Explorations and revelations taking place in the medical school

In a clinical trial published in the *New England Journal of Medicine*, trauma patients who received plasma while in transport via air medical service had a 10 percent reduction in mortality.

BLUE SKY IDEA

A TEST FLIGHT FOR PREHOSPITAL PLASMA | BY KRISTIN BUNDY

Say a STAT MedEvac helicopter is flying a patient, a nonresponsive 44-year-old man, from the scene of a car accident. He is bleeding and at risk of hemorrhagic shock. His systolic blood pressure dips dangerously—below 70. But the patient is still 20 minutes from the hospital.

"Giving early plasma in the emergency department was thought to be beneficial," says Jason Sperry, MD professor of surgery and critical care at Pitt. He wanted to see what benefits a prehospital infusion would give a patient like this. So Sperry and coprincipal investigator Francis Guyette, a Pitt MD associate professor of emergency medicine and the medical director of STAT MedEvac, launched a clinical trial, Prehospital Air Medical Plasma (PAMPer). The results came out in the July *New England Journal of Medicine*.

Collaborating with seven other large trauma centers with busy helicopter units, the team collected data to determine if plasma given in addition to standard resuscitation efforts during a flight would affect 30-day mortality rates post-trauma in a randomized controlled trial.

Funded by the U.S. Army, Sperry and Guyette's study was no small task. Because the patients could not give consent at the time of the intervention, the FDA had to approve the study design under a protocol known as exception from informed consent (EFIC). This was in addition to seeking approval from the Department of Defense (DoD) and the institutional review boards of each study site.

EFIC criteria are stringent. Investigators must first demonstrate that not only is there a potential benefit for the patient, but also that there is no time for the patient to give informed consent and no other way to do the research. Second, the primary endpoint has to represent a significant outcome—in this instance 30-day mortality rates. Third, the researchers have to make the community aware of the upcoming study.

Outreach for PAMPer came via bus ads, newspapers, YouTube and other sites, and radio. People who chose to opt out wore a "No PAMPer" bracelet. In all, it took two and a half years of planning and four years to complete the study, Sperry says.

It was worth the wait. Plasma was shown to save lives (improving the 30-day mortality figures by 10 percent). In addition, it reduced blood transfusion requirements and improved a measure of coagulation.

What's more, benefits were found in a broad patient population. The inclusion criteria required that participants have either one episode of low blood pressure with a high heart rate or an episode of severe low blood pressure at any time before reaching the trauma center via air medical service. Some patients had liver injuries, some had traumatic brain injuries (TBIs), and some didn't have significant injuries at all, Sperry says.

For him, the most interesting finding was the rate at which mortality improved in a subset of patients with TBI. Of the 87 in that treatment arm, two thirds survived, representing a 21 percent relative improvement in survival. "No other intervention on the planet" can do that, says Sperry. "Now, that's a subgroup," and not statistically significant, he says. "It deserves further investigation."

Although they don't know how the plasma works, they have a theory. In addition to treating the bleeding and preventing further bleeding, the plasma might provide some additional protection:

