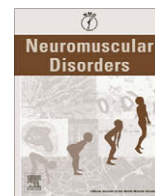


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## Workshop report

# The development of antisense oligonucleotide therapies for Duchenne muscular dystrophy: Report on a TREAT-NMD workshop hosted by the European Medicines Agency (EMA), on September 25th 2009

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On behalf of the meeting, steering committee, and of the TREAT-NMD Network

## 1. Introduction

A workshop entitled The Development of Antisense Oligonucleotide Therapies for Duchenne Muscular Dystrophy, sponsored by the EU funded Clinical Network TREAT-NMD (Translational Research in Europe for the Assessment and Treatment of Neuromuscular Diseases; [www.treat-nmd.eu](http://www.treat-nmd.eu)), Association Francaise contre les Myopathies, Duchenne Parent Project, European Medicines Agency, Muscular Dystrophy Association and Patient Project Muscular Dystrophy, was held on 25th September 2009 at the European Medicines Agency (EMA), London, UK. The 40 active participants represented six countries and included scientists, clinicians, patient representatives, industry and regulators from the EMA.

The workshop opened with a welcome note from Agnes Saint-Raymond (EMA) followed by the TREAT-NMD coordinator Volker Straub leading with an overview of what TREAT-NMD is and he emphasised it was created to relieve the schism impeding translational research for the development of new therapeutic strategies for rare neuromuscular diseases. Francesco Muntoni (chairman) then presented the background to and objectives of the workshop. Exon skipping represents a novel antisense oligonucleotide therapeutic approach for the treatment of Duchenne Muscular Dystrophy (DMD), which unlike existing treatments, directly addresses the lack of functional dystrophin protein that causes the disease. As there is currently no therapy addressing disease pathogenesis for DMD patients, exon skipping to convert the severe DMD disease into a condition analogous to the substantially milder Becker (BMD) is an appealing approach to stabilise disease progression. Recent pre-clinical and clinical trial results of exon 51 skipping

antisense oligomers (AOs) as a therapeutic option to treat boys affected by DMD with deletions bordering exon 51, have fulfilled proof of principle suggesting that ultimately this approach could target >80% of DMD patients [1]. However, to achieve this aim, at least 30 novel AOs will be required. Current regulations stipulate that each novel AO is classed as an individual medicinal product and thus each AO must undergo rigorous safety and efficacy studies prior to clinical use or it must be justified why and how efficacy and safety data can be extrapolated from one AO to another. Since DMD is a relatively rare disease, with only 100 new cases/year in the UK from a range of different genetic mutations in the *DMD* gene, randomised controlled studies may not be feasible if most of the 30 candidate AOs have to be studied separately in such a small patient cohort. The aim of this workshop was thus to seek legislative guidance from both EMA and other regulatory representatives, to identify a rational strategy for developing a personalised AO therapeutic approach in a realistic time-frame for it to be of real benefit to DMD boys.

## 2. The parent's perspective

The first session opened with Elizabeth Vroom, a parent representative for United Parent Project Muscular Dystrophy who presented the parent's and young individual's perspective of living with DMD. She showed videos of DMD boys aged between 4½ and 19 to illustrate the clinical progression of the disease. These boys stated that what they want most in life is to be able to maintain the muscle function they have left, which demonstrates that the patients themselves see retardation of disease progression as a beneficial outcome. She also highlighted the importance of including patients/carers in trial design, so as not to miss apparently minor non-clinical issues which are in fact of considerable burden to patients/carers. Her presentation is reproduced in part below:

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“15 years ago we came forward to organise research meetings where we made it clear we were willing to shoulder responsibility and contribute towards advancing treatments and a cure. We asked for advice about how we might make a difference, for which the reply was to raise money for peer reviewed research and advocate for government support. We achieved these aims in both Europe and the USA, contributing more than 40 million Euros. There are now a series of exon skipping clinical trials planned that patient group’s support and we have helped develop new Duchenne patient registries. Parents and their families have enthusiastically embraced these trials and have come forward to work with clinical teams to test the new drugs. Today we again come as responsible, willing partners, asking you to understand what is at stake and hope to learn from you how we might expedite AOs as a potential therapy. Although there is great enthusiasm among the parent community about these new possibilities we have the following concerns:

- (i) Living with such a progressive disorder is a race against the clock. We as parents are very worried that regulatory and administrative procedures are time consuming. For our sons’ every year, month, day counts and we want to avoid wasting time.
- (ii) Families can see progress with one target exon, but are asking if drugs tailored to meet the other boys needs will be developed and if so when? We fear that pharmaceutical companies will be unable to fund 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, consists of only a small subset of the Duchenne boys. All other AOs treat even smaller subsets, yet companies are eager to develop these treatments with the encouragement of patient advocacy groups. This, under the existing regulations, each new AO might need to undergo several years and many millions of Euros in animal testing and then several more years and tens of millions of Euros in clinical testing. Even for the exons at the bottom half of the priority list, it will be difficult to find enough boys to fulfil all the regulatory obligations. What about the boys who need two different AOs because 2 exons need to be skipped? 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?
- (iii) How can we make sure that boys of the full age range can profit from new medicines? We understand regulatory agencies as well as industry and researchers consider walking tests as a good outcome measure to show the drug is effective in ambulant boys. But this should not result in delay of developing protocols for the other 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.
- (iv) The core message of all laws regarding approval and testing of new drugs and even more specific in children, are focussed on “doing no harm”, which we as parents embrace. No parent wants his child to suffer or die due to the testing or use of new medicines. However in this disease, we as parents worry that the strict regulatory issues may have the opposite effect and harm rather than protect the boys, since doing nothing is a fatal risk. When the letter of the law is applied instead of the concept of the law, our sons are in danger. Together we need to determine ways to streamline

the process so that potential therapies are not delayed, while our sons lose function and die. Again, we are not desperate parents who want to take every possible risk, but we can’t accept that regulatory systems do harm to our children. It is unacceptable when potential treatments are available, but have to sit on the shelf because of regulatory issues!! We are very happy to have the opportunity today to discuss with you the risk/benefit analysis for these boys and how this could be implemented in the next steps of development in the nearby future.

So what are the current risks? So far, widespread testing of the AOs in both normal and dystrophic animals, as well as in patients suggests AOs are well tolerated with no adverse consequences detected. Could what we learn from the testing of one AO with a specific backbone be used for future AOs and reduce the potential risk, so that less pre-clinical work is required?

What would be the benefits? 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 as evidence of benefit for DMD boys. Just slowing down the degeneration is already considered as beneficial by the boys and their families.”

Elizabeth concluded with an analogy: “In our country, as in most European countries, we have speed limits on every road and we consider this as very important for safety reasons. However, when somebody with a life-threatening illness is transported at high speed in an ambulance the outcome of the risk/benefit analysis is different and it is safer to drive faster. Similarly Duchenne is a life-threatening situation. Boys with Duchenne bear significant risk, indeed to life itself. We recognize that your concern is about safety, protecting the patient. We ask that you consider the ‘emergency’ of Duchenne. We are not asking agencies to put safety aside, simply to understand the ‘emergency’, to help design a regulatory approach that fits the circumstances and to find the right process in order to rescue the patient(s) in the most efficient way possible.”

### 3. Clinical overview of DMD and exon skipping

#### 3.1. General overview: What is DMD, its natural history and standards of care available?

The next session was opened by Kate Bushby who outlined the clinical spectrum of patients with mutations in the *DMD* gene, its natural history and summarised the currently available treatment. DMD is one of the most common fatal genetic disorders to affect children on a global basis. Approximately one in every 3500 boys born worldwide is afflicted with DMD, with a global prevalence of ¼ million. Due to prenatal diagnostic screening in known families the incidence is stable. 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. The social and emotional costs and burden of this 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 (2007 figures) 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.

The condition is life-limiting and without supportive ventilation and cardiac therapies death occurs by the early twenties. International guidelines for care have recently been generated [2]. Life threatening complications of DMD include a decline in respiratory function and a progressive cardiomyopathy. Since these are

predictable, they can be proactively treated. Steroids in conjunction with physiotherapy can prolong ambulation, preserve respiratory strength, cardiac protection and are offered when physical performance has reached a plateau. However, steroids may have significant side effects; notably weight gain, height restriction and osteoporosis to name but a few. With such interventions it is possible to extend the life of many affected individuals by at least another decade. Despite this treatment paradigm, none of the currently recommended interventions for DMD 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 therapeutic interventions are badly needed for DMD patients and that AO technology is currently a viable approach to meet this aim.

