OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00142
Title: Non-invasive Delivery of Genes to the Brain: Present and Future perspectives
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COMMENTS TO AUTHORS

The authors present an interesting and topical review on the delivery of gene therapy to the brain. While this is an interesting review it comes across as a superficial coverage of the literature and would be improved by significantly more referencing and a more in-depth coverage of some of the points they cover. Below are some specific points that should be addressed:

Line 11 - "Such diseases are thought to carry a larger social burden compared to other diseases". This is a rather bold statement so some clarification of exactly what they mean by this would be good.

Line 50 - "Viral vectors have been used in many clinical trials in gene therapy". This requires references and in fact there is very little referencing throughout the review. This needs to be rectified.

Makes reference to several cell type specific/selective AAV vectors have been reported and mentions endothelial cells and astrocytes. Makes no reference to other cells oligodendrocytes or microglia which have also been successfully targeted with AAV using specific promoters or serotypes. Also, other targeted evolution attempts have been made to create cell specificity (as an example Juttner, J et al. (2019). Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates and humans. Nat. Neurosci. 22, 1345-1356. doi: 10.1038/s41593-019-0431-2) - why are these not referenced?

In places it is repetitive - e.g talking about neutralising antibodies and high doses. This is mentioned in two separate places within the review.

"Non-viral vectors offer some advantages, including a safer and more flexible (?) route for gene delivery". What do the authors mean here. It is not at all clear how the routes of delivery are really any different between viral or non-viral approaches i.e systemic, intrathecal or delivery direct to the tissue. Clarify what is meant here. Are the authors referring to the text later in this section where they talk about conjugation to functional peptide etc? These are not routes of delivery but methods of targeting the gene therapy. This is not the same thing. The terminology used needs to be carefully checked to avoid confusion/lack of clarity.

More detail could be added so that the review stands alone to describe the field. For example - "Although PC formation on a NP surface may adversely affect targeting, it is possible to control them so as to achieve more effective targeting". A reference is given but a sentence or two to at least briefly describe how this is done would make this so much more informative as a review. Please consider revising other parts of the manuscript with this in mind.

The review then returns to talk about AAV and crossing the blood brain barrier. I feel this information would be better above were AAVs are discussed. Or perhaps have headings so it is clear that this section is discussing gene therapy crossing the BBB.

The authors make reference to Ly6a as the receptor for the AAV-PHP.eB serotype. This serotype has been shown to have relatively poor transduction in non-rodent species. None of the non-rodent species tested with this serotype have been shown to have an ortholog of the Ly6a gene, as far as this reviewer is aware. The presence of the appropriate receptors to cross the BBB following systemic delivery of AAVs is an important consideration and should be addressed here. Same applies to the discussion regarding ApoE-LDLR involvement in the tropism of AAV-PHP.eB. This work has been done in mice, caution should be taken in assuming this will translate to humans. Again, this should be acknowledged.