Author's response to reviews

Title: Sodium vanadate combined with L-ascorbic acid delays disease progression, enhances motor performance, and ameliorates muscle atrophy and weakness in mice with spinal muscular atrophy

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Author's response to reviews: see over
Dear Dr. Lee,

Enclosed please find our revised manuscript (MS: 9610239758122127). We thank the editor and reviewers’ for their constructive and insightful comments. In response to their concerns, we have made some corrections and additions and have consulted our in-house English science editor. Figure 3B has been removed from the manuscript and placed into the “Supplementary Figure” section (Additional file 5). We also added our rationales for choosing this mouse model of late-onset SMA and for how we designed the timing of drug administration and added these details to Figure 3A. In addition, we have added our method for evaluating cell viability to the “Materials and Methods” section and have corrected a citation in response to the reviewer’s comments. We have revised the manuscript according to the reviewers’ suggestions, and our point-by-point responses to the reviewers’ comments are below.

We hope that you will find the manuscript suitable for publication in *BMC Medicine*. We look forward to hearing from you.

Sincerely,
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Our point-by-point replies to the reviewers are below.

Referee #1: (Dr. Chien-Ping Ko)

Major comments:

1. In Figures 1 and 2, the authors showed combined treatment reduced cytotoxicity and increased gem numbers in fibroblasts of SMA patients. However, SMN expression was examined only in NSC34 cells. It is still unclear whether combined treatment could restore SMN expression in SMA patient fibroblasts.

   We agree with the reviewer’s concern and have presented data showing the ability of combined treatment to restore SMN levels in human dermal fibroblasts (paragraph 1 on page 15, lines 13-18 and page 16, line 1; Figure 1I and 1L) WT and SMA HDFs that received combined treatment showed a significant increase in SMN protein levels.

2. The method for evaluating cell viability is not provided in the manuscript.

   We thank the reviewer for pointing out this omission and have included the description in the “Materials and Methods” section (page 8, line 14-17 and page 9, line 1-2) in our revised manuscript.

3. To evaluate the in vivo effect of combined treatment, the authors set off to treat SMA mice from PND1 to PND30, and to conduct phenotypic analysis in SMA mice at PND30 and 90. The authors did not explain why they only treat animals for 30 days. Although the authors did state, “Based on these results, SMA mice received combined treatment for 1 month beginning on PND 1” (page27), they based on results from the more severe SMA mice, which show symptoms much earlier than the mild mice that the authors used in this study. While early treatment is critical for mitigating disease phenotype in severe SMA mice, whether early intervention is also necessary for mild mice with late-onset SMA has not been established. Since the current study is to evaluate the therapeutic potential of combined treatment for late-onset SMA patients, ideally, the authors should have started the treatments later (e.g. from PND 20, instead of PND 1) to mimic the potential benefits of later treatments to type II and type III SMA patients. The authors should at least comment on the feasibility of later treatment in their mouse model in Discussion.

   We thank the reviewer for this insightful and constructive comment. The rationale for using the PND 1-30 treatment window for this study has been added to
Briefly, the therapeutic timing (PNDs 1-30) was selected as several studies have shown that early drug intervention (before PND 5) can target neurons in sufficient numbers to confer some lifespan extension in SMA mouse models [58-60]. Additionally, antisense oligonucleotides administered on PND 1 have been shown to dramatically prolong the lifespan of SMA mice [28], suggesting that transiently increasing SMN protein levels during the first few weeks of life can improve long-term survival of SMA mice. Finally, temporal restoration of SMN levels from birth through PND 28 resulted in no phenotype or abnormal NMJs in SMA mice (Le TT et al. 2011). This provided our rationale for administering the drugs between PNDs 1 and 30.

However, we also agree with the reviewer’s comment that an investigation of the effects of later intervention is crucial to effective treatment of the less severe forms of SMA. We have added a discussion of the importance of these studies to the Discussion section (page 29, lines 14-19, and page 30, lines 1-17).
Referee #2: (Dr. Matthew E.R. Butchbach)

Major comments:

1. Please provide a rationale for using the PND1-30 treatment window for this study.
   Please see our response to comment 3 from referee #1.

2. Why was this model of SMA selected for this preclinical trial instead of one of the early-onset SMA mouse models?
   We thank the reviewer for this comment. We have also attempted this course of treatment in the early-onset SMA mouse model. However, the early-onset SMA mice generally died within 9 days of birth and exhibited a phenotype too severe to be rescued.

3. In the description of the gem analysis results, there are no error measurements provided in the results sections (pg 17). Please provide these error measurements.
   We thank the reviewer for this comment and we have included the error measurements in the main text of the gem analysis (page 18).

4. While the effect of ascorbic acid on rescuing vanadate-induced toxicity is interesting, its prominent placement in the results section could confuse the reader. The figure showing this rescue of vanadate toxicity (Fig 3B) should be removed from the main manuscript and be included as a supplementary figure.
   We agree with the reviewer’s comment and have moved Figure 3B from the main text to the additional files.

5. The scale on the y-axis of Figure 4B is not uniform and it should be. Additionally, the body mass data for ~PND10-PND24 WT mice are missing from the graph.
   We have corrected the text and y-axis of Figure 4B. To clearly show the tail lengths in the SMA groups, a magnified figure is included at the right of the figure.

6. The authors mention that "several studies have demonstrated that early drug intervention yields more promising therapeutic effects in SMA mouse models." One of seminal papers that supports this premise, however, is not referenced (the quinazoline study published in HMG in 2010). This paper
should be cited in this manuscript.
We thank the reviewer for this comment and have added the citation.

Minor comments:

1. The first subsection header in the results section ("L-AA has minor efficacy of SV of inducing SMN protein") should be more clearly worded.
   We apologize for our unclear wording and have modified the subject heading on page 15.

2. In the methods sections, the negative geotaxis assay was first described in reference #48, not #49. Please correct.
   We thank the reviewer for pointing out our error. We have corrected the reference on page 11, line 8.

3. Quality of written English: Needs some language corrections before being published
   The manuscript has been carefully edited by a native English science editor, and we believe the revision has resulted in significant improvements in both quality and clarity.