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P.20.14
Non ambulant patients with deletion treatable by exon skipping 53 present a more severe phenotype than the general Duchenne population

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Exon skipping therapy is an emerging approach in Duchenne Muscular Dystrophy (DMD). Antisense oligonucleotides that skip exon 51, 44, 45, and 53 are currently evaluated in clinical trials. Knowing the precise phenotype of potentially eligible patients is important for designing the clinical trials. It is generally believed that there are few if any phenotypic differences between these different groups of patients or between these patients and the general DMD population. In preparation of exon 53 skipping mediated by an AAV8 construct, we recruited 24 patients aged from 6 to 20 years in an observational study of upper limb strength and function, using the Motor Function Measure (MFM), the Myogrip, the MyoPinch and the MoviPlate, three tools already validated in a large non ambulant DMD population. Data of 14 non ambulant DMD patients (13.8 ± 2.7 years) at inclusion were compared with 14 age and size-matched DMD controls (13.7 ± 2.6 years) at all types of mutation. DMD 53 patients scored significantly lower in the MFM (D3: 66 ± 17% vs. 82 ± 13% for DMD 53 vs. DMD control), had significant hand-grip and key pinch strength on both hands. They had lost ambulation 13 months earlier (Age: 105 ± 20 vs. 118 ± 19 p = 0.04 for DMD 53 vs. DMD control).

In order to rule out that this is due to a selection bias, we compared DMD 53 patients with other DMD patients carrying a deletion not involving the 45–55 region and looked at DMD patient cohort identified at the Cochin hospital’s routine diagnostic laboratory. We found that 91 DMD ex53 patients had lost ambulation at 108 ± 15 months, whilst other 400 non 45–55 DMD patients with a deletion had lost ambulation at 139 ± 54 months.

Taken together, these preliminary data demonstrate that non-ambulant patients treatable by exon 53 skipping present a more severe phenotype than the general DMD population. This must be taken into account in the design of studies concerning this population.

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IMMUNE MYOPATHIES II

P.21.1
Autophagy as a link between immunity and inflammation in idiopathic inflammatory myopathies

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Idiopathic inflammatory myopathies (IMMs) are a heterogeneous group of immune-mediated muscle disorders characterized by muscle inflammation and weakness. They comprise inclusion body myositis (IBM), polymyositis (PM), juvenile dermatomyositis (JDM) and adult dermatomyositis (DM). Autophagic pathway impairment has been well documented in sporadic IBM, where it has been suggested to be responsible for the accumulation of multiple-protein aggregates, typical of the myopathy. The main candidates responsible for this impairment are suggested to be TLRs. We evaluated the autophagic process also in PM and DM, in particular the interaction between autophagosome maturation and innate immune system. LC3 and other autophagic molecules, together with peptides corresponding to unique epitopes generated by exon skipping were tested for the induction or inhibition of autophagy. Gene expression analysis showed a dysregulation of autophagy in all IMM subgroups. A tight correlation between autophagy and innate immunity in myopathic muscles compared

The pre-symptomatic induction of inflammatory cascades and invasion of muscle by immune cells in dystrophin deficient muscle contributes to the pathology of Duchenne muscular dystrophy (DMD). Traditional and RNA-based gene therapy approaches for DMD are progressing through clinical development. Whilst in one study a post-treatment reduction in inflammatory infiltrate after antisense oligonucleotide-mediated exon skipping has been reported; another study, on viral delivered mini-dystrophin, has shown evidence that dystrophin epitopes expressed in revertant fibres can elicit T cell production in untreated patients which may accelerate a post-treatment immune response.

To extend this observation, we are performing annual ELISPOT IFN-gamma assays on patients recruited into a four year DMD natural history study. The study is recruiting 30 ambulant and 30 non-ambulant DMD boys with exon skippable deletions from three clinical centres. ELISPOT assays on all patients are performed with a full length dystrophin peptide set whilst patients with exon 51 or 53 skippable deletions are also assessed with peptides corresponding to unique epitopes generated by exon skipping. This allows us to assess pre-existing immunological responses to dystrophin epitopes in patients prior to inclusion in clinical trials, as well as the likelihood of a post-exon skipping immune response to newly-generated dystrophin protein.

Here we present the results to date from patients recruited during the first year; we correlated data to factors such as age, ambulation status, steroid regime and DMD deletion. The majority of patients did not have significant T cell activity against the full length or unique epitope peptide sets; whilst in those that did (approximately 1/6), the activity was relatively low. These ‘positive’ patients are presented and the responses mapped to specific dystrophin peptides. Our data provides a useful immunological baseline for future DMD clinical trials.

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P.20.15
Assessing T cell-mediated immune response to dystrophin in the natural history of Duchenne muscular dystrophy

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The pre-symptomatic induction of inflammatory cascades and invasion of muscle by immune cells in dystrophin deficient muscle contributes to the pathology of Duchenne muscular dystrophy (DMD). Traditional and RNA-based gene therapy approaches for DMD are progressing through clinical development. Whilst in one study a post-treatment reduction in inflammatory infiltrate after antisense oligonucleotide-mediated exon skipping has been reported; another study, on viral delivered mini-dystrophin, has shown evidence that dystrophin epitopes expressed in revertant fibres can elicit T cell production in untreated patients which may accelerate a post-treatment immune response.

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