### 3.2. Outcome measures for the clinical trials in DMD

Julaine Florence presented an overview of outcome measures (OM) currently used in ambulant and non-ambulant DMD boys.

Examples of OM for ambulatory DMD boys, which give high test–retest correlations, include the 6 min walk test (6MWT), timed functional tests (10 m 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 [3]. Intra-class correlation coefficient (ICC) for the 6MWT was 0.91 [4,5]. The ICC values for the timed functional tests ranged from 0.74 to 0.93 [5,6] while myometry values ranged from 0.74 to 0.91 [5].

In regards to OM in non-ambulatory individuals a variety of tests await validation by regulators. These include a variety of functional scales measures of Quality of Life (QoL), care-giver surveys, assessment of muscle strength with hand-held myometry or fixed quantitative system [7] active and/or passive range of motion, pulmonary function testing, and assessment of upper limb and hand function looking at grip and pinch strength, nine hole peg and Jebsen timed tests [8,9]. In addition, studies are underway to validate outcome measures, previously shown reliable [7,10] in both ambulatory and non-ambulatory DMD to see if they are appropriate proxy for survival.

The EMA have previously recommended the use of 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 used together with the generic PedsQL Core thus combining the benefits of a generic and a disease-specific QoL outcome measure [11]. The relationships between QoL and disease severity show wide variation and how QoL might change in a trial is not necessarily intuitive. The PedsQL NMM and the Core have had limited use in DMD, but its use is now being actively encouraged so that its usefulness can be documented. There is no CGB outcome measure that has been devised for use in children with neuromuscular disease. Guidance is requested from the EMA as to their reasons for wishing to include this as an OM.

Since newborn screening for DMD is currently not generally available and DMD patients are clinically asymptomatic in the first several years with diagnosis in the majority delayed until the age of 4 or 5 years, no great emphasis for the development of OM in the first years of life has yet been established.

Various points were raised by the regulatory authorities on the way ahead for developing more meaningful OM and also on younger boys. One topic was how important are the changes in OM measured as the child ages and how does this translate into every day life. The audience was reminded that in the context of a progressive condition such as DMD, 'no change over age' would qualify

as 'beneficial', since it implies no further deterioration. It was generally agreed that being able to stabilise the disease with AO therapy would be a significant advancement and should be considered an achievable goal. Suggestions were made for additional OM to be considered as this field develops, such as assessing the number of myofibres with functional dystrophin that translates to improvements in muscle strength, as well as OM which examine enhanced cardiac output and lung vital capacity. Finally, it was suggested that a goal attainment scale of OM could be compiled as utilized with Alzheimer patients. For this to be operative goals need to be defined and adhered to. The patients themselves can take an active role in what these goals are since they are the best judges of what enriches their lives. Several members of the regulatory authorities expressed concern about the lack of neonatal screening and thus appropriate OM in the early years, since obviously it would be expected that earlier treatment should conserve muscle mass and lead to an improved prognosis. As Francesco Muntoni reiterated, neonatal screening does not currently occur because there were no early treatment interventions available. However, such screening would be feasible and has been piloted in individual regions by studying serum creatine kinase, an inexpensive and effective test. If permission were granted for AO trials in neonates, then appropriate OMs would be developed to take into account the higher percentage of functional muscle present. However, it will also be important to acquire information related to the natural history of the condition in this age group, something that has not been well described in the literature yet quite simply because most diagnoses are made very much later.

