Reply to Letter 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[1] 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-min 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 Food and Drug Administration (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 magnetic resonance imaging (MRI) and muscle biopsy. This is what is precisely described at our ClinicalTrials.gov website. In addition, regulatory approval in the Investigational New Drug (IND) application (14845) describes both safety and function, all of which is reported in the publication. These outcomes are not post hoc, but rather planned from study conception and initiation. By definition, this is a phase 1/2a clinical trial.

Dr. Greenberg also criticized that three sporadic IBM (sIBM) patients received unilateral injections at a lower dose (2e11 vg/kg) but were not discussed in this paper. 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 were reported to the FDA, permitting us to proceed with this phase1/2a 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 *Molecular Therapy* article[1] 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 the risk assessment corporation (RAC), institutional review board (IRB), and data and safety monitoring board (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 expression.[7] The rationale for immune suppression is particularly relevant because older patients, as those participating in the sIBM clinical trial, demonstrate increasing seropositivity to adeno-associated virus (AAV) that advances with age.[8] In addition, in a very up-to-date gene therapy clinical trial in spinal muscular atrophy (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 affect 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 ameliorated,[9] 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 100 m distances on the 6-min walk test (6MWT) is unlikely. A combinational effect is more realistic given the known increases of plasma follistatin following muscle contraction.[12] 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 2007[13] exclusively accounted for our findings, it is likely that this approach would 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 (Center for Drug Evaluation and Research [CDER] and Center for Biologics Evaluation and Research [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 versus intramuscular) and dosing adjustments.

(2) Dr. Greenberg's criticism undermining the value of historical controls that 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 disease.[14]

(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, which hopefully will benefit by an improved quality of life through follistatin gene therapy.

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