

GENE THERAPY: SCIENCE IN SLOW MOTION

Early-stage human trials of gene therapies that could target some of the world's deadliest diseases are underway. An air of caution lingers, however, as past setbacks remain on investors' minds. Researchers are optimistic about the potential for positive outcomes that could lure biotech companies back. **BY JOHN CARROLL**, Senior Contributing Editor

Back in the early 1980s, a bright, young graduate student at the University of Florida—Gainesville, named R. Jude Samulski went to work on his PhD thesis in a novel field. With his mentor, Nicholas Muzyczka, PhD, he believed that the adeno-associated virus — typically referred to as AAV — could be used to create benign nanoparticles that would carry gene therapies to specific areas in the body. These tiny particles would become a kind of “biologic FedEx package” that could safely deliver a new kind of therapy to treat some devastating diseases.

Last spring, Samulski and a group of collaborators used the first laboratory-derived chimeric AAV to deliver an engineered mini-dystrophin gene into six young volunteers. These subjects signed on to test the theory for the treatment of Duchenne muscular dystrophy

One of a small but influential group of gene therapy researchers, Carl June, MD, is heading work at the University of Pennsylvania aimed at blocking the HIV virus from replicating.

(DMD), a wasting disease that triggers steady deterioration of the muscles.

DMD usually begins in childhood, and is found only among boys, but a DMD diagnosis is almost always initially overlooked. Patients gradually weaken, the result of a missing gene responsible for the protein dystrophin, part of the intricate web of materials vital for muscle development. By their 20s, many victims start to die as their cardiovascular systems shut down. The lone therapy approved for DMD only slows the effects of the disease, but there is no therapy that either slows or stops its progression.

The lead researcher in the project, Xiao Xiao, PhD, was Samulski's first graduate student when he began teaching. Xiao, a professor of molecular genetics and biochemistry, as well as pharmacology, at the University of Pittsburgh School of Medicine, developed a miniature dystrophin gene and put it in the AAV particles. He is presently studying to see if the replacement gene can correct nature's mistake.

Samulski, director of the Gene Therapy Center at the University of

North Carolina—Chapel Hill, is still waiting for the trial to wrap, but already he has had a chance to see how the AAV-borne therapy performed in animal trials. He's hopeful that decades of research work will pay off for patients.

“Most kids with DMD go from ambulatory to wheelchair-bound in the years from 6 to 9,” says Samulski. If you can save 10 percent of their muscles, he says, you might keep them out of the wheelchair. The benefits in their quality of life — “all the cascade of events once you become mobile,” he says — would be enormous.

A DIFFERENT CLASS OF SCIENTISTS

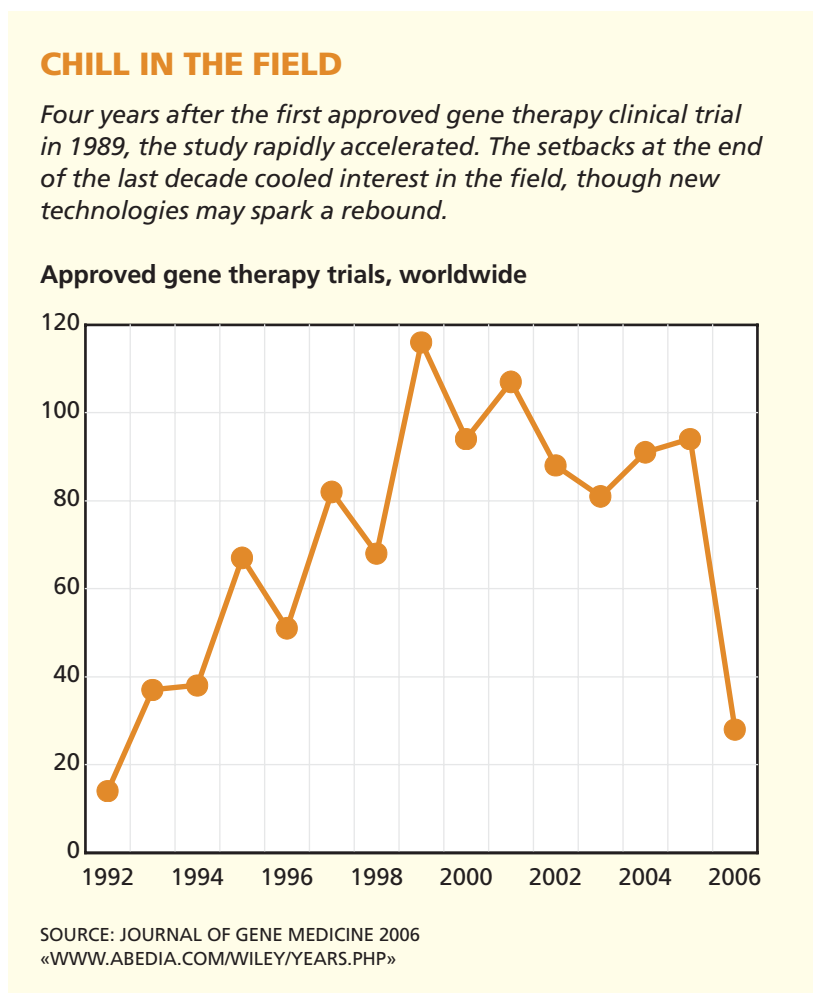
Samulski and Xiao are part of a small yet influential group of researchers scattered around the country whose career paths have intersected at key junctures. This select group is spearheading a series of early-stage human trials of gene therapies and, in several cases, they are gathering the first positive human data for gene therapies that target some of the world's most intractable diseases. Funded largely by grants and contributions, they

