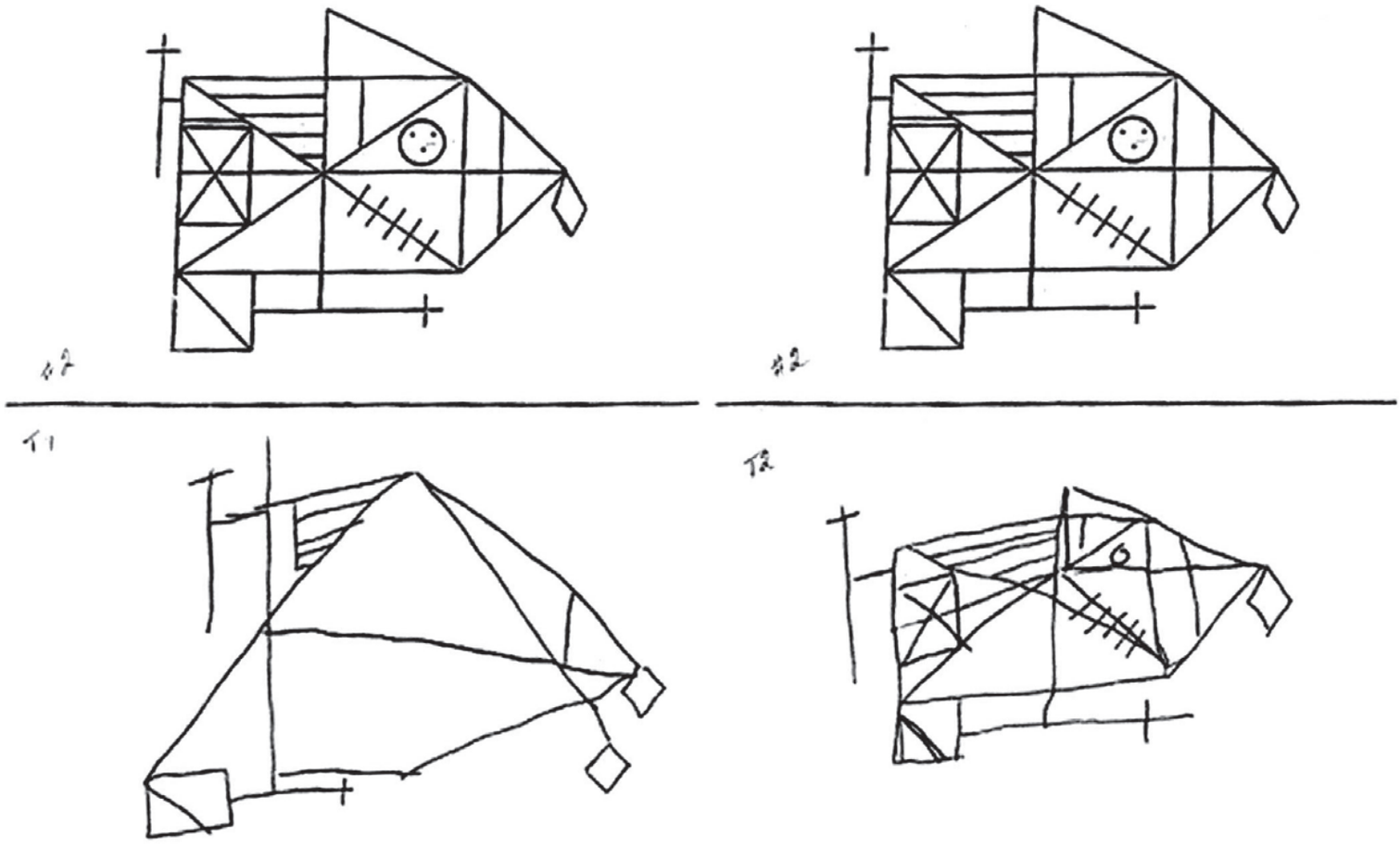
COMPLEX FIGURE TEST



BASELINE **6 MONTHS**

In a recent trial using stem cells that promote healing and reduce inflammation, patients who were treated months or even years after a stroke showed marked improvement in tests similar to this one. Patients were asked to study a complex figure (top row) and then draw it from memory.