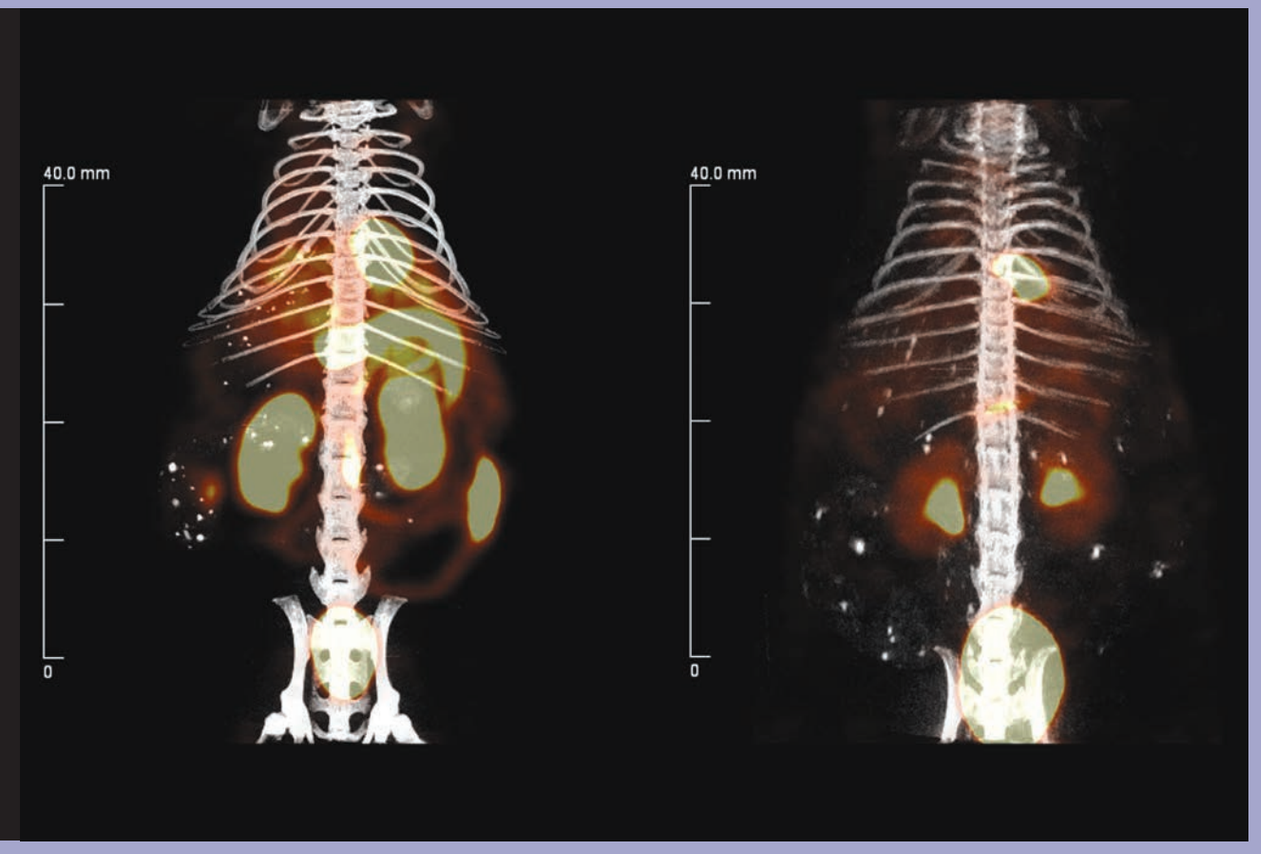
"Newer evidence suggests that plasma may give back," says Sperry. "It may protect the endothelium—the cells that line the blood vessels—which is almost an organ in and of itself. When they're in shock, these endothelial cells release cytokines that cause inflammation, and plasma may have certain pro-endothelial cell mediators that benefit."

Thawed plasma is just one stepping stone along the path of products Sperry is studying to try to improve outcomes after trauma. In addition to further investigating data from the PAMPer study, he wants to learn what advantages other types of plasma may have when given immediately post-trauma.

"This impact—this 10 percent reduction in mortality—could change practice across the country," he says. "It may not be able to be done using thawed plasma, which only has a five-day shelf life, but it could be done with liquid plasma [which has never been frozen]—and in the future, potentially freeze-dried plasma that has no shelf-life or storage limitations."

Because injury is one of the leading causes of death in people ages 1 to 44, the new approach has the potential to save a huge number of lives, notes Sperry. ■

STEVEN MATTO/FROM THE SCENE PHOTOGRAPHY



Zahid aims to reduce the amount of radiation that patients are exposed to during cardiac stress tests. Here, the organs of a mouse injected with traditional imaging agents (left) show higher exposure (yellow) than Zahid's more targeted approach (right).

COURTESY MALIHA ZAHID

A BETTER WAY TO IMAGE THE HEART

BY AMERIGO ALLEGRETTO

CORDONED EXPOSURE

When patients describe chest pain as coming from the heart, they are often prescribed stress tests that use a radiological scan for diagnosis, prognosis, or treatment monitoring.

"During these tests, the radiation goes everywhere—it goes into the liver, the lungs, and the gut, to name a few organs," says Maliha Zahid, assistant professor of developmental biology at the University of Pittsburgh. Because the gut is so close to the heart, she explains, it can sometimes cause images to be difficult to interpret.

So Zahid, a cardiologist, has created a way to alleviate both of these problems: deliver radiation straight to the heart. "By sending the radiation only to where it's needed for imaging, you can reduce the amount of radiation by as much as 80 percent," she says. "We are hypothesizing that this will also lead to higher image quality."

