Perspectives on microdystrophins and delivery

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Muscular dystrophies refer to a group of inherited disorders characterized by progressive muscle weakness, wasting and degeneration. So far, there are no strongly effective treatments but new gene-based therapies are currently being developed with particular advances in using exon skipping and other RNA-based approaches, conventional gene replacement strategies, and cell-based gene therapy. In the case of DMD, a number of groups are testing gene therapy with adeno-associated virus vectors expressing engineered microdystrophins (AAV-MDs). In our hands, highly sequence optimised AAV-MDs are available for use in mouse, dog and humans, expressed using a strong skeletal and cardiac muscle specific synthetic promoter, have been tested in detail in mdx mice, and in the GRMD dog. Here we report the outcome of a study of AAV-canne MD delivery (5 × 1E12 vg/kg) by isolated limb perfusion in GRMD dogs from ages 3 months to 6 months. No immunosuppression was applied. In skeletal muscles of the treated limb at 3-months after vector administration we report widespread vector biodistribution, and sustained high level MD expression (up to 95% of fibres). In addition the treated limb exhibited very significantly improved parameters of muscle degeneration, fibrosis, 1H-MRI and 31P-NMR spectroscopy, and muscle strength. In the context of immune parameters, AAV administration elicited anti-AAV2/8 antibodies and a transient elevation of serum cytokines (wk5–10), but no evidence of cellular immunity to microdystrophin or to the AAV vector was observed. This study strongly supports the hypothesis that the current optimised microdystrophin is highly functional, not only in mice, but also in a large animal model, and that AAV2/8 vector delivery results in sustained expression of microdystrophin without adverse immune responses. The current optimised AAV-microdystrophin configuration thus lays a sound basis for a translation programme towards clinical trials in DMD patients.

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ADVANCES IN THERAPY FOR NEUROMUSCULAR DISEASES

Cell therapy and muscular dystrophy

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Cell therapy was first proposed in the 80’s as a potential treatment for muscular dystrophies, based upon early results obtained in mdx mice: dystrophin expression was restored in this model by intramuscular injections of normal myoblasts. These results were quickly followed by clinical trials for patients suffering from Duchenne Muscular Dystrophy (DMD) in the early 90’s, based mainly upon intramuscular injections of allogenic myoblasts. The clinical benefits obtained from these trials were minimal, if any, and research programs concentrated then on the various pitfalls that hampered these clinical trials. New therapeutic venues were then explored, such as the use of stem cells with myogenic potential, which have been described in various populations, including bone marrow, circulating blood or muscle itself. These stem cells presented the main advantage to be available and not exhausted by the numerous cycles of degeneration/regeneration which characterize muscular dystrophies. However, the different stem candidates have also shown their limits in terms of efficiency to participate to the regeneration of the host. The ideal candidate should
be easily isolated, amplified and injected systemically while targeting the desired site. A state of the art summary of the various ongoing clinical and preclinical trials and of the various cell types that are currently available will be presented.

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TREATMENT APPROACHES FOR MUSCULAR DYSTROPHY

O.11

Personalized therapy in Duchenne muscular dystrophy: An integrated approach

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Exon skipping, a promising new approach for the treatment for Duchenne muscular dystrophy (DMD), relies on the systemic administration of exon-specific antisense oligonucleotides (AONs) to reframe genetic mutations. As the targeted exon is relevant to specific subpopulations of patients with DMD, a number of AONs targeting increasingly smaller patient populations are required. Orphan drug designation and development is ongoing for six compounds, and a comprehensive development program is underway for the most prevalent subgroup, representing approximately 13% of patients with DMD. Such a program is not feasible for less prevalent groups. Discussions with regulators, clinicians, and scientists are underway to find appropriate ways to develop these compounds, building on information gained from the lead molecule. Concepts such as abrogated preclinical development packages, manufacturing design space, and seamless trial designs may facilitate this process. Central to this theme is the patient’s perception of risk, which is being pursued independently by patient advocacy groups.

Here we will discuss the seamless design accepted by the EMA and FDA for two exon skipping programs targeting sub-populations too limited to carry out fully powered, placebo-controlled pivotal Phase III studies and how future programs may be further accelerated in the ‘ultra-orphan’ DMD space.

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O.12

Peptide-enhanced uptake and bioactivity of antisense oligonucleotides in muscle and heart of DMD and DM1 mouse models


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RNA-modulation by antisense oligonucleotides (AONs) represents an interesting therapeutic approach for different neuromuscular disorders, such as Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1). DMD is caused by out of frame mutations in the DMD gene. AON-mediated exon skipping, aimed to restore the disrupted reading frame of the dystrophin transcript, is currently in (pre-) clinical development. In DM1, DM protein kinase transcripts contain a toxic expanded (CUG) n repeat stretch. Suppression of these toxic transcripts by AONs is currently being explored as a potential molecular intervention for DM1. For both diseases, efficient body-wide delivery of the AONs to muscle tissue and heart would enhance their therapeutic effect. The lack of dystrophin in DMD muscle results in more permeable muscle fibers, which, in the mdx mouse model, was demonstrated to promote AON uptake in muscle but to a lesser extent in heart. As the muscle fiber membranes are not impaired in DM1, various delivery-enhancing complexes and ligands are being explored to obtain sufficient muscle and heart uptake of AONs. Here, we present results with a 7-amino acid linear peptide that, conjugated to 2’-O-methyl phosphorothiate AONs, seems to enhance their uptake and bioactivity in muscle and, particularly, heart, in both DMD and DM1 mouse models.

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O.13

Using out-of-frame exon skipping to induce IRES-driven expression of an N-truncated dystrophin isoform for 5’ DMD mutations

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Frame-truncating mutations within the first five exons of the DMD gene typically do not result in Duchenne muscular dystrophy, but instead result in milder dystrophinopathy syndromes. This was first demonstrated in individuals carrying the c.9G > A (p.Trp3X) mutation, a North American founder allele associated with clinical severity ranging from a very mild Becker muscular dystrophy phenotype to a complete absence of symptoms. We have previously shown that the mild phenotype associated with this and similar 5’ exon mutations is due to alternative initiation of translation from methionines encoded in exon 6, a process mediated by the presence of a muscle-specific and glucocorticoid-inducible internal ribosomal entry site (IRES) found within exon 5. The resultant N-truncated dystrophin protein lacks the first calponin homology domain of the canonical actin binding domain 1. Nevertheless, it is highly functional, raising the possibility of the therapeutic use of this isoform.

We hypothesized that disruption of the mRNA open reading frame via exon skipping – resulting in a downstream premature termination codon – would stimulate IRES utilization. The feasibility of this approach is supported by the recent identification of an asymptomatic patient harbouring an exon 2 deletion, and we designed 70mer RNA vectors targeting exon 2 for use in both patient-derived cell lines and in a new DMD mouse model harbouring an exon 2 duplication. Both in vitro and in vivo we can stimulate IRES activity, and by combining exon-skipping and glucocorticoid treatment we are able to restore expression of a properly localized yet N-truncated dystrophin. These results not only provide evidence for the functionality of the dystrophin IRES – and, hence, for the presence of a novel N-truncated dystrophin isoform under yet to be clarified physiologic conditions – but also suggest that out-of-frame skipping is a promising therapeutic approach for DMD patients harbouring 5’ mutations.

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