

Accepted Manuscript

Reply to Letter to the Editor submitted by Steven Greenberg: “Unfounded Claims of Improved Functional Outcomes Attributed to Follistatin Gene Therapy in Inclusion Body Myositis”

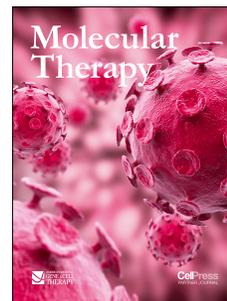
Jerry R. Mendell, MD

PII: S1525-0016(17)30415-X

DOI: [10.1016/j.ymthe.2017.09.003](https://doi.org/10.1016/j.ymthe.2017.09.003)

Reference: YMTHE 4452

To appear in: *Molecular Therapy*



Please cite this article as: Mendell JR, Reply to Letter to the Editor submitted by Steven Greenberg: “Unfounded Claims of Improved Functional Outcomes Attributed to Follistatin Gene Therapy in Inclusion Body Myositis”, *Molecular Therapy* (2017), doi: 10.1016/j.ymthe.2017.09.003.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Reply to Letter to the Editor submitted by Steven Greenberg: “Unfounded Claims of Improved Functional Outcomes Attributed to Follistatin Gene Therapy in Inclusion Body Myositis”

Jerry R Mendell, MD
Nationwide Children’s Hospital
Research Institute
Columbus, OH 43205
Jerry.Mendell@nationwidechildrens.org

To the Editor:

Dr. Steven Greenberg is a well-respected clinician scientist who has limited experience in gene therapy. The premise for his critique of our recent follistatin paper^x is that claims by the authors imply that follistatin “demonstrated clinical and biomarker efficacy for inclusion body myositis (IBM) are unfounded”. He also insists that the primary outcome for this study, i.e., the 6-minute walk test, is a misrepresentation of information provided at ClinicalTrials.gov (NCT01519349). We challenge these grounds for criticism. We must assume from this statement that Dr. Greenberg has not been involved in any first in-human gene therapy trials. Scientists translating first in-human gene therapy trials recognize the well-established FDA criteria: safety must be the primary outcome with secondary outcomes inclusive of improving function. Validation of clinical results also includes the use of biomarkers such as MRI, and muscle biopsy. This is what is precisely described at our ClinicalTrials.gov website. In addition, regulatory approval in the IND application (14845) describes both safety and function, all of which is reported in the publication. These outcomes are not post hoc but planned from study conception and initiation. By definition, this is a Phase 1/2a clinical trial.

Dr. Greenberg also criticized that three sIBM patients received unilateral injections at a lower dose (2e11vg/kg) but were not discussed in this report. Again, the question seems to stem from a lack of experience in gene therapy. For the IND (14845), our discussions with the FDA included and demonstrated a satisfactory safety profile based on pre-clinical studies^{2,3} and the toxicology package included in the IND application. There had been concern that follistatin might be associated with toxicity when expressed in patients. For this reason, the FDA extended the toxicology studies to obtain the IND beyond the usual requirement and we used an alternatively spliced follistatin cDNA, FS344, with minimal effects on the hypothalamic-pituitary-gonadal axis^{4,5}. The IND was approved using this novel, first in-human follistatin isoform, but before we could proceed to clinical trial the FDA reviewer requested the following:

“You propose to deliver your study product to the quadriceps muscle of both legs. However, the safety profile of the product in human is unknown. Therefore, please initiate your trial by delivering the study product to the quadriceps muscle of one leg. If the safety data are satisfactory, you might consider delivery of the product to the quadriceps muscles of both legs.”

The single-limb study was completed safely and the findings reported to the FDA permitting us to proceed with this Phase 1/2a clinical trial. Allegations by Dr. Greenberg suggesting that we were withholding safety data suggest limited insight for initiating a Phase 1/2a clinical study. What we reported in the Journal article¹ regarding the AAV.CMV.FS344 Phase 1/2a clinical trial, employing higher dosing, multiple limb transfection, and long-duration follow up were completely independent from the single-limb safety data that we provided to the FDA and also presented to RAC, IRB, and DSMB prior to starting the clinical trial.

Dr. Greenberg was also concerned with the potential for the use of high-dose prednisone to influence outcome because of a potential “placebo effect”. Two points can briefly be stated in response to this concern. In the planning stage of the trial, the use of prednisone was a specific request of the DSMB to combat the potential for poor transduction efficiency based on concerns related to pre-existing immunity. Two of our prior publications emphasized the importance of this concern: 1) In the micro-dystrophin gene therapy trial we found pre-existing immunity to the transgene (micro-dystrophin)⁶ and in our LGMD2D clinical trial, pre-existing immunity to adeno-associated virus precluded gene expression⁷. The rationale for immune suppression is particularly relevant because older patients, as those participating in the sIBM clinical trial, demonstrate increasing seropositivity to AAV that advances with age⁸. In addition, in a very up-to-date gene therapy clinical trial in SMA achieving motor milestones and function never before seen in this disease, we have used a similar protocol of corticosteroid treatment pre- and post-gene delivery. No experienced Journal reviewer, scientist or FDA reviewer has raised concerns that the results of a short course of corticosteroids accounted for the findings. In the sIBM study, the results were reported for 12-24 months following corticosteroid dosing making it unlikely that a placebo effect that would persist for almost 1-2 years. It should also be noted that in a very large cohort of 136 sIBM patients, it was reported that 91.5% of patients received prednisone and the natural course of the disease was not ameliorated⁹, confirming findings from smaller studies^{10,11}.