also are working with a select number of small biotech companies, including several university spinoffs like Asklepios Biopharmaceutical, also in Chapel Hill and which owns the research being developed by Xiao and Samulski. After the completion of more than nine phase 1 clinical studies using an AAV delivery approach without any adverse events, many are confident that it is emerging as the safest way to deliver gene therapies.

These research teams are being led by some older and more experienced pioneers of gene therapy who stuck with the science in the years after a volunteer fatality occurred during a 1999 clinical trial and the death of 1 of 3 French children who contracted leukemia following an earlier gene transfer trial. The lurid headlines that followed each setback winnowed the field—pulling out any impatient researchers or biopharmaceutical companies but leaving a core of determined investigators who restarted their work with a high degree of caution.

“There was a whole chill on the field that lasted a couple of years,” says Carl June, MD, a gene therapy researcher at the University of Pennsylvania, in Philadelphia, where the 1999 fatal case occurred. “Everyone who was running a gene therapy trial was closed down until the FDA figured out new forms of oversight and additional safety criteria.”

Armed with some promising data released from clinical trials over the past year, these scientists are hopeful that the gene therapy field is once again poised to earn the positive attention of potential biopharmaceutical backers. With that kind of corporate deep-pocket



partnership, the research can return to the fast track as gene therapies emerge from phase 2 and head into late-stage studies.

For now, however, every step taken in these new trials is being carried out in scientific slow motion.

“It’s a different class of scientists,” asserts Samulski about the work being done today. “I think the field paid the price and suffered from a lot of overselling and the expectation of hitting a home run every time it performed one of these studies.”

“Broad, subtle issues don’t sell newspapers,” notes Theodore Friedmann, MD, president of the

American Society of Gene Therapy, Milwaukee. Splashy problems do, however, and “we had our share of splashy problems.”

After the headlines faded, gene therapy for eye diseases continued to make impressive gains, Friedmann says. But in the area of neurodegenerative diseases, he cautions, the science is still at an early stage, with a lot of hurdles left to clear.

“The basic underlying hurdle is that we often don’t know enough about the pathogenesis of disease to know if we’re attacking the right target,” explains Friedmann. “There are a number of targets in Parkinson’s disease, but we don’t know the most effective ones in cancer. Even

in single-gene defects like hemophilia, the target gene is not always clear either.”

Then, there’s the technological hurdle of getting the gene to the right cell in the right development stage with the right vector, all without triggering a potentially lethal response.

“The body may not like what you’re doing and respond in ways that will destroy what you’re trying to do,” Friedmann adds. “Knowledge of immune response is very important.”

Small biotech companies and academic groups are ideal places for working through those issues, says Samulski. When something doesn’t work, they’re flexible — and patient — enough to go back to the drawing board and figure out a new approach.

But money’s tight.

“It’s all grant money and foundation money, and people staying in areas where they have lots of expertise,” says Samulski. In some respects, he adds, the researchers who stayed with gene therapy are paying an unfair penalty. “If you look at the positive results the field has had with retroviruses and the ‘bubble boy’ disease, anyone else who created a therapy with an 80 percent cure rate for an otherwise incurable disease would likely be judged a smashing success” similar to that of imatinib mesylate (Gleevec), used for chronic myeloid leukemia.

“We brought undue scrutiny upon ourselves, and we’re paying the price for it. In one community, we’re the scapegoat, and in another, we would be the next Microsoft coming down the pike,” says

Samulski. “You can put on either hat and it would be justified.”

