Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder, which is characterized by the loss of muscle fibers and replacement by fibrotic tissue due to the lack of the dystrophin protein. Myostatin and Transforming Growth Factor (TGF)-beta play important roles in regulation of muscle differentiation and fibrosis, and are emerging as attractive therapeutic targets for DMD treatment. Both cytokines signal primarily via the overlapping Smad2/3-dependent signaling pathways. In this study we targeted myostatin/TGF-beta type 1 receptors Acvr1b (ALK4) and Tgfbri1 (ALK5) using antisense oligoribonucleotides (AON) targeting regions in the pre-mRNA encoding ligand binding and/or kinase domains of the receptors. Transfection of ALK4 or ALK5 AONs resulted in skipping of the targeted exon, ~50% downregulation of the full length transcript and enhanced myoblast differentiation. In addition, local administration into mdx mice showed considerable downregulation of full length ALK4 (~80%) or ALK5 (~50%), leading to ~50% increase of myogenic genes expression and ~40% decrease of fibrotic markers expression. Furthermore, we used these AONs to study how TGF-beta and myostatin signaling are regulated in different cells. We showed an exclusive requirement of ALK4 for myostatin signaling in myoblasts, but preference for ALK5 in the other cell types, which suggested that at least two co-receptors are involved in this cell type-specific regulation of myostatin signaling. In summary, we were able to use this specific genetic approach to selectively disrupt and dissect the overlapping pathways and revealed a novel muscle specific mechanism of myostatin signaling. Long-term systemic administrations are currently ongoing to further assess the therapeutic benefits of these AONs and to determine the differential effect of ALK4 and ALK5 knockdown on the dystrophic pathology of the mdx mice.

O.14 Bioincompatible nanoparticles as slow-release delivery system of 2′OMePS AON administered both intraperitoneally and orally in the mdx mice: dystrophin rescue and nanoparticles biodistribution

We recently demonstrated that lower doses of 2′-O-methyl-phosphorothioate antisense oligoribonucleotides (AONs) adsorbed to cationic core-shell nanoparticles (NPs) induce widespread dystrophin restoration, even 45 days after intra-peritoneal (I.P) treatment, in mdx mice. Here we describe persistent, albeit low, levels of AON-induced skipped transcript in muscles from mdx mice sacrificed three months after NP-AON treatment, associated with well-maintained dystrophin expression in skeletal muscles, detectable by immunostaining and western blot analysis. Nanoparticles labeled with IR-Dye (Li-COR Biosciences) were used to evaluate biodistribution in mdx mice injected I.P or orally, by using Odyssey Imager–Li-COR Biosciences. Twenty four hours after I.P injection the NPs are still in the peritoneal cavity. After longer time the peritoneal cavity becomes less fluorescent, while the fluorescence diffuses widely in all the body, especially in lymphatic tissues (spleen) suggesting a body distribution via lymphatic vessels. Fluorescence is detectable up to 22 days. The time course of NP-IR-Dye after oral administration demonstrates the persistence of NPs in intestinal lumen for at least 48 h. During this time some fluorescence is also visible outside from the intestine, as in the spleen. The Telethon Italy Grant GGP09093 (to AF, PB, ML, MNM) is acknowledged.

**O.14**

**Interference of myostatin and TGF-beta signaling by antisense-mediated exon skipping in ALK4/5 receptors**

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**O.14**

**Bioincompatible nanoparticles as slow-release delivery system of 2′OMePS AON administered both intraperitoneally and orally in the mdx mice: dystrophin rescue and nanoparticles biodistribution**

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We recently demonstrated that lower doses of 2′-O-methyl-phosphorothioate antisense oligoribonucleotides (AONs) adsorbed to cationic core-shell nanoparticles (NPs) induce widespread dystrophin restoration, even 45 days after intra-peritoneal (I.P) treatment, in mdx mice. Here we describe persistent, albeit low, levels of AON-induced skipped transcript in muscles from mdx mice sacrificed three months after NP-AON treatment, associated with well-maintained dystrophin expression in skeletal muscles, detectable by immunostaining and western blotting. We also administered by oral route NP-AON in mdx mice: rescued dystrophin protein is detectable in intestinal smooth muscle by immunofluorescence and...
Timed 30 foot walk is predictive of time to loss of ambulation (LOA) in patients with Duchenne Muscular Dystrophy (DMD) without corticosteroid treatment. This has not been evaluated with corticosteroid treatment. To evaluate if timed 30 foot walk is predictive of LOA in patients with DMD treated with corticosteroids. Retrospective review, 1/1/1997–5/31/2010. Setting: Tertiary care children’s hospital neuromuscular clinic. Participants: 41 patients with DMD, >5 years old, treated with corticosteroids >6 months, who had lost independent ambulation. Timed 30 foot walk, time to LOA. The mean age at LOA was 11.03 years (6.28–16.60), the mean duration of steroid treatment was 4.17 years (0.92–13.40), and the mean age at steroids initiation was 6.85 years (3.19–11.30). The mean age at LOA was 9.76 (6.28–12.97) for those treated with steroids <4 years and 12.24 (9.29–16.60) for those treated >4 years, p = 0.0002. Most 30 foot walk times remained relatively stable between 4–5 s prior to clinical decline. 77% of patients who took 6–7 s lost ambulation within 4 years. All patients who took >9 s lost ambulation within 3 years and those who took >11 s lost ambulation within 2 years. Ages 7 & 8 were studied separately because this age is concerning for progression of decline in function. All patients at age 7 or 8 (n = 20) who took >8 s lost ambulation within 2 years and those who took >10 s lost ambulation within 1 year. Timed 30 foot walk is predictive of time to LOA for patients with DMD treated with corticosteroids. This is important for counseling patients and families about disease progression and timing for home modifications and acquisition of mobility devices. This data supports studies that show steroid treatment prolongs ambulation by 2 years or more.

