Evaluation of DMD transcripts after golodirsen treatment of MyoD-converted fibroblasts from 4053-101 clinical trial patients

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Introduction: Duchenne muscular dystrophy (DMD) is an X-linked, rare, neuromuscular disease caused by mutations in the DMD gene which result in a substantial reduction or absence of dystrophin protein. The commonest type of mutation is deletion of one or more exons, followed by large duplication; together they occur in 75% of DMD cases. Antisense oligonucleotides modulate exon incorporation, masking the splicing enhancer sequences or splice junctions in the pre-mRNA. This induces exon skipping, resulting in the restoration of the mRNA reading frame and translation of internally shortened dystrophin protein. Several antisense oligonucleotides are now approved, including the phosphorodiamidate morpholino oligomers (PMOs) Eteplisyn, casimersen and golodirsen, respectively targeting exons 51, 45 and 53.

Material & Methods: Proteins and RNAs were collected from fibroblasts, derived from 25 DMD patients enrolled in study 4053-101 at baseline and then differentiated into myotubes by lentivirally-mediated MyoD. Single-system qPCR with TaqMan® systems specific for DMD ex53 and ex55 was used to test the exon 53 skipping efficiency after treatment. FluiDMD cards were used to compare the transcript amount pre and after treatment.

Single-system TaqMan® qPCR experiments

Ex53 skipping efficiency in-vitro evaluation

Bar graph represents the exon 53 skipping efficiency for each patient after in-vitro golodirsen treatment. Each bar corresponds to an individual patient carrying different deletions (X-axis) while the skipping efficiency is expressed as percentage (Y-axis). Exon 55 skipping (DCI) is used to normalize the exon 53 expression (DC) and the DCI in the treated sample was normalized with the non-treated DCI. The full method used for the exon skipping efficiency evaluation is reported by Fortini & Risser, 2012.

In-vitro versus in-vivo ex53 skipping evaluation

The graph represents the ex53 skipping efficiency percentage in the in-vitro study (left graph) and in the in-vivo 4053-101 study (right graph), in which the muscle biopsies of the patients were studied. The ex53 skipping efficiency detected in our in-vitro study was correlated with that reported by Frank et al, 2020 in muscle biopsies from the same patients that had been treated with golodirsen. The differences in the skipping efficiency are likely due to the experimental conditions (in-vitro vs in-vivo) and by the different method to evaluate the skipping: In-vitro, qPCR. Ex53 skipping %: molarity of skipped band/molarity of the skipped band + molarity of the unskipped band*100 [Frank et al, 2020]

FluiDMD card® experiments

DMD transcript amount in treated vs non-treated samples

Bar graph represents the percentage of transcript increase after in-vitro golodirsen treatment. Each bar corresponds to an individual patient carrying different deletions (X-axis) while the differences between treated and non-treated transcript amount is expressed as percentage (Y-axis).

FluiDMD card experiment in treated samples

Graphic visualization of FluiDMD card experiment for one of the in-vitro treated patients carrying the ex53-ex55 deletion. In red, the bar corresponds to ex53-ex54 junction, that is affected by golodirsen treatment. The full DMD transcript coverage by FluiDMD allows us to conclude that golodirsen treatment does not give rise to other unspecific splicing products, in which one or more exons were alternatively spliced out. Each bar corresponds to a specific ex-ex junction or isoform unique region (X-axis) and expression amount for every system is expressed as Ct (Y-axis).

Conclusions: Single-system qPCR in treated versus non-treated patients revealed a high exon skipping rate in all post-treatment cells. The skipping efficiency rate appears higher in the in-vitro compared with the in-vivo study, which may be due to the nature of experiment and the methods used. Nevertheless, it is possible to see a similar skipping pattern between the two studies. While the skipping efficiency does not directly correlate with the transcript amount in treated samples, the majority of patients (76%) show a transcript increase. FluiDMD cards allow us to investigate if antisense treatment is associated with other unspecific splicing products. Our experiments demonstrate that the golodirsen treatment selectively removes ex53 and does not induce skipping of any other exons in the entire transcript of all patients studied.

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