Kristopher Boesen, who broke his neck in a car accident, regained the ability to move his arms and hands after his spinal cord was injected with stem cells.

GREG IGER/KECK MEDICINE OF USC

NEUROSCIENCE

New nerves for old

Stem-cell therapy promises to restore motor function after a stroke or spinal-cord injury, but neurologists are proceeding with caution.

BY KATHERINE BOURZAC

Two years after having a stroke at 31, Sonia Olea Coontz remained partially paralysed on her right side. She could barely move her arm, had slurred speech and needed a wheelchair to get around. In 2013, Coontz enrolled in a small clinical trial. The day after a doctor injected stem cells around the site of her stroke, she was able to lift her arm up over her head and speak clearly. Now she no longer uses a wheelchair and, at 36, is pregnant with her first child.

Coontz is one of stem-cell therapy's "miracle patients", says Gary Steinberg, chair of neurosurgery at Stanford School of Medicine in California, and Coontz's doctor. Conventional wisdom said that her response was impossible: the neural circuits damaged by the stroke were dead. Most neuroscientists believed that the window for functional recovery extends to only six months after the injury.

Stem-cell therapies have shown great promise in the repair of brain and spinal injuries in animals. But animal models often behave differently from humans — nervous-system injuries in rats, for example, heal more readily than they do in people. Clinical trial results have been mixed. Interesting signals from small trials have faded away in larger ones. There are plenty of unknowns: which stem cells are the right ones to use, what the cells are doing when they work and how soon after an injury they can be used.

The field is still young. Stem cells are poorly understood, and so is what happens after a spinal-cord injury or stroke. Yet, there are success stories, such as Coontz's, which seem to show that therapy using the right sort of stem cell can lead to functional improvements when tried in the right patients and at the right time following an injury. Researchers are fired up to determine whether stem-cell therapies can help people who are paralysed to regain some speech and motor control — and if so, what exactly is going on.

SCAR TISSUE

Neurologists seeking functional restoration are up against the limited ability of the human central nervous system to heal. The biology of the brain and spinal cord seems to work against neuroregeneration, possibly because overgrowth of nerves could lead to faulty connections in the finely patterned architecture of the brain and spine, says Mark Tuszynski, a neurologist at the University of California, San Diego. Local chemical signals in the central nervous system tamp down growth. Over time, scarring develops, which prevents the

injury from spreading, but also keeps cells from entering the site.

"It's really hard to fix the biology," says Charles Yu Liu, a neurosurgeon and director of the University of Southern California Neurorestoration Center in Los Angeles. Stem cells seem to promise a workaround.

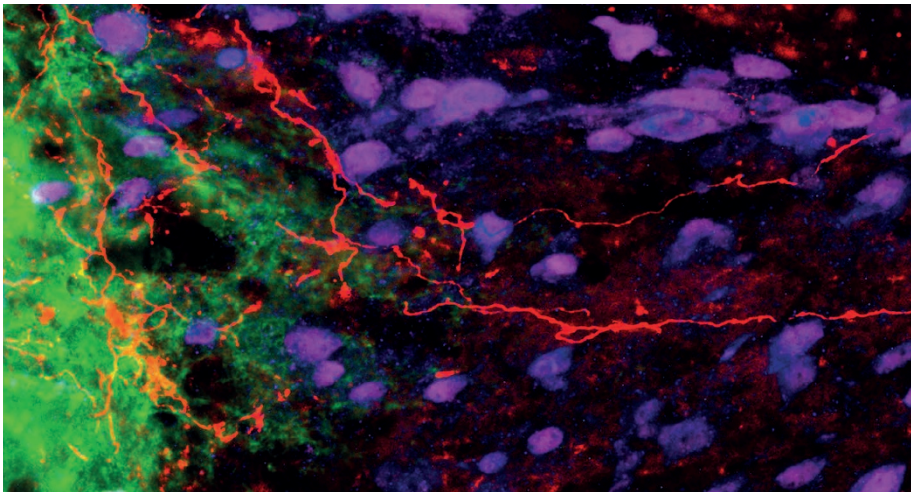
So far, neural regeneration cell therapy has had only anecdotal success, leaving investors and patients disappointed. In people with Parkinson's disease, for example, neurosurgeons replaced dead and dying dopamine-producing neurons with fetal neurons. Although initial results were promising, in larger studies, patients reported involuntary movements. Another effort tried treating people who'd had a stroke with cells derived from tumours; the results were mixed, and researchers were uneasy about the cells' cancerous source.

