Reviewer’s report

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

Version: 1 Date: 26 March 2008

Reviewer: Carmen Bertoni

Reviewer’s report:

The authors have attempted to induce skipping of exon 45 through 55 of the dystrophin mRNA to produce an in frame dystrophin transcript using either a cocktail of antisense oligonucleotides (AONs) or a linked AON. RT-PCR analysis was used as method of detection of in frame products and no differences were detected between treated and untreated samples. They therefore concluded that skipping of multiple exons in this area of the dystrophin mRNA is not achievable using either type of treatment.

Major Compulsory Revisions

1) The results shown in Figure 2 are difficult to interpret maybe due to the primers used. False annealing, amplification of products whose size does not correspond to any particular splicing, amplification of non specific sequences and PCR artifacts (acknowledged by the authors in the test) obstruct the accuracy of the analysis. Furthermore the presence of already large amounts of naturally skipped products lacking exons 45 through 55 makes the detection of differences between controls and treated samples complicated. No conclusion can be made based on the analysis presented or the method used to assess the level of mRNA. A more sensitive assay might be able to discriminate between the levels of exons 44 to 55 skipping naturally present and those induced by AONs treatment.

2) The lack of full length expression in any of the samples analyzed in the middle panel and in the majority of the samples tested in the lower panel is confusing. The non skipped product should be predominant in both treated and untreated sample but is only present in untreated samples (figure 2 upper panel). This seems to suggest the presence of multiple skipping that is not efficiently detected by PCR. A western blot analysis would be more informative and more definitive.

Discretionary revision

The work described here using 2’-O-methyl phophorothioate backbone might have had a completely different outcome if performed using AONs containing different chemical structures and different linkages. Great examples of how such modifications can redirect splicing of the dystrophin gene more specifically and effectively are present through the literature. Similarly, length and sequence specificity has shown to have a profound effect on splicing patterns and predictability. It is worth mentioning that more detailed investigations could help
identify single formulations of AONs that can be used to treat patients affected by different deletions.

Finally the authors should reconsider the statement made in the first paragraph of the introduction. "Antisense-mediated exon skipping is emerging as the most-promising therapeutic approach for Duchenne muscular dystrophy." A comparative analysis of all the approaches currently in trials or approaching clinical trials for DMD has never been done.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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.