REPRINTED WITH PERMISSION FROM WOLTERS KLUWER: C.S. STILLEY ET AL., "CHANGES IN COGNITIVE FUNCTION AFTER NEURONAL CELL TRANSPLANTATION FOR BASAL GANGLIA STROKE," NEUROLOGY, VOL. 63 NO. 7, PP. 1320-1322.

OPENING THE WINDOW

A NEW TIMELINE FOR STROKE TREATMENT

BY JILL NEIMARK

When a stroke occurs, it's a desperate race against the clock. Current treatment can help dissolve clots, improve blood flow to the damaged part of the brain, or limit brain bleeding. But the IV (or, in a minority of cases, endovascular treatment) must be administered within hours of the stroke. And even when patients are fortunate enough to be rushed to the hospital within that brief window, prognosis is grim. Seven out of 10 stroke survivors suffer lasting disabilities.

A new approach—using adult stem cells—could change that, says Lawrence Wechsler, MD professor of neurology and neurological surgery who holds the Henry B. Higman Chair at the University of Pittsburgh and is founding director of the UPMC Stroke Institute. Wechsler was an investigator on two recent multicenter adult stem cell studies, which he helped design. He hopes that an array of targeted therapies could pry that window open by days, months, or even years and ultimately improve patient outcomes.

In separate trials, Wechsler's team looked at two delivery methods—IV infusion in subacute care and brain surgery to infuse stem cells directly into damaged cerebral tissue months or even years after a stroke. Both methods proved safe.

The first, a double-blind, placebo controlled phase 2 safety trial, conducted in collaboration with more than 30 hospitals and universities in the United States and Great Britain, followed 126 acute stroke patients for a year. Sixty-five patients were given an IV infusion of mesenchymal stem cells, and 61 were given a placebo infusion. David Hess of the Medical College of Georgia, Wayne Clark of Oregon Health and Science University, and Wechsler presented the results to the American Heart Association International Stroke Conference in February of 2016.

The researchers used multipotent adult progenitor cells (MAPCs), which are usually derived from bone marrow (these mesenchymal cells can also be found in fatty tissue, dental pulp, umbilical cord blood, and other tissues). MAPCs can be multiplied in the laboratory and stored frozen for several years. They are well tolerated, do not require tissue matching, and appear to have profound anti-inflammatory and tissue repair effects.

As the patients recovered, the researchers looked at their neurological deficits and degree of disability in daily activities. They also imaged the brain via MRI and examined blood for levels of inflammatory chemicals circulating. A higher number of patients treated with stem cells achieved an excellent or good recovery by day 90 poststroke. Hospital stay, time in the ICU, infections, and readmissions went down. The key, the team found, was early treatment—meaning within 36 hours of a stroke—a far cry from currently available therapies. A year out, "there was a clear, statistically significant benefit for treated patients," says Wechsler.

"Stroke has an effect on the immune system itself," he explains. "In the early stages, inflammatory immune cells rush to the site of damage in the brain, and they may actually impede the recovery process. Suppressing that inflammatory reaction appears to be beneficial."

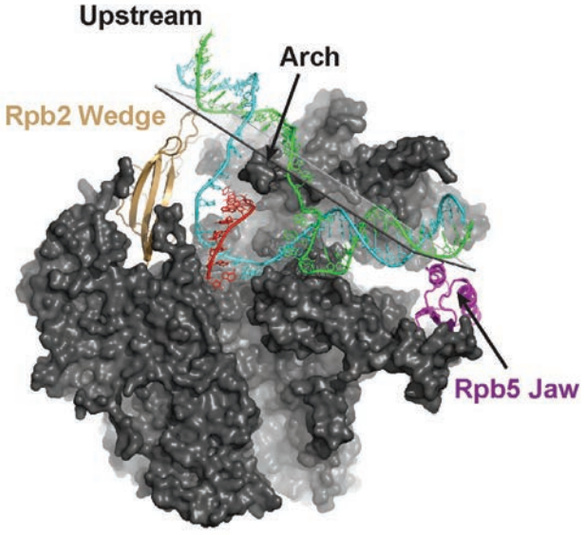
And, in what Wechsler calls "a completely different stem cell approach," 18 patients suffering from chronic stroke underwent brain surgery to infuse another kind of specialized mesenchymal stem cell directly into the damaged area of the brain. Called SB623, these cells secrete factors that protect neurons from hypoxic injury, help repair damaged cells, support neural growth, quell inflammation, and promote blood vessel growth. The study was published online in *Stroke* this June.