In recent years, researchers have had success with stem cells coaxed to develop into particular cell types, such as neural support cells. Tuszynski has showed how well stem cells can work — at least, in animal models[1]. His group implanted neural stem cells derived from human fetal tissue into rats with severe spinal-cord injuries. Seven weeks later, the cells had bridged the gap where the spinal cord had been cut and the animals were able to walk again. The cells used in the study were manufactured by Neuralstem of Rockville, Maryland. The group has shown that other kinds of stem cell, including those derived from adult tissue, also work. Tuszynski has seen similar results in a rat spinal-cord-injury model, using neural stem cells made from the tissues of a healthy 86-year-old volunteer[2].

"Every time we get an experiment done we realize it's more complex than we thought it would be."

But animal studies are also making it clear that simply regrowing the connective wiring of the nervous system to bridge damaged areas is not enough, says Zhigang He, who studies neural repair at the Harvard Stem Cell Institute in Cambridge, Massachusetts. No matter what the animal model is, he says, the axons don't always grow into the right places. It's not enough to have a nerve, that nerve must become part of a functional circuit.

There is growing evidence that besides becoming replacement nerves, stem cells perform other functions — they also seem to generate a supportive milieu that may encourage the natural recovery process or prevent further damage after an injury. Many types of neural stem cell secrete a mix of molecules that unlock suppressed growth pathways in nerves. Earlier this year, Tuszynski reported that any sort of spinal-cord stem cell, whether derived from adult tissues or embryos, from humans, rats or mice, could trigger native neural regeneration in rats[3].



MARK TUSZYNSKI/KEN KADOYA/REF. 3

Regeneration of axons (red) beyond implanted neural progenitor cells (green) in a rat with a spinal injury.

But his success in rats has not yet translated into clinical trials. More work is needed, Tuszynski says, to determine which type of cell will work best for which particular injury.

REINVENTING RECOVERY

For people who have had a stroke or spinal-cord injury, physical therapy is currently the best hope for recovery in the weeks and months after the injury. The brain is plastic and can co-opt other circuits and pathways to compensate for damage and to restore function. Once the inflammation ebbs and the brain adjusts, people can start to regain function. But the window of opportunity is short. Most people don't make functional gains after six months.

That timeline is why the remarkable recovery enjoyed by Coontz and other patients with chronic stroke in the same clinical trial is so surprising, says Steinberg. "This changes our whole notion of recovery," he says. There were 18 people in the trial Coontz took part in, and all were treated using stem cells manufactured by SanBio of Mountain View, California. The company's cells are bone-marrow-derived mesenchymal stem cells. The cells are treated with a DNA fragment that is transiently expressed in them, and causes changes in their protein-expression patterns. In animal studies, these cells promote the migration and growth of native neural stem cells, among other effects.

The trial, which was designed to look at safety as well as efficacy, recruited patients after an ischaemic stroke. During this kind of stroke, a clot cuts off the blood supply to part of the brain, causing significant damage. Patients in the trial had all had ischaemic strokes deep in the brain 7–36 months earlier — past the 6-month window for significant recovery. Each patient was injected with either 2.5 million, 5 million or 10 million of SanBio's cells[4]. Steinberg has followed participants for 24 months; an interim study at 12 months reported that most patients showed functional improvements. Some, like Coontz, achieved almost complete recovery.