### 3.3. The specific issues of personalised medicine in DMD

Annemieke Aartsma-Rus described the genetics underlying DMD, the cellular function of dystrophin, the mechanics of exon skipping and introduced AOs as a therapeutic strategy to treat DMD boys. DMD is caused by mutations in the *DMD* gene which disrupt the open reading frame, completely abolishing the production of a functional muscle protein called dystrophin. Dystrophin cross-links the extracellular connective tissue to the internal cytoplasmic actin filament network, via a collection of protein complexes at the sarcolemma. Its 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 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. Without functional dystrophin–protein complexes, 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. In-frame DMD mutations, generally deletions of single or multiple exons, clinically leads to the less severe BMD, where an internally shortened dystrophin molecule is generated. Thus, protein–protein interactions at its termini are retained and so the structural cross-link is maintained, allowing the shortened protein to function, albeit with less strength. BMD is diagnosed in adolescence or adulthood, and the overall progression of the disease is slower. The majority of BMD patients do not become wheelchair dependent until 10–15 years after diagnosis, although some remain ambulant until late in life. Importantly, BMD patients have a near-normal life expectancy. Therefore exon skipping to convert the severe DMD disease into a condition analogous to the substantially milder BMD, it is an appealing approach to stabilise disease progression.

Antisense-mediated exon skipping modulates 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 one or more exons. The result is to convert the mRNA unable to encode for dystrophin to one that produces an internally shortened form. Specific targeting by AOs can be used to skip most of the 79 exons in the DMD gene, restoring the open reading frame for many different mutations (see Fig. 1 for an example [12]) and theoretically restore the reading frame in 83% of all DMD patients [1]. A low level of spontaneous exon skipping is found in many DMD patients, generating a low percentage of so-called “revertant” dystrophin positive fibres. Therefore, this AO protocol mimics a naturally occurring phenomenon. *In vitro* testing of AO technology on DMD patient-derived cell cultures, and animal models for DMD have confirmed this is a viable approach to treat patients [12,13].

### 3.4. DMD

Mutations occur throughout the gene, but there are ‘hotspots’ so the skipping of some exons applies to more patients than others [10]. Table 1 lists the top 10 most applicable exons. Skipping these exons would restore dystrophin expression in >40% of patients. Mutations affecting the first and last exon, or those involved in the binding to dystroglycan (exons 64–70) and very large deletions (>35 exons) removing functional areas such as the actin binding domains are unable to be corrected. 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

**Table 1**

Overview of exons applicable to largest groups of patients.

| Exon    | All mutations (%) | Deletions (%) | Duplication (%) | Small mutations (%) |
|---------|-------------------|---------------|-----------------|---------------------|
| 51      | 13.0              | 19.1          | 0.3             | 3.0                 |
| 45      | 8.1               | 11.8          | 0.2             | 2.2                 |
| 53      | 7.7               | 11.4          | 0.1             | 1.5                 |
| 44      | 6.2               | 8.8           | 0.4             | 2.7                 |
| 46      | 4.3               | 6.2           | 0.2             | 1.6                 |
| 52      | 4.1               | 5.7           | 0.5             | 2.3                 |
| 50      | 4.0               | 5.6           | 0.2             | 1.9                 |
| 43      | 3.8               | 5.3           | 0.2             | 2.6                 |
| 6 and 7 | 3.0               | 3.6           | 0.1             | 6.3                 |
| 8       | 2.3               | 2.3           | 0.0             | 8.0                 |

mutation ‘hotspot’ located between exons 43 and 55 [14]. Close to 20% of patients require double-exon skipping (notably exons 51 and 45 which applies to 1.1% of patients) and this procedure is effective in patient-derived cells and animal models [12,13].

