Reviewer's report

Title: Modulation of mdm2 pre-mRNA splicing by 9-aminoacridine-PNA (peptide nucleic acid) conjugates targeting intron-exon junctions

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Reviewer: Michael Gait

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Redirection or modulation of splicing by oligos such as PNA is fast becoming a useful methodology for controlling gene expression. The authors target the mdm2 gene with Acr-PNA oligos in order to inhibit splicing or to cause exon skipping. Targeting the mdm2 mRNA by Acr-PNA has already been described by this lab in 2004 (reference 20). In principle the new work describing targeting of the mdm2 pre-mRNA is well conceived and executed and the results are interesting to a wide readership.

Minor essential revisions

1. Figure 6B and 6C should be referred to at the end of page 8. Also there is no description of the data in this Figure and I found it difficult to follow which gel bands indicate the lack of exon skipping of exon 3 and the lack of effects on splicing of adjacent introns. It would be helpful to lead the reader through this Figure with a couple of explanatory sentences on page 8.

2. Page 9. The authors suggest that the Cat-Lip-PNA2968 activity plus chloriquine is lower than the lipofectamine mediated delivery of the corresponding PNA2512. However, studying Figure 8 (Cat-LipPNA2968 +CQ) there is 47% skipping at 2 µM whereas in Figure 7C (PNA2512 + LFA) there is 25% skipping at 2 µM. So there seems to be a discrepancy between the statement and the data. Please clarify.

3. Figure 9. Western blot of Mdm2 protein knockdown by mismatched PNA is rather poor quality and it looks to me that at 4 µm there is significant knockdown of protein (band looks much weaker). If the authors want to claim sequence specificity, a better Western blot should be shown.

4. Figure 10. The authors chose a high concentration of PNA to transfect (6 µM) and then show a logarithmic plot of potentiation of camptothecin-induced cell cytotoxicity by the PNA. It should be stated what the effects of transfection of different amounts of matched and mismatched PNA alone are on the cell viability.

5. Discussion of the results of this study in comparison with their own previously published results of targeting the mdm2 mRNA by Acr-PNA (reference 20) is completely lacking. This would seem to be an important comparison and would help establish if targeting the pre-mRNA is a better route to mdm2 inhibition than
targeting translation.

**Level of interest:** An article of importance in its field

**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