thereafter. Thus cAMP and cGMP may both be involved, with different roles, in myogenesis. Selective PDEs inhibitors will help to better evaluate the role of cAMP and cGMP in regeneration and to validate PDEs as valuable drug targets in muscular dystrophy.

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P4.26

Toward the identification of druggable pathways involved in disease-related fatigue in Duchenne muscular dystrophy: In vivo and ex vivo studies in dystrophic mdx mice

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Along with progressive weakness, fatigability is a typical feature of Duchenne muscular dystrophy patients; in fact the 6 min walk test is a clinical readout parameter (Bushby et al., Lancet Neurol., 2010). Fatigability might result from multiple alterations, such as improper blood supply, metabolic distress and impaired mechano-transduction signals. The clarification of the disease-related mechanisms underlying fatigue may help to identify novel drug targets. To this aim, in vivo and ex vivo studies were performed on dystrophic mdx and wild-type (wt) mice of different ages and underlying or not standard protocols of forced treadmill exercise (Burdi et al. J. Appl. Physiol., 2009). In vivo, fatigue was detectable in mdx mice at 4, 8 and 16 weeks of age with a 30% lesser distance run vs. age-matched wt. Interestingly, fatigue, in parallel with weakness, was significantly enhanced in 8-week old mdx mice, but not in wt, that were chronically exercised for 4 weeks (163 ± 30 m; $n = 6$ vs. 390 ± 25 m of wt mice, $n = 5$; $p < 0.001$). A greater fatigue was observed up to 12 weeks of exercise, although slightly attenuated. In order to correlate the in vivo observations with muscle impairment, contractile parameters of extensor digitorum longus (EDL) and diaphragm were determined ex vivo. Twitch and tetanic force values were lower for both muscles in mdx mice, with a not significant worsening by exercise. However, a greater force drop was found in exercised mdx EDL muscle underlying eccentric-contraction protocols (30% drop vs 19% of sedentary mdx and 9% of wt). Preliminary TaqMan RT-PCR experiments showed that exercise failed to enhance the expression of peroxisome proliferator γ co-activator 1α, an activity-related metabolism modulator, in mdx, but not in wt, gastrocnemius muscle. The results confirm that complex mechanisms underlie fatigability in dystrophic subject; the role of non-muscular components and the hypothesis of a metabolic-mechanical uncoupling deserve further investigations.

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MOLECULAR: POSTER PRESENTATIONS

P4.27

Fourty eight-week follow-up data from a Phase I/IIa extension study of systemic PRO051/GSK2402968 in Duchenne muscular dystrophy: Comparison with contemporaneous controls for 6-min walking distance test

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PRO051/GSK2402968 is an antisense oligonucleotide that induces exon 51-skipping during pre-mRNA splicing and produces novel dystrophin expression in a genetic subpopulation of Duchenne muscular dystrophy (DMD) patients. We report efficacy and safety data for 48 weeks' treatment with PRO051/GSK2402968 from a Phase I/IIa extension study and compare the 6-min walking distance (6MWD) results with contemporaneous control DMD patients. Twelve DMD subjects with mutations correctable by exon 51-skipping completed a dose-escalation study and entered the open-label extension. All subjects were to receive weekly subcutaneous injections of PRO051/GSK2402968 6 mg/kg and were on stable steroid doses during the study. Assessments were performed at baseline and 4-week intervals for safety and 12-week intervals for clinical efficacy. All subjects reported treatment-emergent AEs. The most common AEs were increased urinary á1-microglobulin (100%), mild and variable proteinuria (92%), and injection site reactions (100%). The majority of AEs were considered mild; there were no severe treatment-related AEs. Non-progressive increases in some renal and hepatic parameters were observed. For subjects completing the 6MWD (mean [SD] age: 9.48 [1.88] years), mean (SD) distance at baseline was 384 (121) m. There was an improvement in 6MWD at 48 weeks (mean [SD]: 29 [80] m). Post-hoc assessment of 16 contemporaneous control patients of comparable age (mean [SD] baseline age: 9.64 [1.45] years), disease stage and standard treatment, with mean (SD) baseline 6MWD of 353 (72) m, showed a mean (SD) decline of 53 (77) m over a similar observation period. PRO051/GSK2402968 6 mg/kg administered weekly by subcutaneous injection was generally well tolerated across 48 weeks of treatment. Renal and hepatic function warrant further monitoring. Deteriorations of 6MWD in contemporaneous controls make the improvement in subjects treated with PRO051/GSK2402968 encouraging.

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P4.28

Immunogenicity assay for detection of anti-dystrophin antibodies in serum of Duchenne Muscular Dystrophy patients following therapeutic antisense-induced exon skipping

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Antisense oligonucleotides (AONs) have been shown to induce exon skipping during dystrophin pre-mRNA splicing, and to result in novel dystrophin expression at muscle fiber membranes in Duchenne Muscular Dystrophy (DMD) patients. To monitor whether the novel dystrophin induces a humoral immune response, we developed a Western Blot screening assay to detect the presence of putative anti-dystrophin IgG antibodies in serum. A Western Blot membrane containing human dystrophin protein from healthy muscle protein lysate is hybridised with patient serum. Serum containing antibodies against dystrophin, cross react with the wild type human dystrophin protein on the Western Blot membrane and generate a band at the molecular weight corresponding to that of dystrophin. As part of qualification of this assay it was shown to detect a positive control mouse anti-human dystrophin antibody in serum from different healthy donors and in serum from DMD patients, demonstrating the specificity of the analytical method to detect the analyte (anti-human dystrophin antibody) in the presence of other components in the serum sample. The Western Blot assay was shown reproducibly to be sensitive enough to detect 250–500 ng of positive control anti-dystrophin antibody per ml of serum and thus is appropriate to support clinical studies. Furthermore, routine monitoring is possible, as serum samples in a trial can be easily obtained, stored (frozen) and assayed in batches. In the phase I/IIa clinical study in which 12 boys were treated with AON PRO051/GSK2402968 and dystrophin expression was observed in muscle fibers in post-treatment biopsies of 10/12 patients, this Western Blot assay was

used and no anti-dystrophin antibodies were detected in the serum of the DMD treated boys.