There were also claims in the Greenberg’s critique that exercise accounted for the final reported outcome in our clinical trial. We agree that that exercise-influenced function in FS344-treated patients but accounting for increases of over 100m distances on the 6MWT is unlikely. A combinational effect is more realistic given the known increases of plasma follistatin following muscle contraction¹². Following gene delivery we believe that by increasing follistatin expression in muscle, the recommended exercise protocol likely resulted in a combined effect that neither could achieve independently. In any case, the combinational effect we observed will require further study comparing exercise regimens, cohorts, viral dose, and methods of delivery. It should be pointed out that if Greenberg’s contention that the effect of exercise reported in 2007¹³ exclusively accounted for our findings, it is likely that this approach would be more be more universally applied to IBM. Dr. Greenberg might have also supported his contention that our findings were exercise related by providing his own experience in this refractory disorder. We must assume that he doesn’t have such data and his comment is merely speculative.

Responding to every allegation in Dr. Greenberg’s protracted Letter to the Editor is unrealistic. Most are his personal views on trial design for a gene therapy trial that he has yet to do. There are major differences in approaches for pharmacology-based clinical trials that he may be more familiar with but cell and gene therapy methodologies must remain separate. Thus, the FDA maintains distinct regulatory centers (CDER and CBER) for product approval. Most gene therapy projects are introduced to the clinic using small patient cohorts and a design that adheres to safety as primary outcome with function as secondary. These small patient populations cannot be statistically analyzed and results must be descriptive as we have done in the FS344 report. It is for this reason that we listed all adverse events and displayed functional outcomes on each and every participating patient. The favorable functional responses in the absence of adverse events provide a path forward using this myostatin inhibition. The critique of Dr. Greenberg potentially diminishes the importance of the findings of follistatin gene therapy, undervalues the reviews approving the clinical trial including design, sample size and outcomes and ignores the decisions of knowledgeable reviewers and editorial board members of Molecular Therapy who considered that the observations described should be available to other translational scientists.

In closing, a few additional comments are compelling and important to emphasize.

1) This was a first in-human follistatin AAV gene delivery trial and dosing was based on pre-clinical studies^{2,3}. The safety and efficacy established in this trial will allow this approach to move forward with modifications in methods of delivery (systemic vs intramuscular) and dosing adjustments.

2) Dr. Greenberg's criticism undermining the value of historical controls as we employed in this trial is not appropriate and our use of controls matched for age and severity is fully justified according to International Conference on Harmonisation (ICH) Tripartite Guidelines and the European Medicines Agency's guidelines. Both published guidelines encourage a single-arm design with historical controls in small populations when comparisons to well characterized natural history populations are available. This is particularly relevant to the sIBM FS344 gene therapy trial given that data from the control population used for comparison was generated by the same physical therapists participating in the follistatin clinical trial.

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E10/Step4/E10_Guideline.pdf. 2000);

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003615.pdf 2006.

3) The impact of these initial studies blocking the myostatin pathway to benefit sIBM has influenced translational scientists designing clinical trials in academic- and industry-based centers. It is very timely now to attempt to improve strength and function by inhibiting the myostatin pathway. Most are not using follistatin but other means of inhibiting myostatin is an approach considered for treatment of this challenging disease¹⁴.

4) Dr. Greenberg's repeated references throughout his letter labeling data analyses and outcomes as "post hoc" is grossly overstated and without merit. By definition, post hoc implies that data was looked at after the experiment implying a retrospective analysis. The data protocol and statistical plan were designed and presented as part of the IND, with submissions to RAC, IRB and DSMB before the trial began. There was nothing post hoc about it!

My final comment is that we never said or claimed that we had a cure for sIBM or that this was the definitive treatment for the disease. The findings were significant for demonstrating the potential use of AAV delivered follistatin, especially isoform FS344. We remain encouraged by the safety and initial results of this trial. This is a historic first in-human gene therapy trial for inclusion body myositis, a disease that has met consistent therapeutic failures. We remain committed to advancing treatment for this patient population that hopefully will benefit by an improved quality of life through follistatin gene therapy.