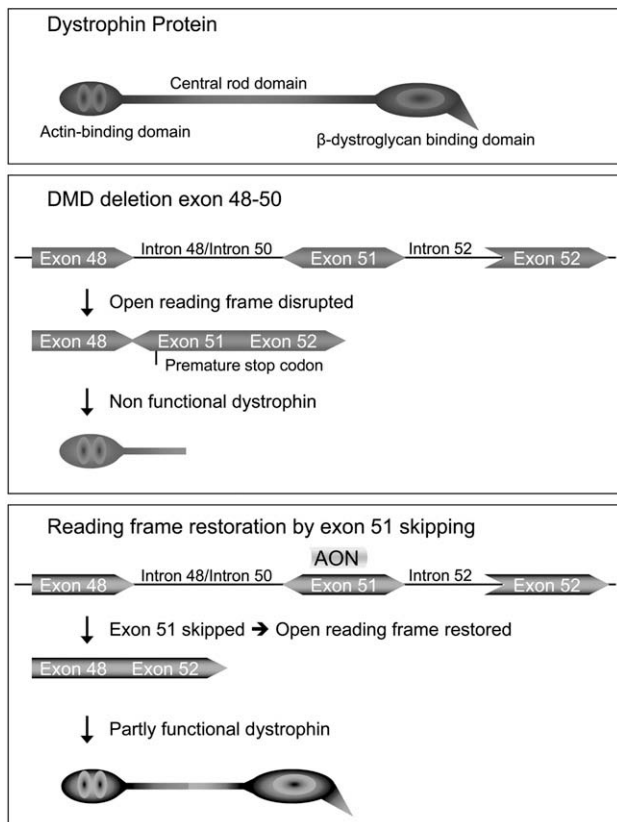
It is understood by TREAT-NMD partners that current trial design for an RCT for one medicinal product in the ambulatory DMD population using current OM would require recruitment of around 150 patients to trial one drug, in which case there would be only enough patients registered in the TREAT-NMD global registry who are suitable to perform clinical trials for the top 4 exons in Table 1. Even these are geographically dispersed and of course not all patients are able or willing to participate in trials. The EMA representatives were thus asked whether smaller numbers of patients could be used to perform subsequent AO clinical trials.

During the general discussion the regulatory authorities corrected the widely held belief that each of these medicinal products would require a full development and that a minimum of 150 patients would always be required to perform a clinical trial. There is a guideline available on clinical trials involving small populations and the EMA would be willing and strongly recommended to be engaged in further discussion for DMD clinical trials before they take place whether or not there are adequate patient numbers. This would mean seeking scientific advice at the EMA as early as possible. The FDA representative upheld this by making examples of randomised studies in USA performed on much smaller patient databases.

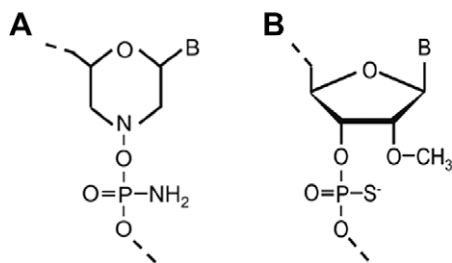
### 4. Clinical applications of antisense oligonucleotides

Art Levin presented a comprehensive study of the history of the development of AO technology, its efficacy in several medical systems, toxicology issues and proposed pathways for taking AO into the clinical setting to treat DMD boys. AO therapy has been in clinical trials for more than 15 years in at least 3000 subjects and in patients suffering from a variety of diseases. There are two oligomer drugs for treating eye disease that have been approved for use in man and approximately 60 currently in clinical trials. Two classes of candidate AOs (see Fig. 2) are currently being studied in patients with DMD, the phosphorodiamidate morpholino oligonucleotides (PMOs) such as AVI-4658 (AVI-Biopharma) and phosphorothioate oligonucleotides (PS oligomers) like PRO051 (Prosensa).

The pharmacokinetics and toxicity of PS oligomers are largely driven by their polyanionic nature and high water solubility. PS oligomers are rapidly absorbed into cells from plasma, with little excreted in urine and faeces because they are low-affinity bound to plasma proteins, which act as carriers. These properties are largely independent of sequence and thus as a class have more pharmacokinetic and toxicologic similarities than differences. The vast majority of oligonucleotides in clinical development belong to this class and therefore the most has been published about the safety of



**Fig. 1.** 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 fulfil 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 out 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.



**Fig. 2.** Structure of the modifications on the AOs referred to in this study. (A) Phosphorodiamidate morpholino oligonucleotide (PMO) e.g. AVI-4658 (B) 2'-O-methyl phosphorothioate (PS oligomer) e.g. PRO051.

