Author's response to reviews

Title: Adenoviral-mediated correction of methylmalonyl-CoA mutase deficiency in murine fibroblasts and human hepatocytes

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Version: 2 Date: 13 March 2007

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March 13, 2007

Dear Members of the Editorial Board,

We appreciate the thorough review of the manuscript “Adenoviral-mediated correction of methylmalonyl-CoA mutase deficiency in murine fibroblasts and human hepatocytes (MS:154991149128912) “ and feel we have addressed all the reviewer and editorial comments. Studies using the viral vector in the knock-out mice are underway and not ready for inclusion in this manuscript.

In response to the reviewer comments, we have made the following changes to the manuscript.

Referee 1 -Major Compulsory Revisions
1. Toxicity of the adenoviral vector was not observed in our studies at an MOI of 1000 but extensive studies were not performed to examine this. Our initial studies with adenoviral delivery used a GFP only expressing adenovirus, which was used to infect MEFs. We observed no increase in C14 propionate incorporation and no gross toxicity at an MOI of 1000 in these studies. We have added a statement to this effect on page 12. The cells were morphologically normal in all the studies after viral infection and we have added this statement on p 13 at the end of the Results section.

2. The adenoviral nomenclature has been changed throughout to second-generation recombinant adenoviral vector as suggested. Vector genomes and PFU assays were used to titer the virus and this is now mentioned in the methods section. Particle and PFU amounts are described in the appropriate sections.

3. A more precise description of the mouse strain is provided on p.8. Studies using the vector in knock-out mice are underway and not available for inclusion in this paper as the experiments are not completed. Figure 2 has been corrected to correspond with the legend and text.

4. A reference to the first presentation of the mouse model has been listed (reference 24) and the enzymatic characteristics of the MEF cells have previously been reported (reference 25). A comment regarding future mouse studies is included at the very end of the discussion as work in progress as detailed in 3 above.

Minor Compulsory Revisions
1. Reference of the Sawada et al paper has been moved from the Discussion to the Introduction as suggested.
2. Figure 1 has been changed as suggested. A reference to the construction method has been added (reference 28).
3. MOI nomenclature has been corrected as suggested.
4. The lane numbering has been corrected as suggested.
5. 12 references were removed and two added to reduce the number of citations as suggested.
6. The figure legend has been augmented to indicate that samples were measured in triplicate and the standard deviation is presented.

Referee 2-Major Compulsory Revisions
1. Figure 2 has been changed to correspond with the legend and text. The photo of the liver has been changed to show green (versus blue as previously) and the sentence has been changed to emphasize that a “higher concentration of signal seen in the lobe that was likely the direct target of injection” on page 9.

Minor Compulsory Revisions
2-5. The changes have been made as suggested.
6. The sentence has been changed (page 11) to state that the transduced cells are “nearly” corrected versus completely corrected.
7. The word Figure has been removed from the Figures.

The MMA patient was enrolled in NIH study 04-HG-0127 “Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders” and signed consents will be faxed to the editorial office. This protocol was reviewed and approved by the National Human Genome Research Institute Institutional Review Board. Hepatocyte isolation from the discarded livers studied in this manuscript were performed after IRB review and approval at the University of Pittsburg. A letter documenting this will be faxed to the editorial office. A parental letter agreeing to the publication of the medical history and use of the liver specimen will be also be faxed to the editorial office. Per a previous email exchange with the office, it is understood that the signed consent forms for the NIH study and parental letter will never be disclosed to insure that the confidentiality of the patient is protected.

Sincerely yours,

Charles P. Venditti MD, PhD