Patients suffering from slow channel syndrome get benefit from molecules reducing the AChR opening time: quinidine and fluoxetine. In our experience, the benefit of these two molecules is very variable, even in patients from the same family and may be disappointing.
Ephedrine or Albuterol, two b2 adrenergic molecules, the mechanism of action in CMS of which remaining unclear, were show to be very beneficial in CMS due to *COLQ* (ACHE), *DOK7*, *AGRN,* b2-laminin genes, even in severe cases. These medications require a careful survey due to cardiovascular risk (hypertension, tachycardia, arrhythmia, cardiac infarct…). The relative efficacy of Ephedrine or Albuterol may differ from one patient to the other. 3,4-Diaminopyridine may be useful in these 2 categories of CMS.
If the causative gene is unknown, the therapeutic strategy will be careful and sequential. If repetitive response to single stimulation, suggesting a Slow-channel or a *COLQ* CMS, cholinesterase inhibitors are totally contra-indicated. If not, cholinesterase inhibitors are proposed as first medication, but the survey is mandatory and if no improvement or worsening, the medication is to be interrupted. Then 3,4-DAP may be proposed and eventually b2 adrenergic.

Table

- AChE (Colq): Ephedrine or Albuterol ; **avoid AChE-inhibitors** ; if necessary add 3,4-DAP
- AChR deficiency: AChE-inhibitors; if necessary add 3,4-DAP
- AChR Fast-channel: AChE-inhibitors; if necessary add 3,4-DAP
- AChR Slow-channel: **avoid AChE-inhibitors,** Quinidine sulfate, Fluoxetine;
- b2-Laminin: Ephedrine; **avoid AChE-inhibitors**
- ChAT: AChE-inhibitors; if necessary, add 3,4-DAP
- Dok7: Ephedrine or Albuterol; **avoid AChE-inhibitors** ; if necessary add 3,4-DAP
- GFPT1, DAPGT1,ALG2,ALG14, GMPPB : AChE-inhibitors , if necessary add 3,4-DAP
- Rapsyn: AChE-inhibitors; if necessary add 3,4-DAP , Albuterol
- MUSK: 3,4-DAP ,Ephedrine or Albuterol
- Agrin : Ephedrine or Albuterol, 3,4-DAP
- Defect in sodium channels : AChE-inhibitors , acetazolamide

Plenary Session- Gene-Based Therapy and Muscular Dystrophies
• *Matthew Wood (UK)* • *Aurelie Goyenvalle (FRANCE)* • *Caroline Le Guiner (FRANCE)*

**Advanced oligonucleotide therapeutics for neuromuscular disease**
*Matthew JA Wood, Professor of Neuroscience and Associate Head, Division of Medical Sciences Anatomy and Genetics, University of Oxford, OX1 3QX, Oxford, United Kingdom.* matthew.wood@dpag.ox.ac.uk

Oligonucleotide-based therapies have potential for treating a range of inherited neuromuscular disorders via modulating gene expression e.g. via splice modulation or RNA silencing. The classical example is Duchenne muscular dystrophy (DMD), where modulation of pre-mRNA splicing of the DMD gene can restore a viable reading frame and the expression of functional protein. This approach is currently being evaluated in clinical trials. However, a major challenge in the application of such approaches to neuromuscular disease is poor delivery to affected tissues including skeletal muscle, heart and to the nervous system across the blood brain barrier. We have developed a range of peptide- and EV-based platform technologies to overcome this challenge. Peptide-oligonucleotide compounds provide greatly improved delivery and enhanced potency and are being developed for future clinical applications in both DMD and for other neuromuscular disorders, such as spinal muscular atrophy. Future prospects will be discussed.

