to the plasma membrane. This demonstrated that the full enchainment of C2 domains is not mandatory to address dysferlin to the plasma membrane. Currently, we investigate the interaction properties of these “mid-dysferlin” constructs with some of the known partners of dysferlin. Finally, based on biochemical and biological studies of these constructs expressed in mouse (C2C12) or Human (HEK) cells, we aim to confirm the functional modularity of dysferlin. Altogether, this approach will improve our knowledge of dysferlin functions and allow us to develop a mini-gene transfer therapeutic strategy.

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T.P.28
Pseudo-exon inactivation of the dystrophin gene: Ideal candidates for exon skipping
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There are many different types of mutations that can inactivate the dystrophin gene and lead to Duchenne muscular dystrophy (DMD). Genomic deletions of one or more exons deletions account for the most common type of dystrophin gene lesion, and a handful of splice switching antisense oligomers could potentially restore the reading frame of some 40% of all DMD mutations. The removal of one or more exons flanking a frame-shifting genomic deletion could restore the reading frame and allow a Becker MD-like gene transcript to be generated from a DMD pre-mRNA. Exon 51 skipping trials are showing great promise as a therapy that may reduce the severity in some DMD patients, but regardless of how efficiently exon skipping can be induced, the resultant dystrophin isoforms will be Becker MD like. There is one type of DMD mutation that could be ideally suited to exon skipping, and although pseudo-exon activation is relatively rare, the application of splice switching to these mutations should result in the generation of a normal, not Becker MD-like, dystrophin gene transcript. A BMD patient was recently diagnosed whose dystrophin defect arose from an as yet uncharacterized mutation in intron 62 that led to the retention of 58 bases of intronic sequence being retained in the mature mRNA. While this pseudo-exon disrupts the reading frame and should have led to DMD, there were low levels of normal dystrophin that reduced disease severity. Antisense oligomers can mask normal exons from the splicing machinery, and we have found that some pseudo-exons are even easier to exclude from the mature mRNA. We describe personalized exon skipping strategies to address two different pseudo-exons, one arising from intron 62 and another from intron 47. In both these cases, normal dystrophin mRNA is induced, and unlike all other splice switching oligomers, compounds targeting these pseudo-exons could be tested in healthy volunteers.

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T.P.29
Categorization of 77 dystrophin exons into five groups by a decision tree using indexes of splicing regulatory factors
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Duchenne muscular dystrophy (DMD), a fatal muscle-wasting disease, is characterized by dystrophin deficiency caused by mutations in the dystrophin gene. Skipping of a target dystrophin exon during splicing with antisense oligonucleotides is attracting much attention as the most plausible way to express dystrophin in DMD. Antisense oligonucleotides (AOs) have been designed against splicing regulatory sequences such as splicing enhancer sequences of target exons. Recently, we reported that a chemical kinase inhibitor specifically enhances the skipping of mutated dystrophin exon 31, indicating the existence of exon-specific splicing regulatory systems. However, the basis for such individual regulatory systems is largely unknown. Here, we categorized the dystrophin exons in terms of their splicing regulatory factors. Using a computer-based machine learning system, we first constructed a decision tree separating 77 authentic from 14 known cryptic exons using 26 indexes of splicing regulatory factors as decision markers. We evaluated the classification accuracy of a novel cryptic exon (exon 11a) identified in this study. However, the tree mislabeled exon 11a as a true exon. Therefore, we re-constructed the decision tree to separate all 15 cryptic exons. The revised decision tree categorized the 77 authentic exons into five groups. Furthermore, all nine disease-associated novel exons were successfully categorized as exons, validating the decision tree. One group, consisting of 30 exons, was characterized by a high density of exonic splicing enhancer sequences. This suggests that AOs targeting splicing enhancer sequences would efficiently induce skipping of exons belonging to this group. Our classification may help to establish the strategy for exon skipping therapy for Duchenne muscular dystrophy.

