scale preclinical Good Laboratory Practices testing of the leading DMD PMO, AVI-4658 are ongoing at present in both rodents and non-human primates, at weekly doses up to and including the maximum feasible dose, which is likely an order of magnitude or more higher than the required effective clinical dose. Preliminary data from these studies demonstrate that AVI-4658 is extremely well tolerated at all dose levels in both mice and Cynomolgus monkeys. Importantly, clinical observations of these animals reveal no evidence of acute systemic or local reactions to the drug, confirming the complete lack of induction of any innate immune response by the PMO drugs.

In clinical trials, monitoring was performed for the ability of drugs of the PMO class to activate complement. In these studies a complete lack of complement activation was observed. With PMOs, as with PS oligomers, plasma clearance is probably dependent on urinary filtration, while the PS oligomers persist in circulation longer because of their binding to serum proteins. There are reports that microscopic evidence of renal uptake in the form of granules can be seen in the kidneys of animals treated with both phosphorothioate (Monteith et al, 1999) and morpholino-based oligomers. (Iversen, 2007). The material comprising these granules is related to the oligomers, the fate of which is likely ultimate elimination via the urine.

2. After a thorough characterization of the toxicity of one or two AOs sequences with a given chemistry, are there abbreviated toxicity programs that could be used to characterize the toxicity profile of the sequences for other exons? What is the minimal length for an acceptable toxicity program? 3 or 6 month studies?

- Does every different AO sequence require a “complete” toxicity characterization from acute effects through chronic administration to carcinogenicity studies?
  The decision on this question may be better left to a case-by-case assessment of the data and a weight of the evidence approach for each new skipping sequence (or chemistry). There are commonalities and how do you best exploit them to make personalized medicine a reality. Some level of testing should be required because we have learned over the years with studies that PS oligomers studies there are some sequences with more than the predicted toxicities. Chronic studies of 3 to 6 months duration should be able to distinguish predictable safety profiles from compounds with unpredictable safety profiles.

- Reproductive toxicology
For charged oligomers there is little evidence that they are taken up by the placenta or transported into the foetus, and there is little or no accumulation of oligomers across the blood-testes barrier. This raises questions as to the utility of studying all oligomers in reproductive toxicology particularly in the light of the disease state. Females will not be directly treated and exposure to developing sperm is limited.

- Carcinogenicity studies
  Representative compounds should be tested but requiring 2 yr bioassays for every sequence might be a burden and not reveal significant new information upon which the compounds will be regulated.

3. What are the best animal models for characterizing toxicity considering the differences in muscle cell permeability between normal and diseased animals?

There has been considerable discussion about the necessity to study toxicity in animals with relevant disease models. It is not clear what the advantages of such studies bring to understanding toxicity. However, dystrophic mdx mice are available in large numbers. They should be considered as a model in which to investigate the possible toxicity of the mechanism of action of the exon skipping drugs in a dystrophic subject, specifically as they relate to the chemistry of each oligonucleotide. Admittedly, only mouse AO sequences to skip mouse exon 23 can be used in this model.

Independent of whether the animals in toxicity studies are disease models or not, the target organs for toxicity are going to be the liver and kidney, and for phosphorothioates, effects related to their potential proinflammatory effects. It has been asked whether there might be immune responses related to the appearance of novel dystrophins. This can be addressed in the studies described above. However there are always low levels of revertant fibres that express dystrophin such that the immune system would not necessarily be seeing new ‘non-self’ proteins, so it is unlikely that the appearance of more truncated dystrophin will elicit an immune response. In addition, animal studies have been notoriously non-predictive for antigenicity effects in man.

While there is every reason to perform pharmacology studies in disease, there does not appear to be the same rationale for these disease models in toxicity studies.

G. Outcome Measures for AO treatment

Appropriate Clinical Outcome Measures are necessary to assess disease progression and/or the effects of therapeutic intervention. The importance of appropriate outcome measures that are standardized and reliable in DMD has been previously documented (Florence et al 1984, Mayhew et al 2007, Mercuri et al 2008). Current models for clinical outcome measures in DMD are being further developed and put to the test in an international multi-centre therapeutic trial of ambulatory boys with DMD. Analysis of the pre-treatment data has demonstrated that we have the capability to perform high quality assessments that are reliable and correlate well with function. A similar model is being implemented to further develop outcome measures for the non-ambulant DMD population.