What is not clear, however, is what the stem-cell injections do in the brain. In animal studies, the SanBio cells do not turn into neurons, but seem to send supporting signals to native cells in the brain. Indeed, preclinical research shows that the cells do not integrate into the brain — most die after 12 months. Instead, the cells seem to secrete growth factors that encourage the formation of new neurons and blood vessels, and foster connections called synapses between neurons. And in rats, the nerve-cell connections that extended from one side of the brain to the other, as well as into the spinal cord, lasted, even though the injected cells did not[4].

But these mechanisms are not sufficient to explain Coontz's overnight restoration of function, says Steinberg. He is entertaining several hypotheses, including that the needle used to deliver the cells may have had some effect. "One week after treatment, we saw abnormalities in the premotor cortex that went away after one month," he says. The size of these microlesions was strongly correlated with recovery at 12 months. A similar effect can happen when electrodes are implanted in the brains of people with Parkinson's, although this deep-brain stimulation quietens tremors for only a short time. The people who'd had a stroke had a lasting recovery, suggesting that both the needle and the stem cells may have played a part.

The SanBio trial was small, and did not have a placebo control; the company is now recruiting for a larger phase II trial. Of the 156 participants that will be recruited, two-thirds will have cells injected — the others will have a sham surgery. Even the trial surgeons, including Steinberg, will not know who is getting which treatment. The main outcome measure will be whether patients' motor-skill scores improve on a test called the Fugl-Meyer Motor scale six months after treatment. Participants will be monitored for

at least 12 months, and will also be evaluated with tests that look for changes in gait and dexterity. Meanwhile, Steinberg plans to study microlesions in animal models of stroke to determine whether they do have a role in recovery.

SMALL SUCCESSES

An ongoing clinical trial evaluating escalating doses of neural stem cells in patients with acute spinal-cord injuries is also looking promising. Asterias Biotherapeutics of Fremont, California, coaxes the cells to develop into progenitors of oligodendrocytes, a type of support cell that's found in the brain and spinal cord and that creates a protective insulation for neuronal axons.

The trial tests the safety and efficacy of administering these cells to people with recent cervical, or neck-level, spinal-cord injury. Interim results for patients who had received the two lower doses were presented at the International Spinal Cord Society meeting in September. After 90 days, 4 patients who received 10 million cells showed improved motor function; a fifth patient had not reached the 90-day mark yet. At one year, the three patients receiving a lower dose of two million cells showed measurable improvement in motor skills.

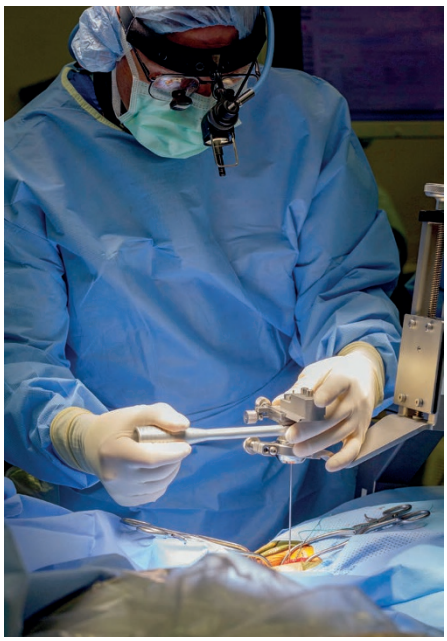
These cells were initially developed by Geron, a biotechnology company that has since moved away from regenerative medicine. Before spinning out Asterias in 2013, Geron had run a safety trial of the cells in people with a chronic lower-back injury. No issues were identified, and the US Food and Drug Administration agreed to let the company test the cells in patients who'd been recently injured. Asterias focused the current trial on patients with cervical injuries because these are closer to the brain, so new nerve cells have a shorter distance to grow to gain functional improvements. People with severe cervical spine injuries are typically paralysed below the level of the damage. The company's hope is to restore arm and hand function for people with such injuries, potentially making a tremendous difference to a person's independence and quality of life.