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**Tricyclo-DNA: highly promising antisense oligonucleotides for splice switching therapeutic approaches**
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Antisense oligonucleotides (AON) hold promise for therapeutic splice-switching correction in many genetic diseases; however, despite advances in chemistry and design, systemic use of AONs is still limited due to poor tissue/cellular uptake. This talk will describe a novel class of AONs made of tricyclo-DNA (tcDNA), which displays unique pharmacological properties and unprecedented uptake in many tissues after systemic administration. These outstanding properties have been demonstrated in different mouse models of genetic diseases such as Duchenne muscular dystrophy (DMD) and Spinal muscular atrophy (SMA). DMD is a neurogenetic disease typically caused by frame-shifting deletions or nonsense mutations in the gene encoding dystrophin and characterized by progressive muscle weakness, cardiomyopathy, respiratory failure and neurocognitive impairment. While current naked AONs do not significantly enter the heart or cross the blood brain barrier, systemic delivery of tcDNA-AONs allow high levels of dystrophin rescue in skeletal muscles as well as in heart and to a lower extent in the brain. Our results demonstrate for the first time physiological improvement of the cardio-respiratory functions and correction of behavioural features linked to the emotional/cognitive deficiency associated with the lack of dystrophin.
These properties, together with the safe toxicology profile of tcDNA make this chemistry particularly attractive for future therapies in DMD patients as well as in other neuromuscular disorders or diseases eligible for splice-switching approaches requiring whole-body treatment.

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**rAAV vectors as potential therapeutics for Duchenne Muscular Dystrophy**
*Caroline Le Guiner, PhD Atlantic Gene Therapies, INSERM UMR 1089, Nantes, France & The "AFM-sponsored Duchenne consortium", which includes Atlantic Gene Therapies (Nantes, France)/Genethon (Evry, France)/Institut de Myologie (Paris, France)/Royal Holloway (Londres, UK)*

Among vector systems that allow efficient *in vivo* gene transfer, recombinant Adeno Associated Virus vectors (rAAV) hold great promise and are currently evaluated in multiple clinical trials for the treatment of inherited diseases. In particular, gene-therapy of muscle diseases rapidly gained attention because delivery of rAAV vectors of several serotypes results in very

efficient transduction of skeletal muscles. Duchenne Muscular Dystrophy (DMD) is an example of a devastating muscle disorder without strongly effective treatment, which could benefit from the reconstitution of a deficient protein after rAAV-mediated gene transfer. DMD is a X-linked inherited muscle-wasting disease primarily affecting young boys with a prevalence of 1:5,000. The disease is caused by loss-of-function mutations in the gene encoding for the Dystrophin protein and is characterized by systemic, progressive, irreversible and severe loss of muscle function. Using a large network of laboratories with complementary skills, we are developing two rAAV-based gene therapy strategies for DMD. The first one stands on the constitutive expression of antisense oligonucleotides to promote, in the injected muscles, correction of the dystrophin messenger by exon skipping. The second one is based on the constitutive expression of a cDNA coding for a microDystrophin (µDys) protein. For our exon skipping approach, we use a gene therapy product consisting in a rAAV vector from serotype 8 (rAAV2/8) carrying a modified U7snRNA sequence promoting exon skipping to restore a shorter albeit functional quasi-dystrophin transcript. After having determined the therapeutic dose, the precise injection protocol and the toxicological/biodistribution patterns of this product in exhaustive pre-clinical studies, we are now in the phase of preparation of a Phase I/II clinical trial. This clinical trial will consist in the locoregional injection of the therapeutic rAAV2/8-U7snRNA vector in one forelimb of non- ambulant DMD patients. In parallel, our conventional gene-therapy approach is focused on the evaluation of a rAAV vector encoding a µDystrophin protein, for the treatment of DMD patients, whatever their genetic status. Using this vector, we injected a total of 12 Golden Retriever Muscular Dystrophy (GRMD) dogs, the canine model of DMD. We recently demonstrated that single-dose intravascular delivery of rAAV2/8-Spc512-µDys, in absence of immunosuppression, led to long-term transduction of distant muscle groups and extended lifespan (up to 2 years). Profound improvement of multiple clinical features was observed, including gait and respiratory parameters, and no toxicity or deleterious humoral and/or cell-mediated immune responses were observed.
The recent results and the specific developments of these translational projects will be presented.