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P4.29

Differential tissue expression and decay of dystrophin mRNA and protein

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Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy and it is caused by out-of-frame mutations in the dystrophin encoding *DMD* gene. Given the fact that the *DMD* gene is expressed in all skeletal muscles and heart, therapeutic strategies need to address all involved muscles in order to ameliorate or positively impact the phenotype. We have studied the expression of dystrophin on RNA and protein level in wild type and *mdx* mice, in control and patients' cell lines and in patients' muscles. Similar amounts of dystrophin protein were found in all skeletal muscles tested, diaphragm and heart. At the RNA level we show here that the *DMD* gene is significantly more expressed in the heart compared to skeletal muscles, both in wild type and *mdx* mice. This clearly impacts the quantification of exon-skipping percentages in the heart where normally lower values are observed compared to skeletal muscles. The differences between mRNA and protein levels can be explained either by different translation efficiency or protein turnover between skeletal muscles and heart. More detailed analysis of transcripts revealed no difference in expression in skeletal muscles between wild type and *mdx* mice upstream and around the mutated exon 23, while we describe a significant reduction of transcript leves in the heart of *mdx* mice compared to wild type mice. Finally *mdx* mice showed significantly reduced expression at the 3' of the gene compared to wild types, suggesting a selective degradation of the full-length mutated transcript in the *mdx* mouse. The expression profile has also been studied in antisense oligonucleotides (AON) treated mdx mice and in patient-derived transfected cell lines. Expression studies in patients' muscle samples and investigations about the decay mechanism are ongoing.

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P4.30

Multiple exon skipping strategies to by-pass selected dystrophin mutations

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Specific manipulation of dystrophin pre-mRNA processing can remove or compensate for protein-truncating mutations that would otherwise lead to the most common and severe form of childhood muscle wasting, Duchenne muscular dystrophy. The most common type of DMD-causing mutation is a frame-shifting deletion of one or more exons, hence initial exon skipping trials are focusing on the most common dystrophin exon deletion subtype that would have the reading-frame restored by the excision of exon 51. Many DMD-causing mutations will require the removal of more than one dystrophin exon to restore the reading frame, and in most cases, two overlapping exon excision strategies are available to restore some functional dystrophin expression. However, in some mutations involving small intra-exonic insertions/deletions or splicing defects, a third and simpler strategy is available: the skipping of a single, flanking, frame-shifting exon can sometimes restore the reading-frame disrupted by the small insertion/deletion. An example of this approach involves an exon 21 acceptor splice site mutation that led 2 bases of intron 20 being retained in the mature dystrophin mRNA. "Conventional" exon skipping to address this defect would involve targeting either exons 20 + 21 or 21 + 22, but a third and simpler approach relies on skipping only exon 20, where 2 bases are lost from the reading-frame, compensating for the retention of 2 nucleotides from intron 20. Such an approach is only possible if no premature termination codons are encountered between the excised exon and the gene lesion, generally limiting this strategy to insertion/deletions at the beginning or ends of a frame-shifting exon. However, such an approach would only require removal of one exon for some mutations, offering a simpler, cheaper and more feasible exon skipping approach to the dual exon skipping routinely considered.

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P4.31

Transient mouse models for the preclinical evaluation of therapeutic dystrophin exon skipping strategies

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Mutations that ablate dystrophin expression lead to Duchenne muscular dystrophy (DMD) an X-linked, relentlessly progressive muscle wasting disorder with a predictable course and limited treatment options. Corticosteroids are effective in stabilizing muscle strength in the short term but do not address the primary etiology of DMD, the absence of dystrophin. The majority of DMD cases are caused by frame-shifting deletion of one or more exons in the dystrophin gene, while- in frame deletions that do not disrupt the dystrophin reading frame generally cause the milder allelic disorder, Becker muscular dystrophy (BMD). Antisense oligomer (AO)-mediated splicing manipulation can remove specific exons during transcript processing, to by-pass DMD-causing dystrophin gene lesions and generate shorter, partially functional BMD-like dystrophin isoforms, and is showing promise as a therapy for DMD. Dystrophin gene structure in mildly affected and asymptomatic BMD patients indicates templates for a number of functional dystrophin isoforms, however, in-frame deletions in some regions of the dystrophin gene, particularly downstrean of exon 55 are rare, and the consequences of exon exclusion in this region are not known. The mdx mouse is a widely used dystrophinopathy model and has a nonsense mutation in dystrophin exon 23. AO induced-excision of this exon from the mRNA removes the mutation without disrupting the reading frame, resulting in functional dystrophin expression and amelioration of the phenotype. We now report that systemic administration of AO combinations to wild-type mice can remove dystrophin exons to generate DMD- and BMD-like dystrophin isoforms for functional evaluation. Assessment of contractile properties of the muscle reveals that some in-frame exon combinations confer near normal function, while others result in muscle susceptible to contraction-induced damage.

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P4.32

Are large in-frame duplications of dystrophin functional?

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Antisense mediated exon skipping to restore the open reading frame in dystrophin transcripts is a promising approach to therapy for Duchenne muscular dystrophy based on the results to date from cell culture, animal models and human clinical trials. It is also clear that whilst it is possible to skip one or two exons at relatively high efficiency, as is required to restore the open reading frame in patients with deletions and point mutations, it is