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Duchenne muscular dystrophy (DMD) is a severe recessive X-linked inherited muscle disease characterized by a progressive loss in muscle strength and respiratory muscle involvement. After 12 years of age, lung function declines at a rate of 6–10.7% per year in patients with DMD. Steroid therapy has been proposed to delay the loss of motor function and also the respiratory involvement. In this uncontrolled, prospective study, the objectives were to assess the pulmonary function of patients with DMD on steroid therapy and to evaluate the influence of chronological age, age at onset of therapy, and walking ability (ambulant or non-ambulant) on lung volumes. In 21 patients with DMD aged between seven and 16 years, the forced vital capacity (FVC) and the forced expiratory volume in one second (FEV1) were evaluated at three different times during a period of 2 years. We observed in this period of evaluation the maintenance of the FVC and the FEV1 in this group of patients independently of chronological age, age at onset of steroid therapy, and walking capacity. We concluded that the steroid therapy has the potential to stabilize or delay the loss of lung function in DMD patients even if they are non-ambulant or older than 10 years, and in those in whom the medication was started after 7 years of age (Supported by CAPES).

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Duchenne Muscular Dystrophy (DMD) is a severe recessive X-linked form of muscular dystrophy characterized by rapid progression of muscle degeneration leading to loss of ambulation and pulmonary function impairment. Motor Function Measure (MFM) is a new measurement tool for assessing motor function in different neuromuscular disorders. It includes 32 items that evaluate three dimensions of motor performance: D1, axial strength and transfers; D2, axial and proximal motor capacity; and D3, distal motor capacity. The items are scored and summed to comprise a total score in which the maximum represents normal motor function. The objective of this study was to assess the evolution of motor function in patients with DMD treated with steroids (prednisolone or deflazacort) through the MFM. Thirty-three patients with DMD (22 ambulant, six non-ambulant and five who lost the capacity to walk during the period of the study) were assessed using the MFM scale on six times over a period of 18 months and in ambulant patients D2 improved during a period of 6 months; an improvement in D3 was noted during the total follow-up. D1 and total score were useful to predict the loss of the ability to walk. The use of the MFM in DMD patients confirms the benefits of the steroid treatment for slowing the progression of the disease (Supported by CAPES).

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Glucocorticoids (GCs) slow decline in Duchenne Muscular Dystrophy (DMD). GCs are started in the early ambulant phase; and different regimes-associated profiles of efficacy and side effect exist. With the aim to optimise and standardise the care of ambulant DMD boys on GCs, the neuromuscular clinicians in the UK collect data in the NorthStar database. Through the NorthStar database, clinical data was analysed for the period 2004–2011. Ambulant DMD boys aged 3–15 years attending 20 UK neuromuscular centres were recruited. Clinical longitudinal data was available for 334 boys: 113 on daily prednisolone (DP), 122 on intermittent prednisolone, 10-days-on/10 days-off (IP). Moderate to severe side effects included: behavioural changes in 40% of DP and 26% of IP boys; weight gain 48% on DP and 29% on IP; Cushingoid features 28% DP and 11% IP; height restriction below the 9th centile 38% DP and 32% IP; and vertebral fractures 6% and 3% respectively. Longitudinal NorthStar Ambulatory Assessment (NSAA) data could be analysed for 75 boys on DP and 61 on IP. One-way ANOVA demonstrated group difference (p < 0.0001) favouring DP: delay in disability progression became significant between 10–12 years of age with a median loss of ambulation at 13 years in DP versus 10 in IP (Kaplan–Meier, p = 0.01), and a median advantage of 1.9 years before decline from peak %FVC (Kaplan–Meier, p = 0.027). This study demonstrates the importance of collecting information on clinical course and response to GCs in ambulant boys. The NorthStar network offers a unique opportunity to collect updated natural history data on a large