Clinical outcome measures currently being performed by evaluators in this international multi-centre therapeutic trial in ambulatory DMD include the 6 Minute Walk Test (6MWT), Timed Functional Tests (10 meter walk/run, stair climb, stair descend and supine to stand, along with grading the quality of the performance of these timed tests), muscle strength testing of shoulder abduction, elbow flexion and extension and knee flexion and extension with hand-held myometry, and StepWatch™ activity monitoring (McDonald, Coleman et al 2009). These tests reflect aspects of function that are important in everyday life and are clinically meaningful to the patients and families.
With appropriate outcome measure design, description, training and implementation the above listed outcome measures have been documented as reliable demonstrating high test-retest correlations. Intra-class correlation coefficient (ICC) for the 6MWT was 0.91. (McDonald, Henricson et al 2009, Florence et al 2009). The ICC values for the timed functional tests ranged from 0.74-0.93 (Eagle et al 2009, Florence et al 2009) while myometry values ranged from 0.74-0.91. (Florence et al 2009).

Good correlations existed between the above listed tests and stratification factors used at the initiation of the clinical trial were supported by the outcome assessments. The results of the analysis of this pre-treatment data allow us to conclude that the assessments are reliable, correlate well and that the predefined stratification factors, known to predict for disease severity, separated the study population as expected (Florence et al 2009). This data supports these outcome measures as appropriate for clinical trials in ambulatory DMD. Further analysis of the treatment data will allow us to determine the sensitivity and validity of the above listed outcome measures.

In regards to outcome measures in non-ambulatory individuals with DMD a variety of assessments have been seriously considered, discussed, recommended and are currently being studied or protocols are awaiting funding to assess the appropriateness of these measures for implementation in clinical trials. These recommended assessments include a variety of functional scales (Brooke, EK, TREAT-NMD upper limb scale), measures of quality of life, caregiver surveys, assessment of muscle strength with hand held myometry or fixed quantitative system (Mayhew et al 2007), active and/or passive range of motion, pulmonary function testing, and assessment of upper limb and hand function looking at grip and pinch strength, 9 hole peg, Jebsen timed tests (Hiller & Wade 1992, Wagner et al 1993) and activity monitoring.

Studies are underway to validate outcome measures, previously shown reliable (Mayhew et al 2007) in both ambulatory and non-ambulatory DMD, in an ongoing longitudinal study of the natural history of DMD. Analysis of data from this longitudinal study will enable us to determine and confirm if any of these outcome measures will be an appropriate proxy for survival. Previously published data suggests maximal vital capacity and its’ rate of decline predicted survival in DMD (Phillips et al 2001).

In a previous workshop (October, 2008) on outcome measures in Spinal Muscular Atrophy the EMEA recommended the potential use of Quality of Life (QoL) and Care Giver Burden (CBG) scales in clinical trials of neuromuscular disorders. There is only one muscle disease specific QoL measure, the PedsQL Neuromuscular Module (PedsQL NMM) which is intended to be used together with the generic PedsQL Core thus combining the benefits of a generic and a disease specific QoL outcome measure. The PedsQL NMM is submitted for publication but not yet published. The PedsQL NMM and the Core have had limited use in DMD and there is as yet no information on responsiveness. Their use in any DMD trial will obtain information on responsiveness and should therefore be encouraged. However there needs to be recognition that the relationships between QoL and disease severity show wide variation and how QoL might change in a trial is not necessarily intuitive. These QoL outcome measures, to our knowledge, are not suitable for the calculation of cost effectiveness e.g. QALYS.

There is no CBG outcome measure that has been devised for use in children with neuromuscular disease. Whether existing CBG measures are suitable or can be adapted depends on the rationale for including this outcome measure in a DMD clinical trial. We would appreciate guidance from the EMEA as to their reasons for wishing to include this as an outcome measure in a DMD trial as this will help focus the choice of the CBG measure. However the probability is that a suitable CBG outcome measure will require adaptation or de novo construction either of which will require extensive resources.

As newborn screening for DMD is not yet a reality in most countries or states there has not yet been great emphasis on the development of outcome measures for the infant or very young child with DMD. As children with DMD are clinically asymptomatic in the first several years of life the development of an outcome measure is difficult but specific neurologic examination with emphasis on
tone and movement have been recommended leading into other specific assessments of motor function as the boys become toddlers. As newborn screening becomes a reality greater emphasis and resources will be directed toward outcome measures for the infant and very young child with DMD.

We would like to emphasize that well established clinical trial networks (TREAT-NMD & CINRG), that have been involved with developing and promoting appropriate clinical outcome measures in DMD, (Mayhew et al 2007, Mercuri et al 2008) are currently planning to collaborate under the terms of a recent memorandum of understanding further promoting the harmonization of therapeutic clinical trial networks in DMD.