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T.P.30
Characterisation of two DMD-causing splice-site mutations and development of personalised exon skipping strategies
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We describe two different donor splice site mutations that lead to Duchenne muscular dystrophy through a common mechanism: retention of the entire following intron in the mature mRNA. Consequently, RT-PCR scanning of the patient transcript was unable to generate amplicons in the region of the mutation, when tens of kilobases of intronic sequence must also be amplified. Despite the superficial similarities in the type of mutation in the donor splice site, and similar intron retention, the responses to splice switching oligomers were found to be distinct. The dystrophin exon 16 donor splice mutation led to the retention of ~20,370 bases, the entire intron 16 in the mature mRNA, accounting for the inability to amplify across dystrophin exons 15–18 in RNA extracted from this patient. Similarly, the exon 45 donor splice site mutation resulted in retention of intron 45 (~36,110 bases) in the mature mRNA, again rendering conventional RT-PCR analysis impossible. The loss of exon 16 does not disrupt the reading frame, and has been reported to not even raise serum CK levels, indicating this should be a highly amenable target for exon skipping. A previously optimized splice switching oligomer targeting exon 16 was found to be very effective at excising this exon and intron 16. In contrast, optimized oligomers designed to excise exon 45 only resulted in limited exon skipping, despite these compounds being very efficient when applied to the normal dystrophin gene transcript. The loss of only
Exon skipping using antisense oligonucleotides (AONs) is a promising therapy for Duchenne muscular dystrophy (DMD) which aims to geno-copy the milder Becker muscular dystrophy (BMD) by restoring the reading frame of an out-of-frame deletion. The less severe phenotype of BMD patients suggests that internally truncated dystrophin retains some functionality. We previously demonstrated that dystrophin and dystrophin-associated protein expression is correlated to clinical severity in BMD patients with in-frame deletions relevant to current exon skipping strategies (exons 51 and 53), and that BMD patients with deletions ending in exon 51 have significantly higher dystrophin levels than those ending in exon 53. We have now studied deletions relevant for skipping exons 44 and 45 which are now being considered for clinical trials. We studied a total of 25 patients; 11 patients had out-of-frame mutations correctable by skipping exons 44 or 45, and 14 patients had in-frame deletions corresponding to the skipping of exon 45. We quantified dystrophin and dystrophin-associated protein expression using immunohistochemistry and total dystrophin mRNA levels using qRT-PCR. DMD patients skippable for exons 44 and 45 had high levels of revertant and trace dystrophin expression (mean: 14% of control) with 6/11 out-of-frame patients presenting with an intermediate phenotype. Corresponding in-frame deletions presented with phenotypes ranging from mild to severe BMD, and showed remarkably lower dystrophin levels (~42% of control) than patients with deletions modelling the skipping of exons 51 or 53 (80% and 56% of control, respectively). Our study provides valuable insights into the stability and/or function of internally truncated dystrophin suggesting that higher levels of the dystrophin produced by skipping exons 44 and 45 are required to achieve the same level of functionality observed with exon 51 and 53 skipping studies.

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T.P.31
Biochemical and clinical variability of Becker muscular dystrophy: Predicting optimal target exons for exon skipping therapy in Duchenne muscular dystrophy
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Exon skipping using antisense oligonucleotides (AONs) is a promising therapy for Duchenne muscular dystrophy (DMD) which aims to geno-copy the milder Becker muscular dystrophy (BMD) by restoring the reading frame of an out-of-frame deletion. The less severe phenotype of BMD patients suggests that internally truncated dystrophin retains some functionality. We previously demonstrated that dystrophin and dystrophin-associated protein expression is correlated to clinical severity in BMD patients with in-frame deletions relevant to current exon skipping strategies (exons 51 and 53), and that BMD patients with deletions ending in exon 51 have significantly higher dystrophin levels than those ending in exon 53. We have now studied deletions relevant for skipping exons 44 and 45 which are now being considered for clinical trials. We studied a total of 25 patients; 11 patients had out-of-frame mutations correctable by skipping exons 44 or 45, and 14 patients had in-frame deletions corresponding to the skipping of exon 45. We quantified dystrophin and dystrophin-associated protein expression using immunohistochemistry and total dystrophin mRNA levels using qRT-PCR. DMD patients skippable for exons 44 and 45 had high levels of revertant and trace dystrophin expression (mean: 14% of control) with 6/11 out-of-frame patients presenting with an intermediate phenotype. Corresponding in-frame deletions presented with phenotypes ranging from mild to severe BMD, and showed remarkably lower dystrophin levels (~42% of control) than patients with deletions modelling the skipping of exons 51 or 53 (80% and 56% of control, respectively). Our study provides valuable insights into the stability and/or function of internally truncated dystrophin suggesting that higher levels of the dystrophin produced by skipping exons 44 and 45 are required to achieve the same level of functionality observed with exon 51 and 53 skipping studies.

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T.P.33
A cutting-edge approach to Spinal Muscular Atrophy treatment using antisense oligonucleotides
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Spinal Muscular Atrophy (SMA), a severe neuromuscular disorder characterised by the loss of motor neurons, is typically caused by deletion of the Survival Motor Neuron (SMN) 1 gene. The loss of the SMN1 gene product is incompatible with life, however in humans the presence of one or more copies of the near-identical SMN2 modifies disease severity. A single C>T base change in exon 7 of SMN2 results in abnormal splicing with the majority of transcripts missing exon 7 that encodes a crucial oligomerisation domain, and therefore results in a non-functional protein. The C>T variation simultaneously abrogates an exonic splicing enhancer, and creates a silencing element that leads to the exclusion of this exon from the mature gene transcript. Hence, redirecting splicing of SMN2 pre-mRNA has become a potential therapeutic target with the aim of re-enforcing exon 7 recognition and inclusion to increase levels of the full-length transcript and functional protein. Antisense oligomers (AOs) can interfere with splice site recognition by the spliceosome, and depending upon design can promote exon skipping or exon inclusion in the mature transcript. In treating SMA, efforts have focused on either enhancing exon 7 recognition, or masking splice silencing motifs located in introns 6 or 7. We present a third strategy where targeting the acceptor site of exon 8 with AOs induced exon and intron 7 inclusion in the transcript. The retention of intron 7 occurs after the normal stop codon and results in a longer than normal 3’ untranslated region. Western blot analysis demonstrated increased SMN expression in various SMA patient fibroblasts transfected with these AOs. “GEMs” are intranuclear aggregates of SMN and are barely quantifiable in fibroblasts from SMA patients. Immunofluorescent GEM staining showed substantial increases in abundance of GEMs in the AO treated SMA fibroblasts, indicating this strategy has considerable therapeutic potential.

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