Chinese regulators, meanwhile, have not been as distrustful of the process as those in the United States. Taking research that was originally done in the United States, Shenzhen SiBiono Gene Technology launched Gendicine, the world’s first gene therapy for head and neck cancer, three years ago. In the United States, however, gene therapy trials are still limited to extremely small numbers of patients.

FASTER, BETTER, SAFER

Terence Flotte, PhD, a geneticist at the University of Florida’s College of Medicine, also relied on an

Regarding three early-stage gene therapy studies for Parkinson’s disease, “Part of the enthusiasm is that this is something potentially disease modifying. It’s tremendously exciting.”

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THE MICHAEL J. FOX FOUNDATION FOR PARKINSON’S RESEARCH

AAV vector to begin delivering a gene that can produce the protein alpha-1 antitrypsin. In an experimental trial that ended in late 2006, Flotte and colleagues reported that the AAV delivery approach was safe, with initial signs of alpha-1 protein production, although not in large enough amounts for therapeutic purposes.

A large segment of Flotte’s work also has taken place at the Univer-

sity of Florida, but he’s done years of his own work on AAV, and believes he can advance these vectors to a whole new level in gene therapy.

“We have another version of the virus that appears in animal studies to be close to a thousandfold more potent at making protein,” Flotte says. “That’s very encouraging to us. So the next trial, which has already begun, uses the new version of the virus and take patients through a similar range of doses, in a very similar scheme, to see if we can maintain the safety while pumping up the efficiency of the protein production.”

There are more than 200 variations of the AAV virus, says Flotte, and it turns out that some do work better than others. By finding types that can more effectively dock with proteins on the cell surface, scientists can get the desired DNA into the cells more efficiently.

“That has been experimentally demonstrated in mice,” says Flotte, who hopes to see the same results in humans as his studies in gene therapy advance. His research team is now testing AAV type 1 in a new phase 1 study that is already underway.

About 100,000 people in the United States who suffer from alpha-1 antitrypsin deficiency stand to benefit from any clinical successes. An absence of the protein eliminates a key protection against inflammation of the lung. Most patients are not diagnosed until their 30s, by which time they have already suffered from severe lung damage. Emphysema and cirrhosis can result, and can be fatal.

If Flotte is right, the University of Florida also stands to benefit from

the work. His research has been spun off into Applied Genetic Technologies Corp., Alachua, Fla., in which the university, along with a group of venture capitalists and other investors, holds an interest.

TURNING CELLS INTO FACTORIES

Nobody knows what triggers the progressive physical deterioration of Parkinson's disease. But it's clear that loss of dopamine-producing cells is behind Parkinson's — the second-most-common neurodegenerative disorder in the United States, exceeded only by Alzheimer's disease. Until now, treatments have been focused only on moderating symptoms, which include slowness of movement, stiffness of muscles, tremor, and problems with gait and balance.

CERE-120 may help change that.

Last year, William Marks Jr., MD, associate professor of neurology at the University of California–San Francisco, led a team that directly injected neurturin into the putamen — a section of the brain that serves as a hub for Parkinson's disease — of 12 volunteers with Parkinson's disease. By putting the growth factor into the AAV 2 vector, Marks' team was able to directly deliver the gene therapy where it was needed at two different dose levels. After 9 months, his team recorded a 40 percent overall reduction in symptoms, along with the early scientific data that gave patients with Parkinson's disease a glimpse at what could be a future method of head-on attack — by stopping the loss of those cells and possibly reversing the damage.

“Cells become factories for growth factor,” says Marks, who leads an experienced team conducting deep brain stimulation therapy for Parkinson's patients.

CERE-120 is being developed by Ceregene, a small, San Diego-based biotech company that helped pioneer the work in primates. Given gene therapy's troubled history, however, the human trial process is being played out more slowly than for most other experimental biologics. In his investigation, Marks dosed the first patient, waited for a month to ensure safety, then went on to the next two patients, and so on. As patients demonstrated no harmful side effects and some benefits, the researchers lined up the next two volunteers, until everyone had been dosed.

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Neurturin is a member of the GDNF growth factor family of proteins that has been studied for many years, says Jeffrey Ostrove, PhD, Ceregene's CEO. It also has long been recognized for its ability to keep dopamine-expressing neurons alive and healthy. The tough part, however, has been getting the therapy to the exact part of the brain where it is needed.

“We're combining the power of growth factors with gene delivery

technology that puts the factors exactly where we need them,” says Ostrove.

The beauty of the AAV vector system is the level of growth factor expression seen in animal models, he adds. It is the same after two years as it is after one month; some scientists have tracked expression for six years without seeing a diminution. “We're thinking of this at this point as a one-time treatment,” says Ostrove.

