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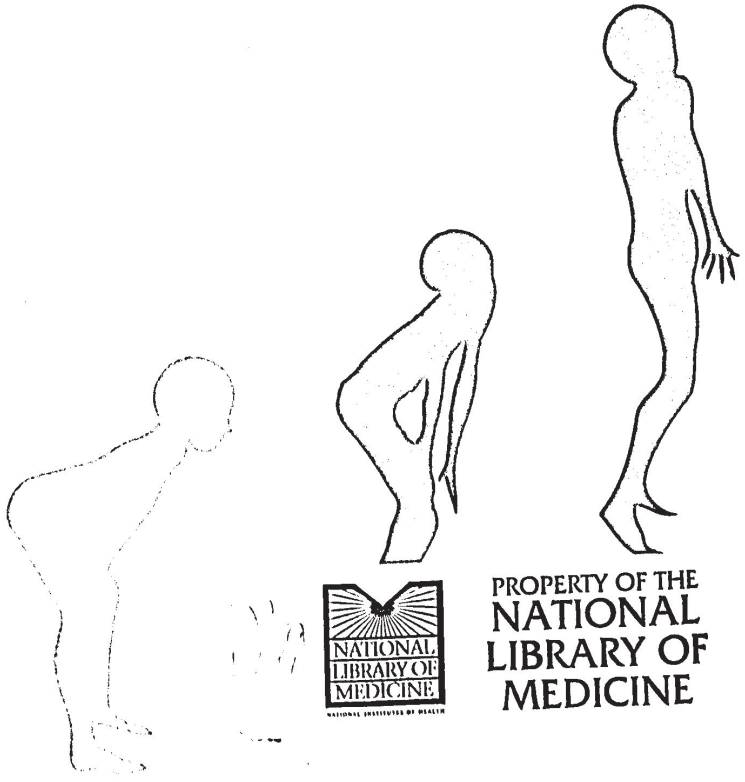

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Workshop report

149th ENMC International Workshop and 1st TREAT-NMD Workshop on: "Planning Phase I/II Clinical trials using Systemically Delivered Antisense Oligonucleotides in Duchenne Muscular Dystrophy"

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1. Introduction

Thirty-one participants from 7 countries (Australia; England; France; Germany; Italy; The Netherlands; USA) attended the second ENMC workshop on antisense oligonucleotides in Duchenne muscular Dystrophy (DMD). The topic of this workshop was on "Planning Phase I/II Clinical Trials Using systemically delivered Antisense Oligonucleotides in Duchenne Muscular Dystrophy (DMD)" and followed a similar workshop held in 2004 focused on intramuscular administration of antisense oligonucleotides or AONs. The workshop was organized with the support of the TREAT-NMD EU Network of Excellence (www.treat-nmd.eu) and Parent Project Muscular Dystrophy (PPMD), and was attended by representative of the two companies involved in the current intramuscular injection trials, Prosensa for the 2-*O*-methyl phosphorothioate RNA modified and AVI Biopharma for the phosphorodiamidate morpholino oligomers (PMOs or more commonly "morpholinos"). Although, the PMO backbone is based on synthetic subunits, not regular nucleotides, for the sake of simplicity we will refer to all antisense oligomers as AONs.

Specific aims of the meeting were for 2 consortia, one from the Netherlands and one from England, currently involved in intramuscularly administered AON trials, to present the progress of their respective studies; and for members of these consortia and representatives from other international groups at different planning stages of the use of AONs in DMD, to discuss various aspects related to the best strategies to plan future systemic AON trials. Issues discussed also included methodological issues on the different backbone of the AONs used (2-*O*-methyl phosphorothioate modified [2*O*MePS] and morpholino) oligomers; safety, regulatory, and ethical aspects.

DMD is a severe muscle wasting condition with onset in early childhood, progressive muscle weakness and disability and ultimately reduced life expectancy. It is caused by mutations in the DMD gene that lead to the failure to produce the corresponding muscle protein called dystrophin. Most of these mutations are out-of-frame deletions. Laboratory studies over the last decade have shown that the addition of small molecules named antisense oligonucleotides or oligomers (AONs) to cultured patient muscle cells, and their injection into muscles of the *mdx* mouse model for DMD can restore the production of the protein dystrophin [1,2]. Although this correction is only temporary, it induces improved function of the patient cells and mouse muscle. More recently, the repeated systemic (intravenous) administration of AONs was shown

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to be capable of restoring a sustained dystrophin expression in the mouse model of DMD, and this was followed by a significant functional improvement of the mouse muscle function [3–6]. If safe and equally effective in people, the repeated systemic administration of AONs could therefore be an effective tool to slow down the disease progression in DMD boys.