Symposium- Parallel Symposium LGMD
• *T. Brand (UK)* • *Volker Straub (UK)* • *Isabelle Richard (FRANCE)*

**A mutation in the cAMP-binding domain of *POPDC1* is causing a limb-girdle muscular dystrophy and cardiac arrhythmia**
*Thomas Brand, Heart Science Centre, Imperial College London, United Kingdom*

The Popeye domain-containing (POPDC) genes encode a novel class of cAMP effector proteins, which is abundantly expressed in muscle and heart. In animal models (zebrafish and mouse), *Popdc1* and *Popdc2* are essential regulators of the structure and function of cardiac and skeletal muscle. However, until now, mutations in *POPDC1* or any other POPDC gene have never been associated with cardiac and skeletal muscle disease in patients. We recently identified a homozygous missense variant (c.602C>T, p.S201F) in POPDC1 by whole-exome sequencing in a family with cardiac arrhythmia and limb-girdle muscular dystrophy (LGMD). This allele was absent in known databases and segregated with the pathological phenotype in this family. The POPDC1$^{S201F}$ allele was not found in a further screen of 104 patients with a similar phenotype, suggesting this mutation to be very rare. Serine 201 is located to the cyclic nucleotide-binding cassette in POPDC1 and a reduction in cyclic nucleotide binding was therefore predicted. Compared with WT protein, POPDC1$^{S201F}$ mutant protein displayed a 50% reduction in cAMP affinity. Significantly, the mutant protein displayed a significantly altered subcellular localisation. In skeletal muscle from patients, the mutant POPDC1$^{S201F}$ mutant protein as well as POPDC2 displayed impaired membrane trafficking and an enhanced perinuclear localisation was observed. Aberrant membrane trafficking and gating was observed when the mutant protein was co-expressed with TREK-1, a two-pore domain potassium channel in *Xenopus* oocytes. Forced expression of POPDC1$^{S201F}$ in a murine cardiac muscle cell line (HL-1) increased hyperpolarization and upstroke velocity of the action potential. In zebrafish, expression of the homologous mutation (popdc1$^{S191F}$) caused heart and skeletal muscle phenotypes that resembled those observed in patients. Our study therefore identifies *POPDC1* as a novel and very rare autosomal recessive LGMD disease gene causing a mild muscular dystrophy and a severe AV-block by affecting protein trafficking and ion channel gating.
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**Trial readiness for patients with limb girdle muscular dystrophy**
*Volker Straub, The John Walton Muscular Dystrophy Research Centre, Newcastle University, Newcastle upon Tyne, UK*

The limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of rare autosomal recessive and dominant diseases that clinically present with progressive weakness and wasting of shoulder and pelvic-girdle muscles. Over the last 20 years, the underlying genetic defects for many of the LGMDs have been identified and insight into pathomechanisms has been gained. Since we have entered an era of translational research for some of the more common forms of LGMD, the need for precise molecular diagnoses, a thorough understanding of the natural history of the diseases and guidelines for standardized assessments of the patients become even more relevant. There are a number of specific challenges at every stage of the translational research pathway. They include the incomplete knowledge and understanding of disease prevalence and disease course, genotype-phenotype correlation, modifying factors, as well as difficulties in identifying patients and accessing patient biomaterials. Moreover, traditional trial designs may be inappropriate or simply not feasible, the tools to measure clinical response to therapy may be lacking, and the relative costs of development and marketing are high. Next Generation Sequencing (NGS) and other Omics technologies, in combination with powerful IT infrastructure and sophisticated bioinformatics tools, now allow the deciphering of the entire genetic (exome, genome) profile of any LGMD patient, and the integration of this information with clinical data (deep phenotyping, ontologies, electronic health records), as well as data from natural history studies, interventional trials and biomarker studies. In this context, our team has started a project called MYO-SEQ, that focuses on the application of NGS, in particular whole exome sequencing (WES), in a large cohort of patients with unexplained limb-girdle weakness. Focusing on undiagnosed patients with a clearly defined clinical