These specific stress tests are "the biggest contributor to radiation that patients receive in the course of medical treatment," notes Zahid. A study published in 2011 by the National Center for Biotechnology Information showed that such scans performed annually in the United States could result in 7,400 additional future cancer cases.

Her research led to the creation of a novel non-naturally occurring 12-amino-acid peptide—peptides are molecules made up of links of amino acids that are the building blocks of proteins—called the cardiac targeting peptide. It intravenously transports radioisotopes needed for stress test scans and is designed to be taken up only by cells that make up the heart muscle (cardiomyocytes). The radiation, therefore, doesn't spread to other organs and, unlike the course for current stress tests, the peptide then leaves the heart and is excreted through the kidneys, sparing the liver.

Zahid says this took about two years and six attempts to get right.

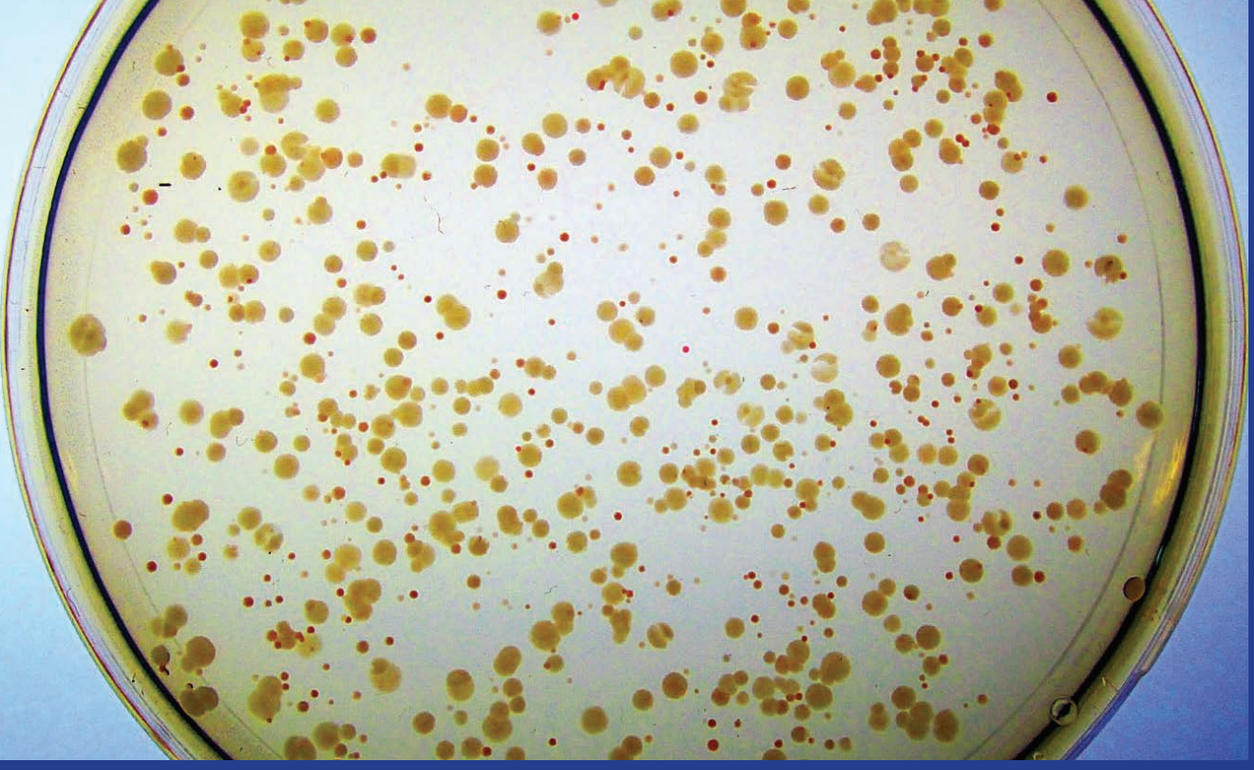
"The last attempt, I told myself, 'If this doesn't work, I'm going to give up,'" she says. "It worked, and we found a peptide that we were able to manufacture in the University's peptide synthesis facility."