these molecules [15–17]. It is clear that there are species-specific toxicities. In rodents, PS oligomers have profound pro-inflammatory effects with cellular infiltrates observed in multiple organs partly due to stimulation of innate immunity. However, since both receptors and mechanisms of innate immunity differ between rodents and primates, regulators feel toxicity data on rodents over-predict for humans. Cynomolgus monkeys are a better model for man, but even these are more sensitive than human beings to complement activation [18] and stimulation of innate immunity in primates is weaker, but not absent. In primates, low levels of cytokine release that tend to reverse over time after dosing are 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. Liver and kidney accumulate the majority of PS oligomers until steady-state is achieved and thus any toxicity effects, which are minor for most sequences, are dose-dependent and will plateau. Many of the accumulation based effects are due to both phagocytic and endocytic cells accruing oligomers to such an extent that they can be visualized microscopically. Few metabolites of PS oligomers accumulate in tissues, but metabolites are found in urine suggesting that metabolites (shorter forms of the parent drug) are excreted very quickly. In monkeys and man, liver has not proven to be a major target organ in either acute or chronic administration with either AO class. However, kidney proximal tubular cells accumulate AOs to the greatest concentration, with much of the oligonucleotide sequestered in phagolysosomes and the kidney can accumulate concentrations in excess of 1500  $\mu\text{g/g}$  without significant adverse effects [19]. Haematological effects such as mild anaemia or a reduction in platelet number can occur unrelated to any accumulation effects. Reproductive effects are not applicable to the female since these will not be treated and there is little or no accumulation of PS oligomers across the blood-testes barrier or across the placenta. Chronic studies of only 3–6 months duration should be able to distinguish predictable safety profiles from compounds with unpredictable safety profiles.

In contrast to PS oligomers, the PMOs are uncharged and as a result do not bind to plasma proteins and are rapidly cleared from plasma and excreted in urine. Although the majority of the administered PMO is lost, they are very stable, so once a PMO manages to enter a cell it potentially has a long-lasting effect. The high excretion rate implies low toxicity, but this issue requires additional investigations. The uncharged property of PMOs may result in significant differences in chemical behaviour between PMOs of different sequence and this would have important implications for both their regulation and registration as drugs. PMOs fail to activate the complement pathway, and like PS oligomers renal uptake is observed [20].

Art Levin ended his presentation with a proposal for an abbreviated toxicity programme as a logistical way forward. He put forward the concept that after a thorough characterization of the

toxicity of two AOs sequences with a given chemistry in animals and proven functional efficacy in clinical trials, could we then go first into man with a third AO sequence with the proviso of 2–4 week studies to confirm similar toxicity profiles.

A wide-ranging discussion on the safety, specificity and mechanisms of application of AO therapy to treat DMD boys then ensued. One concern was whether the AO would be retained in muscle cells long enough to ensure functional effects. Delivered AO are sufficiently stable to allow a pool of dystrophin to accumulate. Dystrophin also has a long half-life. Furthermore, dystrophic muscle is 'leaky' and thus uptakes more AO than normal muscle. As the DMD muscle is reverted to a Becker-type it will still take up enough AO since Becker muscle is adequately leaky for uptake. Another concern raised by the regulators was that even the slightest possibility of an AO mistargeting to another mRNA would be unacceptable. Art Levin reassured the audience that non-specificity risks are overcome using bioinformatic technology to design each AO in conjunction with the testing the specificity of an AO in cell lines prior to clinical trial. Art Levin further commented that an Oligonucleotide Safety Working Group exists which is examining what effect, if any, AOs have on miRNA function, which would also be viewed as an off-target effect. Pre-clinical trials would also detect any AO unable to bind to its target sequence arising from mRNA sequence-specific 3D structures which may affect AO accessibility. Circumventing the testing of the efficacy of each AO could be achieved by the simultaneous treatment with 2 or more AO spanning the target region, but it was not thought to be technologically feasible at the present time. Annemieke Aartsma-Rus replied to these points by saying that to date all the AOs they had tested on patient-derived cells had worked and thus there was currently no supporting evidence that these problems would arise. There was a disagreement between laboratories whether the delivery mechanisms and thus target specificity, differ between *in vivo* and *in vitro* models, with evidence for and against being presented. This suggests sequence-specific AO variation effects may exist. Indeed, Art Levin confirmed that some AO sequences do have atypical toxicity profiles and these need to be screened out; hence the necessity for toxicity studies for all sequences even those with minimal numbers of potential beneficiaries.