H. Ethical considerations for treating DMD patients with AOs

Restrictive approaches to the inclusion of children in clinical trials places the individual in a position of “double jeopardy” (Hagger and Woods 2005). Children with a diagnosis of DMD are disadvantaged both from the rarity of their condition and from the opportunity for a long life of good quality. These disadvantages may be compounded if restrictive regulatory approaches are adopted requiring each potentially therapeutic AO compound to go through the process of placebo controlled clinical trials. In DMD, there is a specific problem in that there are not enough patients with the rarest mutations to render the required phases of study feasible, limiting the research to fewer than 6 exons (Aartsma-Rus et al 2009). Therefore some flexibility in the way that the candidate AO compounds are researched is needed.

(i) Patient age at participation

Clinical trials on DMD patients will initially involve 5-12 year olds since they are clinically less severely affected, but there is also an imperative to include older boys because the immediate impact is likely to be life saving as well as life extending. These cohorts raise different ethical concerns. This history of using young children in clinical trials is overshadowed by abuse (Lederer and Grodin 1994, Lederer 2003). There is also a contemporary trend to restrict inclusion to children who have sufficient maturity and understanding, which is not necessarily related to how well they will benefit clinically. However ongoing AO studies have demonstrated that the ethical challenges of involving younger children in this kind of research have been met. Moreover it should be noted that the use of younger children in invasive clinical studies has now become routine in the field of paediatric oncology and this provides a relevant moral parallel to DMD research. In theory there is less potential benefit to treating older children as their muscle damage is deemed irreversible. However this view is flawed. Regaining the use of a hand, translates to greater independence for the older boy since he will be able to control his own wheelchair and manage a computer. Patient organisations are emphatic that what appears to be a modest benefit to the overseer is in fact a major advance for the individual patient’s welfare and should be given proportionate weight as outcome measures.

(ii) TREAT-NMD strategy for patient recruitment

There is some evidence to suggest that patients and parent organisations are at times less likely to weigh rationally the risks and benefits when invited to join a clinical trial (Henderson 2009). TREAT-NMD takes these concerns very seriously and as a consortium has developed a number of strategies and policies to ensure good practice. Examples of these strategies and policies include:

a) The establishment of a Global Patient Registry for DMD – with an international oversight committee which includes clinicians, scientists, ethicists and patient representatives. The Registry provides a resource to trial designers whilst protecting the interests and confidentiality of patients (see registry Charter: http://www.treat-nmd.eu/patients/patient-registries/toolkit/). The TREAT-NMD Registry also provides training for those seeking to establish new national registries and this includes advice and guidance on governance and good practise.

b) International training events on research ethics

c) An ongoing research programme into the social and ethical aspects of translational research.
d) An ongoing programme of patient communication which includes the translation of information into multiple languages with a view to improving patient/parent knowledge of the disease, its treatment and research.

I. Bibliography

Papers related to exon skipping

1. Laura van Vliet1, Christa L de Winter1, Judith CT van Deutekom2, Gert-Jan B van Ommen1 and Annemieke Aartsma-Rus. Assessment of the feasibility of exon 45–55 multiexon skipping for duchenne muscular dystrophy. BMC Medical Genetics. This article is available from: http://www.biomedcentral.com/1471-2350/9/105


Reviews on AO therapies


Papers related to Efficacy and Toxicology of AOs


3. Scott P. Henry, Tae-Won Kim, Kimberly Kramer-Stickland, Thomas A. Zanardi, Robert A. Fey, and Arthur A. Levin CHAPTER 12: Toxicologic Properties of 2′-O-Methoxymethyl Chimeric Antisense Inhibitors in Animals and
Papers related to Outcome Measures


**Papers related to Ethical Issues**


**Papers related to Clinical Trials in Rare Diseases**


2. Madhusmita Behera, MS, Ambuj Kumar, MD, MPH, Heloisa P. Soares, MD, Lubomir Sokol, MD, PhD, and Benjamin Djulbegovic, MD, PhD. *Evidence-Based Medicine for Rare Diseases: Implications for Data Interpretation and Clinical Trial Design*. Cancer Control April 2007, Vol. 14, No. 2


**General**

**Morning Chair: Francesco Muntoni (TREAT-NMD, University College London)**

8:30 Arrival and Registration

9:00 Welcome and Introduction
   - Agnes Saint-Raymond (EMEA)
   - Elizabeth McNeil (FDA)
   - Volker Straub (TREAT-NMD, Newcastle University)