Zahid's peptide won first place at Pitt's Clinical and Translational Science Institute 2016 Pitt Innovation Challenge (PInCh); the $100,000 in winnings will advance Zahid's research findings toward clinical application. Zahid received assistance in translational research strategy from sciVelo, a Pitt Innovation Institute program aimed at accelerating life and health sciences translational research and commercialization.

And she recently also received an American Heart Association Scientist Development Grant for $231,000.

Pitt, Zahid, and her co-inventor, Paul Robbins, a former Pitt faculty member who's now a molecular medicine professor at the Scripps Research Institute in Jupiter, Fla., have been granted a U.S. patent on the peptide technology and are planning a startup company, CardioTrak.

"The past year has been fun and fast-paced like a roller coaster ride," Zahid says. ■



COURTESY ANN DONNELLY/HECHT LAB/PRINCETON UNIVERSITY

Donnelly constructed Syn-F4, the first-ever artificial enzyme capable of working in a living organism—in this case, *E. coli*. Pictured here are engineered *E. coli* lacking the Fes enzyme, either expressing Syn-F4 (large beige colonies) or a control protein (small red colonies).

A LIFE-SUSTAINING CREATION

BY ALLA KATSNELSON

ENZYMES FROM SCRATCH

Enzymes are the workhorses of the cell—and of all biological systems. These specialized proteins set into motion all the cells' chemical reactions, making sure they run fast enough for life on a biochemical level to proceed. Each enzyme precisely aligns with the cellular processes it regulates. Thanks to evolution, our world is full of these meticulously well-suited proteins.

A study published earlier this year by Ann Donnelly, a research specialist in the Department of Biomedical Informatics, has revealed that scientists can also make working enzymes from scratch.

The study was e-published in January in *Nature Chemical Biology*. Donnelly, who came to Pitt in 2017, did the work as a PhD student in the lab of Michael Hecht at Princeton.

"We showed that you can take novel protein sequences that nature has never seen before and put them in natural systems—and they can function," Donnelly explains.

The findings hint at some fascinating dimensions of our primordial history, she says: Namely, the reactions that governed early cellular processes were much more flexible than they are now. "The enzymes we see today have a lot of evolutionary baggage and have been really refined to do the things they do," Donnelly says.

But evolution's solutions weren't the only ones, it turns out. "Our work suggests it's possible to replace what we have now with something completely different."

The synthetic enzyme Syn-F4 was one of a big batch made in Hecht's lab a decade ago. The group routinely produces synthetic proteins, designing them to conform to a folding pattern called a four-helix bundle and then testing them in mutated strains of the bacterium *Escherichia coli*. The idea is to see whether any of the synthetic enzymes they make can replace the functions of *E. coli* genes that have been knocked out. And sometimes the artificial versions do work. Mostly they come into play in a pinch—by switching on cellular processes that can have functions similar to what's called for.

"But the case with Syn-F4 was a little different," Donnelly says.

Syn-F4 and its synthetic cohort were evolved to fill in for an *E. coli* enzyme called Fes that had been hobbled by a mutation. The job of Fes is to release iron from a compound in *E. coli* that nabs the metal from the environment so that it can be used for healthy growth in the cell. Without Fes, the bacterial colonies grow poorly, speckling with red as iron builds up around them. But when Donnelly added Syn-F4 to these sickly colonies, the red began to disappear, returning the *E. coli* to its healthy state. "It was clear as day," she says. "It was unbelievable for me to see this happening in real time."

So unbelievable, in fact, that she kept mum about it until she had repeated the finding several times. In addition to testing the synthetic protein in living bacteria, she also mixed it directly with its iron-grabbing substrate and biochemically analyzed the ensuing reaction.

Later, she tweaked the substrate by chemically reversing its orientation. She found that this prevented Syn-F4 from working its magic, demonstrating its specificity and supporting the idea that it was working as an enzyme.

What's interesting, says Donnelly, is that the natural enzyme and the artificial one look completely different. The natural one is about four times as big, and it is known to connect to the substrate through a site that includes the amino acid serine. The artificial enzyme, though, has no serine residue in it whatsoever.

"It's difficult to pinpoint exactly how it's working, but at minimum we know that they are not catalyzing the reaction the same way."

These days at Pitt, in the lab of Erik Wright, an assistant professor of biomedical informatics, Donnelly's using her ingenuity to focus on understanding how pathogens evolve to become resistant to antibiotics. ■