The regulators remarked that they would require full documented pharmacodynamic, pharmacokinetic and toxicology properties on more than one AO, before they could recommend a therapeutic framework. The audience was reminded that to treat this disease we are looking at first in child, rather than first in man and regulations would need to accommodate an extended treatment time as the treatment would potentially be given over a whole life-time. There was also a request for more pharmacodynamic data to ascertain an optimal dose and whether the maintenance and starting doses would need to be the same. EMA representatives stated the need to find what AO dose is required to produce enough functional dystrophin for patient benefit with minimal toxicity. Moreover, to date the longest exposure in man is 1 year and so the favourable safety record may be insufficient to conclude for a treatment that may be over decades. More extensive documentation on safety in the target organs needs to be gathered, but some of this is generally gathered post-authorisation. Finally the regulators commented that clinicians often hope that when faced with a small patient group size, leniency will win their cause; where in fact it is the functional patient benefit which is of importance and the design of the experiments should objectively show this. It also needs to be ascertained whether dystrophin is the most appropriate surrogate marker, by screening for other candidates.

Two industrial partners presented their ongoing clinical trial work on skipping exon 51 in DMD boys, using a PS oligomer (PRO051) or a PMO (AVI-4658). Giles Campion representing

Prosensa Therapeutics summarised their data on PRO051 where both local and systemic proof of concept trials have been completed and the local study published [21]. Briefly they demonstrated stable, *de novo* expression of dystrophin a favourable pharmacokinetic profile, no adverse events and no safety concerns. A systemic extension study is currently underway which involves SC injection once weekly over 2 years at a dose of 6 mg/kg into 12 DMD patients over an age range of 5–14 years. Finally, he pointed out that Prosensa felt there were a lack of well-validated OM in DMD and that historic data are lacking in DMD patients to guide safety monitoring. Help from organizations such as TREAT-NMD would be very welcome in this regard. Steve Shrewsbury (AVI Biopharma) summarised data from 17 studies with 5 different PMOs against West Nile Virus, restenosis, Hepatitis C, P450 metabolised drugs and DMD boys to a total of 417 patients with a maximum dose of 600 mg AO delivered either IV/IM/SC or orally with a maximum exposure duration of 28 days. The dystrophin positive fibre count was 44–79% across 5 patients in the local single intramuscular dose study [22]. The current study involves escalating doses to a maximum single dose of 300 mg so far and several DMD boys have complete 12 × weekly doses of AVI-4658. 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 2000 mg without obvious adverse effects to date. The Regulators reminded all participants of the need to ensure proper trials able to demonstrate efficacy and safety are performed, and that scientific advice should be sought for this purpose before the trials start.

## 5. Ethical considerations for trials in DMD

Pauline McCormack raised ethical issues associated with research into DMD. Children with a diagnosis of DMD are disadvantaged in several ways, the rarity of their condition, its life-limiting-nature and from the lack of effective therapies. The need for research is therefore paramount but as a group children are the most vulnerable potential research subjects. Yet the precautionary approach to research this vulnerability requires can also be a further disadvantage [23]. The protection afforded to these children needs to be balanced against the disadvantage of delaying research leading to effective treatment thus potentially harming by omission. It is therefore necessary to make a realistic assessment of the risks involved in conducting research but to also consider the benefits and weigh the risks of being too cautious with regard to research. So the first imperative is that affected children must be involved in clinical trials.

Since only affected children can be used as research subjects it is wrong to limit this to clinical trials involving young children on the premise that they are clinically less severely affected and because there is the potential for most benefit. A second imperative is that older children with more advanced disease should also be included in clinical trials on the grounds of beneficence and justice. Older children also stand to benefit both directly and indirectly. For older and more severely affected boys the potential to stabilise the disease and retain the use of a hand is a major benefit as it will mean they will be able to control their own wheelchairs 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 an OM. The indirect benefits of trial participation should also be considered since patients have testified that the social utility of participating in a trial, making a contribution to the fight against the disease and the feelings of self-worth and value this engenders is a major benefit quite independent of whether the trial results in personal benefit. So the third imperative is to

weigh the risks of research against the direct and indirect benefits of participation.

