Reviewer's report

Title: Assessment of the feasibility of exon 45-55 multiexon skipping for Duchenne Muscular Dystrophy

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Reviewer: Sylvie Tuffery-Giraud

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This paper is from one of the leader group in the field of the development of new therapeutic strategies in Duchenne muscular dystrophy based on antisense oligonucleotides (AONs). The use of AONs induces the specific skipping of one or multiple exons in order to correct the reading frame of a mutated transcript so that it can be translated into a partially functional protein. In this study the authors tested the feasibility of inducing multiexon 45-55 skipping of the dystrophin gene as this skipping would be beneficial for a large series of DMD patients.

However the paper does not add substantial data to the results previously published by the same group. They have previously shown that multiexon-skipping stretches, such as exons 17-51, exons 42-55, and exons 45-59 were difficult to achieve probably because they are dependent on cotranscriptional splicing and the size of the flanking introns. In this paper, they report the negative result of the multiexon 45-55 skipping assay.

Major compulsory revisions

1- Some RT-PCR results seem inconsistent and difficult to interpret. Figure 2, middle panel: we notice that the mutated exon 44-56 transcript is not anymore detected after treatment with the cocktail of AONs (contrary to the upper panel, not transfected cells) indicative of a probably good efficiency of transfection leading to several exon skipping events. However the intermediate skipping products are not detected (except in one lane). Is there any explanation about the higher intensity of the exon 45-55 skip band observed in the non treated cells compared to the transfected cells (even though the PCR conditions are not quantitative)?

Overall, the exon 45-55 event seems to be more frequent in the non-treated cells than in the treated ones while AONs are specifically used to reach this objective. Could the RT-PCR product detected in the not transfected cells correspond to a non-specific RT-PCR product or an artifact due to the sequence primers? Has the RT-PCR 44-56 band been verified by sequencing? (it is mentioned in the legend of Figure 2 that PCR artifacts are obtained in some occasions).

2- Would it not be more reliable to assess the true efficiency of the AONs treatment by dystrophin staining rather than by RT-PCR only? In the patient (del48-50) the level of naturally occurring multi-exon 45-55 skipping is expected to give rise to a barely undetectable level of dystrophin in the cells since the
patient has a Duchenne phenotype. Dystrophin staining could help to distinguish between exon 45-55 skipping resulting from natural alternative splicing (which may occur at low level) and skipping induced by of the AON treatment (in the treated cells).

Minor essential revisions
1- Results section, line 11: "we tested a cocktail of AONs targeting each individual AON (change into exon) from exon 45 to exon 55"
2- background section, last paragraph: "indicating that the acceptor splice sites of intron 45 and 56 can compete => change into acceptor splice sites of exon 45 and 56 or intron 44 and 55.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests