The first U.S. gene therapy trial for Duchenne muscular dystrophy gets off the ground. The therapy uses a miniature gene engineered by a Pitt researcher.
A MINIGENE FOR MUSCULAR DYSTROPHY

CLINICAL TRIALS TO START

BY JOE MIKSCH

A boy is born. Much like any other little one, he gurgles and cries. Eventually, he grins and rolls around. Then, sometime before he turns 3, the boy's legs weaken. He begins to lose control of his muscles.

The worried parents take their son to the doctor. A muscle biopsy confirms what the physician feared—the little boy has Duchenne muscular dystrophy (DMD), a genetic disease marked by continual disintegration of muscle tissue. There's no cure for the disease, which affects one in every 3,500 boys born, and there is no effective treatment. The boy will almost certainly die before he sees 30.

University of Pittsburgh investigator Xiao knows such stories and is intent on changing their endings. This year his work will result in the first gene therapy trial for Duchenne muscular dystrophy in the United States.

His collaborators include gene-vector production expert R. Jude Samulski of the University of North Carolina-Chapel Hill and clinical scientist Jerry Mendell of Ohio State University.

Xiao, associate professor of orthopaedic surgery, has, for almost two decades, been exploring the efficacy of a once-ignored virus, adeno-associated virus (AAV), as a delivery system for gene therapy. AAV, the easy-going and avuncular Xiao jokes, should stand for "almost a virus," because it doesn't cause disease, explaining why it was given little attention until the 1980s. AAV does, however, have a couple of things going for it as a treatment vector—it can infect all kinds of cells and does not provoke an overt immune response.

A defect in the dystrophin gene—which spurs the production of the dystrophin protein, a major player in a protein chain that keeps muscle cells intact—causes DMD. Xiao and his lab mates decided to insert a healthy dystrophin gene into AAV and inject the virus into the muscle tissue of a DMD-affected mouse. This act, Xiao thought, might return dystrophin production to normal levels.

One problem. The dystrophin gene is huge. About three times larger than the payload of AAV. In 2000, Xiao and colleagues found a way to pare down the dystrophin gene to the point where only the essential components needed for dystrophin production remained. The resulting therapy proved effective, reversing DMD in mice and showing promise in dogs. Proving efficacy in people is the next step, one Xiao expects to take sometime in the first half of this year in clinical trials.

As Xiao's DMD gene therapy moves into the clinical stage, he has turned his attention toward gene therapies for other forms of muscular dystrophy.

In the case of congenital muscular dystrophy (CMD), the laminin alpha-2 gene doesn't work. A lack of laminin alpha-2 protein causes similar symptoms to DMD, though more severe. Doctors typically diagnose the disease shortly after birth, and children afflicted die soon thereafter.

Because the laminin alpha-2 gene is also far too large to fit inside AAV, Xiao's lab substituted a smaller and similar gene (called agrin). In mice, agrin gene therapy improved cell-structure integrity, muscle function, and life span. Yet this therapy, Xiao says, is not a potential cure, because the researchers weren't able to replicate the exact function of the laminin alpha-2 gene—in particular, its cell structure-maintenance duties. To correct this shortcoming, Xiao and colleagues are working on what they hope will be an effective mini laminin alpha-2 gene.

Xiao has also found a gene therapy that's effective in hamsters with limb girdle muscular dystrophy (LGMD). LGMD affects skeletal muscle and often causes congestive heart failure. The therapy Xiao developed uses another variant of AAV (known as AAV-8) that is able to transverse the blood vessel barrier. The other AAV therapies he's testing need to be injected directly into affected muscle areas; AAV-8, however, can be infused into the bloodstream, which carries it to all muscle groups. (A much more practical approach than giving a series of injections, Xiao says.)

Xiao doesn't know the mechanism that allows AAV-8 to cross the blood vessel barrier, but he does know this: "This is almost a cure for the hamster."

The animals' heart function returned to near normal, and other muscular dystrophy symptoms abated.

With all these achievements in animal models of muscular dystrophy, Xiao is enamored of gene therapy's potential to treat these disorders in people. And his method has been deemed safe to pursue experimentally by the FDA and other oversight organizations.

Still his optimism is laced with caution: "Gene therapy works super at the preclinical stage," he says.

When applied to a human—a more complicated system compared to a rodent breed to develop a disease—potential hurdles come up, Xiao explains.

"With people, everything is different."
RODNEY AND ME

EXOSOMES GET RESPECT

BY JOE MIKSCH

As far as minuscule fragments of biologic material go, the exosome is a bit like the late Rodney Dangerfield. Historically, it has gotten precious little respect.

The tiny vesicle, emitted from all manner of cells, was first noticed in 1978. The most significant purpose anyone could divine for the exosome was to get rid of cellular junk. Few scientists' curiosity was piqued by a cellular garbage man.

Today, the exosome, about the size of a virus, is still not fully understood. However, it is gaining prestige. Pitt scientists are examining its role in cancer, immunosuppression, and as a treatment for autoimmune disease.

The exosome—a former afterthought—is now on a few Most Wanted lists.

Theresa Whiteside, professor of pathology and of otolaryngology at the University of Pittsburgh and director of the Immunologic Monitoring and Cellular Products Laboratory at the University of Pittsburgh Cancer Institute, is one of the investigators pushing exosomes to the forefront. In the case of cancer, the exosome is a nasty little devil; Whiteside explains that it helps tumor cells thwart the immune system's protective instincts.

Whiteside says there may be a couple of ways to stop this wholesale slaughter of anti-tumor cells. Find a method to remove these particular exosomes or develop protections for the immune cells affected by these marauding microvesicles. But such an antitumor vaccine is far from a reality today, she says.

Exosomes can manipulate the immune system in other ways as well.

Paul Robbins, Pitt professor of molecular genetics and biochemistry, got interested in exosomes by accident. He had been looking at modes of gene therapy for autoimmune diseases. After injecting a therapeutic dose into the paw of a mouse afflicted with rheumatoid arthritis, Robbins noticed a couple of things. As he had hoped, the condition at the site of the injection improved. But something else befuddled him. The paw opposite the injection site got better, too.

The injected gene did what was expected. It helped suppress the immune response that was causing rheumatoid arthritis. (You may have read about this breakthrough in our August 2005 issue.) Then the affected dendritic cells emitted exosomes that traveled through the bloodstream. The exosomes seemed to carry news through the circulatory system to the other joint that the immune cells shouldn't call for the destruction of the mouse's joints.

Robbins is now working on generating exosomes from immune cells in vitro and injecting the resultant immunosuppressive exosomes into mice. He considers this method a potentially viable treatment for all manner of autoimmune disease. But why use exosomes rather than cells?

Exosomes, Robbins says, don't change. An exosome is an exosome is an exosome. Whereas the immunosuppressive attributes of a cell can be reversed in the body, those of exosomes cannot, it seems. They're a more stable and, he believes, safer method of achieving immunosuppression.

Immunosuppression is also the province of Adrian Morelli's research. The assistant professor of surgery and member of the Thomas E. Starzl Transplantation Institute hopes to use exosomes to eliminate the need for immunosuppressive drugs, which often have serious side effects, after organ transplantation.

Exosomes, he says, carry MHC (major histocompatibility complex) molecules, which allow the immune system to differentiate between self and nonself. Imagining a transplantation scenario, Morelli sees taking exosomes from the organ donor and injecting them into the transplant recipient. The exosomes would be internalized by the recipient's dendritic cells, which would process them with the cell's MHC molecules, creating a hybrid molecule that recognizes both the donor tissue and recipient tissue as self. Morelli plans to graft donor tissue to a hybridized mouse later this year. Clinical trials, though, are a long way off. "This is a futuristic view," Morelli says. "But two years ago, I didn't know much about exosomes at all. We're making a lot of progress."
Caring for the System

A New Course Focuses on Day-to-Day Practice Dilemmas

By Hattie Fletcher

Uncover a disease mechanism. Identify a compound that targets it just so. Develop a drug. Get it past clinical trials, and voilà—the hard part is done, right? Next stop is the reward for all that persistence and intellectual sweat: effective treatments and cures. Maybe. From here on out is not necessarily a free and clear road to dispensing good health. Many obstacles can stand in the way of getting the right pill into the right patient's mouth. Here's a short list: How big is the market for the drug? Will anyone manufacture it? What if a hospital staff member misreads the doctor's handwriting on an order for it?

Yet if medical schools offer discussions of ways to address such implementation issues, or examine how the profession is systemically changing, they tend to do so only informally or in electives.

Pitt med's class of 2008, however, will have a head start on understanding the science behind implementing good modern care. That's because it's the first class to take a required course, "The Basic Science of Care," developed by a team from the medical school as well as from Pitt's nursing, public health, health and rehabilitation, dental, and pharmacy schools.

The course focuses on subjects that threaten to compromise the quality of care and patient safety, even seemingly simple things like illegible handwriting. These are also the kinds of subjects that can enhance care and safety if given proper attention, says Loren Roth, one of the course designers. Roth is chief medical officer and senior vice president of quality care at UPMC as well as Pitt's associate senior vice chancellor, health sciences.

In fall 2003, Vladimir Manuel (Class of '06) and several of his classmates began organizing dinner lectures on malpractice insurance and other aspects of the healthcare system not addressed in depth in the curriculum.

Roth was a guest lecturer at some of those meetings. For some time, he and other faculty members (including the chairs of medicine and anesthesiology and head of the medical center's electronic records system) had wondered whether there was a curricular way to cover some of the topics Manuel had identified.

While planning, Roth and others were joined by Allison DeKosky (Class of '08). DeKosky had worked in U.S. Senator Arlen Specter's office as a health policy adviser and in the private sector as a consultant. In her experience, many doctors knew the medical issues involved in policy but didn't always have a clear understanding of the bigger picture—of how the system worked. When DeKosky started at Pitt, she learned that planning was under way for a required course and worked closely with the course directors, sharing her policy perspective.

The resulting course has generated a buzz among students trickled out of the lecture hall in twos and threes, weighing the pros and cons of attending a possible lung cancer by e-mail and threes, weighing the pros and cons of learning about a possible lung cancer by e-mail and threes.

In a discussion that followed, students raised a host of questions for a panel that included Fischer and Tashbook.

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