The fourth imperative is the need to adopt collaborative approaches to trial design and consent processes. Informed consent is one of the foils against the possibility that enthusiastic investigators, parents and patients will not weigh the risks involved in research before consenting. TREAT-NMD takes these concerns very seriously and as a consortium has developed a number of strategies and policies to disseminate good practice and provide sources of clear and comprehensive information. TREAT-NMD agrees with EMA that more can be done to involve parents and patients in research design and along with FDA and other will seek to have more inclusive approaches towards children's participation including allowing for the assent of young children alongside parental consent.

The EMA representatives commented that children should be more involved in the design of the trial and it should not be necessary for them to understand all aspects. The FDA commented that in USA assent can occur as young as 9, where compassionate trials exists in tandem with normal trial protocols to take into account the particular requirements of the disease in question.

## 6. General discussion

Edward Connor chaired this session where a representative from each organisation summarised the take home messages relevant to their own involvement. Francesco Muntoni said it is now clear that TREAT-NMD needs to address the quantification of dystrophin levels and their functional correlates, and this can be addressed with international collaboration. There is also a need to collect natural history data across the full age range of DMD and thus provide the data for the development of more relevant OM as well as determining the potential value of goal attainment scales. Again, this can be done on the basis of an international collaborative effort. It is interesting to note that with appropriate adaptive trial design and carefully considering the number of patients to be recruited in trials, there should be necessary numbers of ambulant children to perform the necessary first pivotal trial(s). Leone Atkinson (PTC Therapeutics) summarised the perspective of the industrial partners. Today's meeting had been monumental in suggesting how collaboration between industry, advocacy groups, clinical teams and regulators could efficiently accelerate clinical development and patient benefit and make use of natural histories. This will contribute to long term clinical trial planning. Pat Furlong (Parent Project Muscular Dystrophy) said the parents were very grateful to have this meeting which was clearly the first step in extending their son's lives by reviewing the clinical trial regulations. Daniel Brasseur (Chair of the Paediatric Committee, EMA) reminded the audience in the face of a progressive disease that you should keep in mind the word 'anticipate' and come and see the regulators sooner rather than later with experimental protocols, so that advice can be offered from guidelines in place on other models. Spiros Vamvakas (EMA) highlighted the need for more natural history on DMD patients since this has been shown to be relevant in developing treatment guidelines on other orphan diseases. There is the need to develop new OM and involve patient representatives in the protocol discussions. Elizabeth McNeil (FDA) commended the enthusiasm of everyone present to move treatment forward and that there may only be one phase II/III study in which to work from. It is always impossible to obtain all the necessary safety data on such a small population before drug registration. It is of the utmost importance that we hear from the patients as to what benefit they want and incorporate it into the protocols. Stabilisation of such a progressive disease is a clear

benefit and should be sufficient for demonstrating efficacy of a therapeutic agent in a progressive disease.

## 7. Outcomes of the meeting

The meeting was considered a tremendous success for all parties involved. The regulatory authorities were both amenable and positive towards the data presented and not only illustrated helpful ways forward but made clear they would welcome closer and more frequent contact to ensure a speedy therapeutic stratagem. The main outcomes of the meeting were:

### (A) Trial design and outcome measures

- Randomised clinical trials might involve fewer than 150 patients where there is no other option.
- Both the children and their parents/carers shall be involved in the design of the protocols and OM since they are in the best position to know what improves their life.
- Natural histories and OM need to be revised and expanded to include neonates, non-ambulant boys, and functional relevance of increased dystrophin levels, patient aging, cardiac output and lung vital capacity.
- A goal attainment scale could be used for OM provided it's validated.
- Objectively show that the increase in dystrophin level achieved is a surrogate marker of functional patient benefit or whether another surrogate marker needs to be followed.
- Disease stabilisation is a reasonable therapeutic option.

### (B) Toxicology, safety and class of drug

- More pharmacodynamic, pharmacokinetic and toxicology data may be required on more than one AO to allow extrapolation of data.
- More toxicology data on the target organs required.
- Pharmacodynamic data needs to ascertain the optimal dose and whether the maintenance and starting doses would be the same.
- AO therapeutic treatment in DMD would represent first in child, which is different to first in man and the safety data has to reflect the extended treatment time.

### (C) General

- Neonatal screening should be considered for DMD so treatment can begin earlier with a view to increasing the benefit for the patients.

#### 7.1. Participants

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