2. Phase I/IIa trials on intramuscular AON administration

Two representatives of the Dutch Consortium, Jan Verschuuren and Judith van Deutekom, presented the data of the recently completed IM injection trial of a 2OMePS AON to induce skipping of exon 51, funded by the Dutch Duchenne Parent Project, SenterNovem (funded by the Department of Economic Affairs) and ZonMw (Dutch MRC, funded by the Department of Health) and Association Française contre les Myopathies (AFM) and sponsored by Prosensa. This study was aimed at defining safety and local dystrophin restoration of IM administered 2OMePS AON in a single muscle in DMD boys [7]. As part of the pre-screening program, boys had a muscle MRI to document adequate preservation of the target muscle, the tibialis anterior (which was invariably the least affected muscle in the lower leg of affected boys), and a skin biopsy for MyoD transfection and myogenic conversion of fibroblasts and in vitro testing of response to the administration of AON. Four boys were included between the age of 10 and 13, carrying deletions of exons 50, 52, 48–50 and 49–50. They received a single dose of 0.8 mg of the 2OMePS exon 51 AON in one tibialis anterior muscle using an EMG guided needle. A muscle biopsy of the same tibialis anterior was performed 4 weeks after the injection of the AON and dystrophin protein and transcript analysed in detail. The results in all 4 boys were extremely encouraging with robust levels of dystrophin skipped transcript visible 1 month after the AON injection, and the percentage of dystrophin positive fibres in the tibialis anterior biopsy comprised between 64% and 97% in the 4 children studied. In view of the unequivocal positive results in these 4 patients, the decision was made not to recruit additional patients into this study — the original plans were to recruit a total of 4–6 DMD boys. Reassuringly the intramuscular administration of the 2OMePS was well tolerated with no apparent inflammatory response to the administration of the AON.

Two representatives of the MDEX consortium, Francesco Muntoni and Maria Kinali, illustrated the status of the study in UK. This study is funded by the Department of Health, sponsored by Imperial College and run in collaboration with AVI Biopharma. This study is similar to the Dutch trial, the main differences being that (i) the AON injected will be a 30mer morpholino, (ii) the study is a dose escalation study; (iii) one extensor digitorum brevis (EDB) of older children (12–17 yrs) will receive the PMO AON administration while the contralateral EDB will receive a sham injection. At the end of the study an open biopsy will be performed on both muscles to allow quantitation and differentiation of dystrophin production following the administration of the AONs from the background of dystrophin that the patient might produce, including revertant fibres. Nine DMD boys will be studied, three receiving the lowest dose, 3 an intermediate dose and 3 the highest dose, with the recruitment of this latter group only considered in case the results from the previous patient groups are equivocal. A muscle MRI protocol has been devised to pre-screen patients in order to confirm the preservation of the EDB, and preparatory studies have indicated that most DMD boys up to the age of 16 have sufficiently well preserved EDB muscle to be eligible for the study. Also the MDEX consortium protocol requires for each patient to be studied by MyoD transfection of skin fibroblasts and subsequent AON treatment to confirm feasibility of AON-induced dystrophin restoration in vitro; in addition a detailed neuropsychiatric questionnaire was devised in order to be able to monitor expectations and impact of the trial on individuals. At the time of the workshop the study was in the process of completing regulatory authorization.

As part of the preparatory studies, the MDEX consortium has studied whether revertant fibres increase with age in DMD boys. Previous studies performed in the *mdx* mouse have suggested that this could be the case. Twelve boys who have had a muscle biopsy at diagnosis and which was available for further evaluation were recruited into this study; these boys had muscle biopsies during planned surgical procedures of either the EDB muscles (9 cases) or paraspinal muscles (3 cases) on average 7 years following the original diagnostic quadriceps muscle biopsy. In none of these 12 patients was there an increase of the frequency of revertant fibres with age, at least as far as the studied muscles were concerned. This information is helpful as it suggests that any dystrophin produced following the AON administration is the likely result of the AON-induced exon skipping and not naturally occurring revertants, provided that the number of revertants in the original muscle biopsy is negligible (a threshold of 5% was arbitrarily agreed).

3. Preclinical studies focused on systemic AON administration

Judith van Deutekom presented the recent results of the systemic administration of a 2OMePS AON against exon 23 in the *mdx* mouse. These studies focused on different mode of administration (IV, subcutaneous [SC]; intraperitoneal [IP]) and results were validated not only using semiquantitative assays, but also by an AON-specific hybridization assay to measure tissue levels of 2OMePS AON. This method developed by Prosensa allowed assessment of the biodistribution of the 2OMePS in a number of organs (including liver and kidney) serum and muscle. While there were significant differences in the pharmacokinetics of the AON in several organs following

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