9:15 Aims of the meeting and questions for the regulatory authorities
   - Francesco Muntoni (TREAT-NMD, University College London)

9:25 Parental / Patient Perspective
   - Elizabeth Vroom (United Parent Project Muscular Dystrophy)

**Session I: Clinical overview of Duchenne Muscular Dystrophy and Exon Skipping**

**EMEA co-chair: Kerstin Westermark (COMP)**

9:40 Presentation 1- General Overview: What is Duchenne Muscular Dystrophy
   - Natural History and Standards of Care
     - Genetic Basis of Duchenne and Becker Muscular Dystrophies
     - Standards of Care for DMD
     - Kate Bushby (TREAT-NMD, Newcastle University)

10:05 Presentation 2- Outcome measures for clinical trials in DMD
   - Outcome Measures currently used in ambulant DMD boys
   - Outcome measures in non ambulant DMD boys
   - Outcome measures in young boys
   - Julaine Florence (Washington University School of Medicine, St. Louis)

10:30 Presentation 3- The Specific Issues of Personalised Medicine in Duchenne Muscular Dystrophy
   - What is exon skipping in Duchenne Muscular Dystrophy?
   - How does it work?
   - How many exons should be targeted?
How long it would take to develop AOs approach for the first 10 exon to skip using current procedures?
Are additional tests required for double exon skipping?

Annemieke Aartsma-Rus (TREAT-NMD, Leiden University Medical Center)

11:00  Tea / Coffee

Session II – Clinical Applications of Antisense Oligonucleotides

**EMEA co-chair: Cristina Sampaio (CHMP)**

11:15  Presentation 4 - Overview of experience with different AON chemistries in clinical trials to date
A historical perspective on antisense technologies: successes and disappointments
Efficacy / equivalency dose
What is an appropriate toxicology package for AON
Art Levin (Levin Biosciences and Santaris Pharma, Hørsholm)

12:00  Discussion and consolidation (led by Dominic Wells)

12:30  Lunch break

**Afternoon Chair: Volker Straub (TREAT-NMD Coordinator, Newcastle University)**

Session III – Clinical Applications of Antisense Oligonucleotides –II Part

**EMEA co-chair: Gopalan Narayanan (CAT)**

13:30  Presentation 5- Toxicological issues in development of AOs as a medicinal product
Long term toxicity
Toxicology package for combinations of AOs
Pathway for subsequent AOs to be taken to clinic
Art Levin (Levin Biosciences and Santaris Pharma, Hørsholm) and Dominic Wells (Imperial College London)

14:00  Industry Presentation 1: Prosensa (Giles Campion)

14:10  Industry Presentation 2: AVI (Steve Shrewsbury)

14:20  Discussion and consolidation (Led by Art Levin)

Session IV – Ethical Aspects

**EMEA co-chair: Agnes Saint-Raymond (EMEA) and Michael Wilks (CPME)**

15:20  Presentation 6 – Ethical considerations for trials in DMD
Funding for trials
Personalised medicine
Fragmentation of the patient community based on 'have and have not's' of specific exons.
Recruitment of patients into sequential trials
Quality of life and burden of disease
Improvement vs stabilisation of condition for regulatory approval?
Simon Woods (TREAT-NMD/PEALS, Newcastle University)

15:40 Discussion and consolidation (led by Simon Woods and Elizabeth Vroom)

16:00 Tea / Coffee

Session V – Summary Discussion

Session Chair: Edward Connor (Children’s National Medical Center)
EMEA co-chair: Daniel Brasseur (PDCO) and Spiros Vamvakas (EMEA)

16:15 EMEA / FDA Feedback and suggestions to the community

17:00 Summary and Discussion
Francesco Muntoni (TREAT-NMD)
Leone Atkinson (PTC)
Pat Furlong (Parent Project Muscular Dystrophy)
Daniel Brasseur and Spiros Vamvakas (EMEA)
Elizabeth McNeil (FDA)

17:15 Concluding Remarks
Daniel Brasseur and Spiros Vamvakas (EMEA)
Elizabeth McNeil (FDA)
Francesco Muntoni and Kate Bushby (TREAT-NMD)

17:30 End of meeting

The organizers would like to thank the following organizations for their financial support of this workshop:

AFM  MDA  Parent Project Muscular Dystrophy

The elements originating during the discussion will not constitute a formal specific advice on a particular product or class of products. The positions expressed by the Experts and the EMEA during this workshop will not be regarded as binding in relationship to any aspect of subsequent institutional work before to be endorsed by the CHMP and/or the PDCO.