Asterias seems to have realized this hope in at least one patient who received one of the higher doses. Kristopher Boesen, who is 21, has had a dramatic recovery. In March, Boesen's car fishtailed in a rainstorm; he hit a telephone pole and broke his neck. About a month later, Boesen was still paralysed below the injury, and his neurological improvements seemed to have plateaued. His doctors at a trauma centre in Bakersfield, California, were in touch with Liu, who is an investigator in the Asterias trial. As soon as he was stable, Boesen travelled to Los Angeles to join the trial.

Liu injected Boesen's spinal cord with Asterias's cells in April. Two days later, Boesen started to move his hands, and in the summer, he regained the ability to move the toes on one foot.

Liu is excited about Boesen's response. "He was looking at being quadriplegic, and now he's able to write, lift some weights with his hands, and use his phone," says Liu. "For somebody to improve like this is highly unusual — I want to be jumping out of my shoes." But Liu cautions that this is still a small trial, and that Boesen's response is just one anecdotal report. Until the results are borne out in a large, placebo-controlled clinical trial, Liu will remain earthbound.

The trial is currently recruiting between 5 and 8 patients for another cohort that will receive a doubled dose of 20 million cells. As the trial goes on, Asterias hopes to find clues about the underlying mechanism. "We're looking at changes in the anatomy of the injury," says the company's chief scientific officer, Jane Lebkowski. She says that there is some evidence that axons have traversed the injury site in patients who have recovered function. Preclinical work suggests that the cells might be sending growth-encouraging chemical signals to the native tissue. And, as support cells, the astrocytes may also be preventing more neurons from dying in the aftermath of the acute spinal injury.



ASTERIAS BIOTHERAPEUTICS

A surgeon prepares to inject stem cells to treat a spinal injury as part of Asterias's clinical trial.

Not all clinical trials have performed so well. The SanBio and Asterias results are positive signals in a sea of negative or mixed trials. For example, StemCells of Newark, California, terminated its phase II trial of stem cells for the treatment of spinal-cord injury in May, and shortly afterwards announced that it will restructure its business. The company declined to comment for this article.

HOPE NOT HYPE

Physicians such as Liu and Steinberg temper their public enthusiasm about stem-cell therapies, so as not to give false hope to desperate patients. People with paralysing injuries or those who have a neurodegenerative disease are easy marks for unscrupulous stem-cell clinics, whose therapies are not only unproven, but also come with risks.

"Patients say, 'Go ahead, doc, you can't make me any worse,'" says Keith Tansey, a neurologist and researcher at the Methodist Rehabilitation Center in Jackson, Mississippi, and president-elect of the American Spinal Injury Association. Unfortunately, that is not the case. Cell therapies given at a clinic, outside the context of a clinical trial, can lead to chronic pain, take away what little function a patient has left and render a patient ineligible for future studies, says Tansey. He has seen the consequences in his clinical practice. "I treated a kid who had two different tumours in his spinal cord from two different individuals' cells," he says.

Many unanswered questions remain about whether stem cells can heal the central nervous system in people, and how they might do it. Researchers also don't know what cells are the best to use. Is it enough for them to grow into supportive cells that send friendly growth signals, or is it better that they grow into replacement neurons? The answer is likely to differ depending on the site and nature of the disease or injury. If the stem cells are producing supportive factors that encourage growth and repair, it might be possible, says He, to discern what these are and give them directly to patients. But biologists are not yet close to deciphering the recipe for such a cocktail.

Tansey agrees that there are many unknowns — and these seem to be multiplying. "Every time we get an experiment done we realize it's more complex than we thought it would be," he says. Tansey thinks that the best way to resolve such uncertainties is with carefully regulated clinical trials. Rat models will only tell us so much — the human nervous system is much larger and is wired differently. If stem cells help patients such as Coontz and Boesen to regain their speech and give them greater independence without adverse effects, then it makes sense to continue, he says, even without knowing all the details of how they work.

Until these positive, but small, results are replicated in larger, controlled clinical trials, neurologists are containing their optimism. "I'd like to hear of any clinical trial that has more than an anecdotal benefit," says Tansey. And Liu is anticipating the day when he won't need to control his elation. In a few years, perhaps there will be a genuine opportunity to jump for joy. ■

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