References

1. Mendell, J.R., Sahenk, Z., Al-Zaidy, S., Rodino-Klapac, L.R., Lowes, L.P., Alfano, L.N., Berry, K., Miller, N., Yalvac, M., Dvorchik, I., Moore-Clingenpeel, M., Flanigan, K.M., Church, K., Shontz, K., Curry, C., Lewis, S., McColly, M., Hogan, M.J., and Kaspar, B.K. (2017). Follistatin Gene Therapy for Sporadic Inclusion Body Myositis Improves Functional Outcomes. *Mol. Ther.* 25:870-879.
2. Haidet, A.M., Rizo, L., Handy, C., Umaphathi, P., Eagle, A., Shilling, C., Boue, D., Martin, P.T., Sahenk, Z., Mendell, J.R., and Kaspar, B.K. (2008). Long-term enhancement of skeletal muscle mass and strength by single gene administration of myostatin inhibitors. *Proc. Nat. Acad. Sci. USA.* 105:4318-4322.

3. Kota, J., Handy, C.R., Haidet, A., Montgomery, C.L., Eagle, A., Rodino-Klapac, L.R., Tucker, D., Shilling, C.J., Therlfall, W., Walker, C.M., Weisbrode, S.E., Janssen, P.M.L., Clark, K.R., Sahenk, Z., Mendell, J.R., and Kaspar, B.K. (2009). Follistatin Gene Delivery Enhances Muscle Growth and Strength in Nonhuman Primates. *Sci. Transl. Med.* 1:6ra15.
4. Lin, S.Y., Morrison, J.R., Phillips, D.J., and de Kretser, D.M. (2003). Regulation of ovarian function by the TGF-beta superfamily and follistatin. *Reproduction.* 126,133–148.
5. Sugino, K., Kurosawa, N., Nakamura, T., Takio, K., Shimasaki, S., Ling, N., Titani, K., and Sugino, H. (1993). Molecular heterogeneity of follistatin, an activin-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. *J. Biol. Chem.* 268, 15579–15587.
6. Mendell, J.R., Campbell, K., Rodino-Klapac, L.R., Sahenk, Z., Shilling, C., Lewi, S., Bowles, D., Gray, S., Chengwen, L., Galloway, G., Malik, V., Coley, B., Clark, R., Li, J., Xiao, X., Samulski, J., McPhee, S.W., Samulski, R.J., and Walker, C.M. (2010). Dystrophin Immunity Revealed by Gene Therapy in Duchenne Muscular Dystrophy. *N. Engl. J. Med.* 363:1429-1437.
7. Mendell, J.R., Rodino-Klapac, L.R., Rosales-Quintero, X., Kota, J., Coley, B.D., Galloway, G., Craenen, J.M., Lewis, S., Malik, V., Shilling, C., Byrne, B.J., Conlon, T., Campbell, K.J., Bremer, W.G., Viollet, L., Walker, C.M., Sahenk, Z., and Clark, K.R. (2009). LGMD2D gene therapy restores alpha-sarcoglycan and associated proteins. *Ann. Neurol.* 66:290-297.
8. Harrington, E.A., Sloan, J.L., Manoli, I., Chandler, R.J., Schneider, M., McGuire, P.J., Calcedo, R., Wilson, J.M., and Venditti, C.P. (2016). Neutralizing Antibodies Against Adeno-Associated Viral Capsids in Patients with mutant Methylmalonic Acidemia. *Hum. Gene Ther.* 27:345-353.
9. Benveniste, O., Guiguet, M., Freebody, J., Dubourg, O., Squier, W., Maisonobe, T., Stojkovic, T., Leite, M.I., Allenbach, Y., Herson, S., Brady, S., Eymard, B., and Hilton-Jones D. (2011). Long-term observational study of sporadic inclusion body myositis. *Brain.* 134(Pt 11):3176-3184.
10. Lotz, B.P., Engel, A.G., Nishino, H., Stevens, J.C., and Litchy, W.J. (1989). Inclusion body myositis. Observations in 40 patients. *Brain.* 112, 727–747.
11. Amato, A.A., Gronseth, G.S., Jackson, C.E., Wolfe, G.I., Katz, J.S., Bryan, W.W., and Barohn, R.J. (1996). Inclusion body myositis: clinical and pathological boundaries. *Ann. Neurol.* 40, 581–586.
12. Hansen, J., Brandt, C., Nielsen, A.R., Hojman, P., Whitham, M., Febbraio, M.A., Pedersen, B.K., and Plomgaard, P. (2011). Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. *Endocrinology.* 152, 164–171.
13. Johnson, L.G., Collier, K.E., Edwards, D.J., Philippe, D.L., Eastwood, P.R., Walters, S.E., Thickbroom, G.W., and Mastaglia, F.L. (2009). Improvement in aerobic capacity after an exercise program in sporadic inclusion body myositis. *J. Clin. Neuromuscul. Dis.* 10, 178–184.
14. Lloyd, T.E. (2010). Novel therapeutic approaches for inclusion body myositis. *Curr. Opin. Rheumatol.* 22, 658–664.