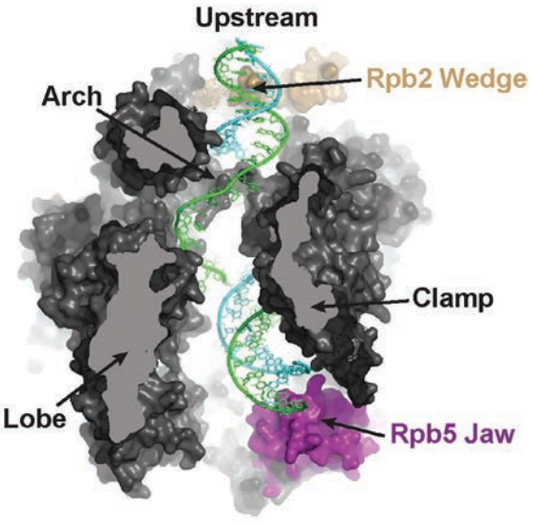
These patients were six months to several years out from a stroke, and the natural recovery process had plateaued. To rekindle healing, the team delivered stem cells every 5–6 millimeters along a track in the damaged area of the brain.

"The cells secrete a variety of growth factors to enhance recovery at a later stage," Wechsler says.

There were no complications directly related to the cells. All patients had at least one treatment-related adverse effect—from headaches to, in the worst case, a seizure related to the surgery. But those complications completely resolved. Moreover, the participants experienced gains possibly related to the treatment, such as improvement in ability to stand and the disappearance of tremor.

It's early yet—more patients will need to be studied for the researchers to be confident of the results (a large, multicenter, phase 2 study is now under way). Wechsler is optimistic. This new approach to therapy has "tremendous promise for enhancing stroke recovery," he says. ■





Calero and collaborators demonstrate the inner workings of Pol II, the RNA polymerase protein. Pol II's "wedge" and "jaw" hold the double helix together above and below the region being transcribed as the protein moves upstream. Pol II's "arch" coordinates the process by which the transcribed strand recombines with the other strand to re-form the double helix.

CALERO SHOWS HOW TRANSCRIPTION HAPPENS

BY ALLA KATSNELSON

RNA IN ACTION

Structural biologists the world over have an unofficial motto: Structure is function. Figure out a protein's shape, in all its nooks and crannies, and you'll find meaty clues to the role it plays in the cell. It sounds straightforward, but there's a constraint. In order to determine a protein's structure with the traditionally used technique, X-ray crystallography, you have to coax it to crystallize. It's a state that many proteins resist—particularly large or otherwise complex ones, or groups of proteins interacting in some sort of cellular tango.

Such complicated proteins or protein groupings are the specialty of Guillermo Calero, an MD/PhD assistant professor of structural biology at the University of Pittsburgh. Calero decided to tackle a mystery of RNA polymerase II, or Pol II for short. Pol II is one of the most important players in the cell; its job is to read the cell's DNA, transcribing it into messenger RNA. Accuracy is essential, because that RNA will eventually travel to the ribosome and get decoded into the proteins that keep almost every single cellular process running.

Pol II had been studied extensively, and scientists had even solved its structure, but it still wasn't clear how exactly it latched onto a section of unzipped DNA and got down to business. Calero and his lab team set out to catch the polymerase in the act.

Their initial efforts fell flat, however, because, predictably and unfortunately, the crystals wouldn't grow.

So the researchers decided to dive in for a closer look: They picked through the detritus of their failed attempts and found tiny aggregates they were able to examine with electron microscopy, which uses electrons rather than light to image a specimen super close-up. "We were able to look at precipitates that most researchers would claim were useless and find that they did indeed contain very small crystals with nice lattices," he says.

Growing crystals involves fiddling with a set of parameters—the concentration of the protein, for example, or various reagents in the solution. By optimizing the conditions under which the electron microscope showed nanocrystals, Calero and his colleagues were able to grow those tiny nuggets larger. "It's essentially electron microscopy–guided crystal growth," he says.

