

Following up on follistatin

By *Tim Fulmer, Senior Writer*

Last year, a team at **The Ohio State University** and **Nationwide Children's Hospital** showed the potential of follistatin gene therapy to treat Duchenne muscular dystrophy in mice.¹ The same group now has shown that the approach did not impair major organ function or elicit an unwanted immune response in healthy macaques, dispelling concerns that the safety and efficacy shown in rodents would not hold up in larger animals.²

Although the paper shows positive outcomes only in healthy animals, the researchers hope to move the gene therapy into a Phase I/II trial to treat musculoskeletal diseases by mid-2010.

Myostatin, a member of the transforming growth factor- β 1 (TGFB1; TGF β 1) superfamily, is expressed in developing and adult skeletal muscle tissue and blocks proliferation of muscle cells. Inhibiting myostatin thus could increase muscle growth and improve muscle function in patients with muscle-related diseases.

Follistatin is a naturally occurring protein that binds myostatin and antagonizes its function. The Ohio State and Nationwide researchers thus have focused on delivering the follistatin gene to degenerating muscle tissue. In 2008, the team showed that a single administration of the follistatin gene increased hind limb muscle mass, total body mass and hind limb grip strength in mouse models of Duchenne muscular dystrophy.

The problem is that the mouse models have little predictive power for multiple reasons, including the high regenerative capacity of diseased muscle fibers in these animals as compared to those in human patients.³

As the next test case, the researchers settled on healthy cynomolgus macaques, which are commonly used in safety studies of musculoskeletal diseases.

The group saw an increase in muscle mass and strength in the healthy animals.

Neither follistatin nor the adeno-associated virus serotype 1 (AAV1) vector used to deliver the gene generated an interferon response over five months following injection, suggesting the therapy did not elicit an unwanted immune response.

Hematological and biochemical tests also showed that the therapy produced no abnormal changes in liver, kidney or muscle function or in hematopoiesis.

Female macaques maintained normal menstrual cycles during the treatment, male macaques showed normal sperm counts and levels of sexual hormones remained within normal range for both sexes. Finally, the hearts of treated monkeys were of normal size, showing that the

Box 1. Preclinical safety endpoints relevant to adeno-associated viral (AAV)-based gene therapy from EMEA guidelines.

- AAV vector does not persist in and impair the function of nondisease tissue.
- AAV vector does not integrate into the host genome and trigger insertional mutagenesis of host genes.
- AAV vector shows no risk of germline transduction.
- AAV vector concentrations in the serum and other bodily fluids are low enough to avoid viral shedding into the environment.
- AAV vector does not increase the potential for viral reactivation if the host is infected by the wild-type virus and a helper virus.
- AAV vector expresses only the correct form of the therapeutic protein, not variants that could have impaired function.

gene therapy did not cause unwanted hypertrophy of cardiac tissue.

The findings were reported in *Science Translational Medicine* by Jerry Mendell, director of the Center for Gene Therapy at Nationwide and professor of neurology, pathology and pediatrics at Ohio State, and Brian Kaspar, associate professor of pediatrics at Ohio State and principal investigator at the Center for Gene Therapy at Nationwide.

A commentary accompanying the article described a laundry list of safety benchmarks that regulatory agencies may require before allowing a gene therapy of this sort to proceed into clinical testing (see **Box 1, "Preclinical safety endpoints relevant to adeno-associated viral (AAV)-based gene therapy from EMEA guidelines"**).⁴

Maria Cristina Galli, author of the perspective, concluded that the latest study requires more experiments "to fulfill EU requirements for preclinical studies on gene therapy medicinal products (GTMPs) before their first clinical use."

Galli, professor of cell biology and neurosciences at the **Superior Health Institute**, emphasized the importance of additional studies on the presence, persistence and activity of the AAV1 vector in organs other than muscle to help rule out toxicity potentially associated with long-term treatment.

Kaspar told *SciBX* that the safety studies will indeed include looking at the potential of vector persistence in tissues outside of muscle.

Strength in numbers

Kaspar's team thinks the results from the healthy animals provide strong hints that follistatin gene therapy will be disease modifying.

Quadriceps injected with the follistatin gene therapy had significantly greater mass at eight weeks after delivery than noninjected control quadriceps ($p=0.01$). The enhanced muscle mass was stable and lasted from week 20 to week 60.

The increased mass corresponded with improvements in muscle strength. Indeed, treated mice showed increases in twitch force and

tetanic force—two standard measures of muscle strength—compared with untreated mice.

The researchers are now "performing formal toxicology and bio-distribution studies that were outlined at a pre-IND meeting with the FDA," said Kaspar. "We have also begun GMP production of the AAV1-follistatin gene therapy construct in support of the planned IND. Pending completion of the safety studies and manufacturing, we anticipate entering the clinic in the middle of 2010."

The researchers initially plan to focus on muscle diseases characterized by quadriceps muscle weakness.

"The two disorders with preferential quadriceps muscle weakness are sporadic inclusion body myositis and Becker muscular dystrophy," said Kaspar. "Those should be two well-defined clinical populations for inclusion into the clinical study. In the trial, we plan to use a six-minute walk test as an outcome measure for prolongation of ambulation following treatment."

Kaspar told *SciBX* that Nationwide "has filed multiple patent claims in both the U.S. and Europe covering the use of follistatin and gene therapy

to enhance muscle mass and muscle function to treat musculoskeletal and neuromuscular disorders."

Nationwide is seeking partnerships and collaborations as well as licensing opportunities for the IP, he said.

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REFERENCES

1. Haidet, A. *et al. Proc. Natl. Acad. Sci. USA* **105**, 4318–4322 (2008)
2. Kota, J. *et al. Sci. Transl. Med.*; published online Nov. 11, 2009; doi:10.1126/scitranslmed.3000112
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3. Fulmer, T. *SciBX* **1**(10); doi:10.1038/scibx.2008.226
4. Galli, M. *Sci. Transl. Med.* **1**, 6ps6 (2009)

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