Years of research are still needed to test that theory, however.

The phase 2 trial is designed to be larger, with 34 patients receiving the higher dose of CERE-120 and 17 patients given placebo injections at eight different centers. Marks has already begun his mid-stage work, and he's hopeful that

some significant progress will be seen.

“In my mind,” says Marks, “the goal of therapy is to stabilize the degenerating system and decelerate disease progression.”

That's the kind of statement that draws muted cheers from patient advocates, who are anxious to see real progress but equally cautious to avoid hyping data that will take years to come to fruition.

But in a field where there is no disease-modifying alternative to compete against, gene therapy also could take a different, faster track.

After phase 2, there's a “possibility of moving to a larger phase 3 trial for FDA approval,” says Marks. “Or, who knows? The results could be so compelling the FDA might say, ‘Let's release this, collect post-marketing data, and see where this goes.’ If efficacy is less than phase 1,

however, it could be back to the drawing board.”

Headquartered in New York, The Michael J. Fox Foundation for Parkinson's Research is closely watching three early-stage gene therapy studies for Parkinson's disease. The foundation partially funded the Ceregene study.

“Part of the enthusiasm is that this is something potentially disease-modifying,” says Katie Hood, deputy CEO of the Foundation. “It's tremendously exciting. The bar is high, and the failure rate is high for efforts to modify the progression of the disease.”

The best-case scenario for seeing a treatment approved for general use, she adds, is somewhere between 4 and 6 years. But given the vagaries of clinical studies and the extreme caution that characterizes them, she hastens to add that such a timeframe is just her best guess.

A NEW APPROACH TO HIV

Not all gene therapy trials rely on classic AAV vectors. Researchers at Penn say they've had promising results using an HIV-based lentiviral vector approach to treating the virus that has claimed more than 25 million lives worldwide. Taking the body's natural infection-fighting T cells from five patients who had already failed at least two classes of antiretroviral drugs, June and his colleagues genetically modified patient T cells with a gutted version of HIV that had been engineered to cripple the health threat of the pathogenic virus by blocking its ability to replicate. These HIV-resistant T cells were then multiplied and administered so that each of the volunteers received about 10 billion of their resistant T cells,

which represents about 10 percent of their total cells. VIRxSYS Corp., in Gaithersburg, Md., is commercially developing the therapy and is conducting a phase 1 clinical trial at the University of Pennsylvania with financial support from the National Institute of Allergy and Infectious Diseases. A follow-on phase 2 multi-site clinical trial is also being sponsored by the company.

HIV multiplies in a classic viral fashion, infecting cells that in turn create thousands of new copies. The gene therapy approach used by June and his team created a virus that blocks the virus or spawns sterile copies.

Over nine months, the viral load of the patients in the trial either stabilized or dropped, with one of the patients showing a massive drop in his viral load after receiving the genetically altered cells. T-cell counts stayed the same or went up in the other four volunteers. The patients saw improvement either because of the antiviral effects or the increased number of immune cells, or both.

VIRxSYS has completed enrollment in its phase 2 study evaluating multiple doses of VRX496 modified cells in the same patient population as the phase 1 trial. The phase 1 study of VRX496 at the University of Pennsylvania also uses multiple doses and targets a healthier patient population that is still sensitive to antiretroviral drugs, with the hope that patients enrolled in the study will be able to stop their medications after VRX496 treatment and stay off any antiretroviral therapies for an extended period of time.

In most small biotech companies, launching phase 2 trials would trigger a round of talks with potential drug development partners who

could fund more expensive, late-stage studies. But in gene therapy, the caution that has tempered each new step in the clinic also has slowed down any matchups that could fund bigger, more convincing studies. Several companies have decided not to fund studies as it became clear that the 12-year average for bringing a new therapy to the point of an FDA approval was likely to require an even longer germination period in gene therapy.

The chill over gene therapy may be on the verge of thawing, however. There have been signs that some bigger pharmaceutical companies are cautiously getting back into the field, June adds, but they're doing it very quietly to avoid investor backlash.

Research foundations are hoping that the shift happens soon. There's a limit to what a foundation can do, says Hood, at the Michael J. Fox Foundation. They can provide funds to get a therapy through phase 1 and toward midstage studies, but at some point, deeper pockets will need to be wooed back into the field.

“The assumption is that pharma is watching and getting ready to pick the winners that emerge,” says Hood.

Samulski believes that the bigger companies won't be able to resist gene therapy when the hard data once again demonstrate just how much potential the field has.

If a major breakthrough occurs, June says, “there will be a big return back into the market. They're all waiting.” **BH**

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