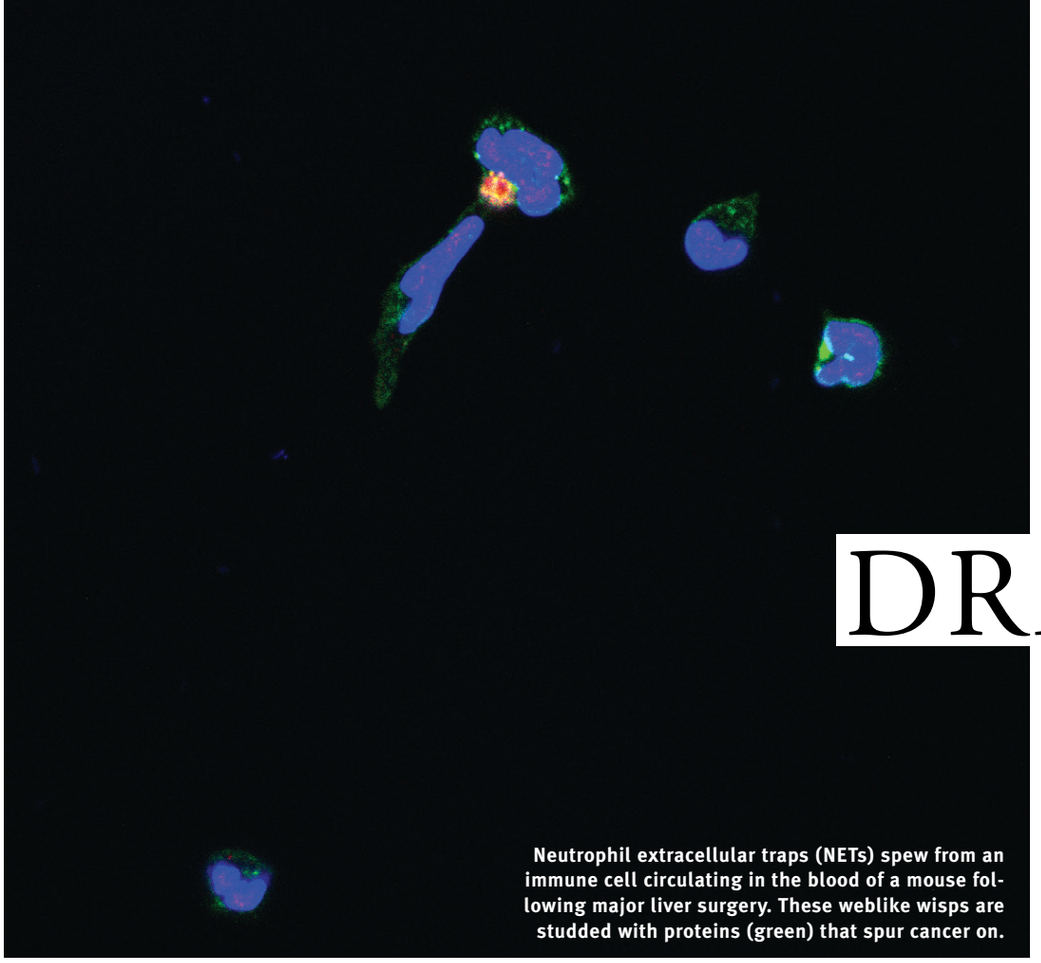
The structure they determined of the RNA polymerase complex in yeast cells, published in *Molecular Cell* in July 2015, provided the first complete look at a transcription bubble passing through a polymerase protein. "It's like if you were able to go to the nucleus of the cell and open your eyes and look for a polymerase molecule," he says.

They could image the protein as it simultaneously grabbed both the upstream and the downstream part of the DNA, using its main subunits to keep the transcription bubble open at one end while coordinating the DNA's annealing of the other end. More recently, Calero and his colleagues used the same approach to solve the structure of the HIV protein Vpr bound to three human proteins. In August, they revealed how Vpr interferes with DNA repair in human cells by inactivating one of the three proteins, and later by earmarking it for degradation.

Calero is now taking his nanocrystal detection technique a big leap farther by teaming up with a beam-wielding collaborator, Tamir Gonen at Howard Hughes Medical Institute's Janelia Research Campus. Gonen recently devised a technique called MicroED for generating high-resolution structures; he puts nanocrystals in an electron microscope and shoots them with an electron beam to diffract them.

"You can actually obtain a structure with only three crystals that are super small—we're talking less than one micron," says Calero. "It's a completely new way to get structures."

REPRINTED FROM MOLECULAR CELL, VOL. 59, BARNES ET AL., "CRYSTAL STRUCTURE OF A TRANSCRIBING RNA POLYMERASE II COMPLEX REVEALS A COMPLETE TRANSCRIPTION BUBBLE." PP. 258–269, © 2015, WITH PERMISSION FROM ELSEVIER.



COURTESY TSUNG LAB

Neutrophil extracellular traps (NETs) spew from an immune cell circulating in the blood of a mouse following major liver surgery. These weblike wisps are studded with proteins (green) that spur cancer on.

CATCHING A CULPRIT IN CANCER'S SPREAD

BY ERIN HARE

DRAGNET

Surgery is the only possible cure for metastatic colorectal cancer, the third most common and third most deadly cancer in the United States. "But there has always been anecdotal evidence suggesting that [surgery] can also cause the cancer to recur quicker," says surgeon Allan Tsung, codirector of the UPMC Liver Cancer Center, Pitt's Roberta G. Simmons Professor of Surgery, and vice chair of research for the Department of Surgery.

In March, Tsung's team published a paper in *Cancer Research* that helps to explain this paradox—a finding that *The Scientist* later highlighted as an editor's choice in cell and molecular biology.

It all started when Tsung, curious about the link between the immune response after surgery and subsequent cancer, was examining serum samples from his post-op patients and noticed something that piqued his interest: Weblike structures known as neutrophil extracellular traps (NETs) were flooding their blood.

Historically, the presence of NETs had been thought only advantageous. During infection, they capture bacteria—like dragnets catching fish—to help the body clear away pathogens and heal. "We weren't sure initially whether NETs were beneficial or harmful in cancer progression," Tsung says. He thought it was possible that NETs just trap tumor cells and help immune cells get rid of them, but they found the opposite. The release of NETs actually supported tumor growth.

First, the team compared the prevalence of NETs in colorectal cancer patients who had undergone surgery on their livers—that's the site where colorectal tumors most often metastasize (the liver is where the primary cancer's lymph fluid and blood drain). The researchers found that patients who had more major liver-resection surgery formed more extensive NETs—and also had a four-fold higher rate of recurrence compared to patients with little NET formation. (They also looked at healthy controls with no NETs.)

Patients with colorectal cancer often have cancerous cells and undetectable tumors throughout their bodies, Tsung explains. Thus, he hypothesized that the released NETs—which are known to be triggered by inflammation—may interact with cancer cells he couldn't remove in the OR. Unfortunately, because the inflammatory response is global, NETs form everywhere. "Any time you have any surgery, everyone thinks, *Oh it's just localized to that organ.* But in fact, multiple parts of your body actually can go through changes" after surgery, Tsung says.

Next, using surgery to induce NET formation in a mouse model of cancer, the team watched via *in vivo* imaging as NETs corralled free-floating tumor cells into clumps—which spelled trouble. Divided, the tiny tumor cells had floundered, but in the inflammatory aftermath of surgery, the milieu seemed to change. Gathering strength in numbers in the NETs, the cells became much more likely to invade tissue and spread.

The team then tried pharmacologically inhibiting NETs with DNase—an enzyme that disbands their DNA backbone—and were pleased to see drastically reduced tumor formation. NETs seemed critical for cancer recurrence initiated by tumor cells that evade a surgeon's scalpel.

Further, they also found that NETs actually changed the behavior of tumor cells via cellular-signaling pathways. NETs are studded with proteins that interact with a receptor called TLR9 on cancer cells to make them more aggressive, Tsung says. When the team manipulated the cancer cells to lack TLR9, the mice grew far fewer tumors in the post-op period.

Having shown in mice that DNase blocked NET formation—and in turn cancer progression—Tsung's team recognized DNase as a potential cancer therapy. DNase is already FDA approved as a treatment for cystic fibrosis; whether it will be safe for people who just underwent major surgery remains to be seen. Tsung expects clinical trials could begin in the next couple of years. ■