

Pitt investigators are illuminating a kind of cell death called ferroptosis, which likely plays a role in many acute and chronic diseases. Here, in a model of traumatic brain injury, cells dying of ferroptosis glow in green.

COURTESY BAYIR LAB AND CENTER FOR BIOLOGIC IMAGING

IRONS



UNDERSTANDING DEATH

BY ELAINE VITONE

IN THE FIRE

On a recent afternoon, the University of Pittsburgh's Valerian Kagan explains the tolerance of living things. Life, in its many forms, only has so much wiggle room, he says.

"You'd be surprised to see a polar bear in Miami, right? And similarly, nobody has reported on hummingbirds on the North Pole?"

Birds, bears, whatever earthly inhabitants you might imagine—all life is defined by two things, he continues: one, the raw material imparted by genetics; and two, "everything else." Food, microflora, temperature. Viruses, pollutants, and heaven forbid, the ionizing radiation from a nuclear bomb. All of the above interact with genomes and their translations, and that, essentially, is life—at least from his particular scientific perspective. Kagan is a Graduate School of Public Health professor and vice chair of environmental and occupational health and School of Medicine professor of radiation oncology.

Well, that's life until, of course, it's not. Once a given biochemical point of no return is crossed? "Bye-bye." Cells die. "It's difficult to recreate the chicken from a boiled egg, right? Did you try it? No? Well, I tried several times. Don't waste your time."

Death always has a reason, he says.

"Hello—nice to meet you," says Kagan's coinvestigator, Pitt Med professor of critical care medicine Hülya Bayır, walking in a couple of minutes later after driving from UPMC Children's Hospital of Pittsburgh, where she directs pediatric critical care medicine research. Her accent is Turkish. Bayır first came to Pitt as a pediatric critical care fellow in 1999 and cut her teeth as an investigator under Kagan's tutelage. They've been collaborators ever since. For her work in the fields of pediatric neurocritical care, traumatic and ischemic brain injury, and redox biology, Bayır has been elected to the esteemed American Society for Clinical Investigation and won several honors from the Society of Critical Care Medicine.

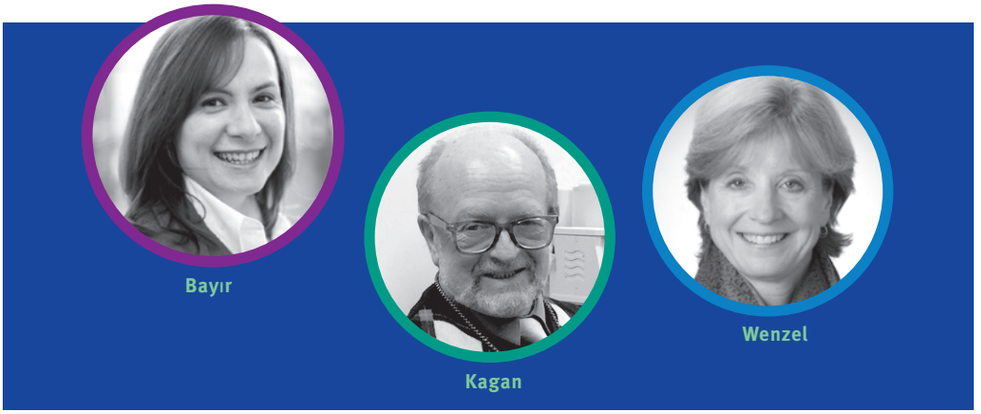
Where were we? Oh yes—death. It always has a reason.

You've got your very quick, smash-into-a-truck ways for cells to die—mechanical reasons. That's called necrosis. And then, as has been discovered over the last couple of decades, there are a dozen other types of cell death that occur naturally, preprogrammed by genetics as a normal part of our life cycle and general upkeep. Balance is key, though. Too much death is not good, for obvious reasons, and too little death is a recipe for cancer.

Usually, programmed cell death is first sparked by factors outside of the body, Kagan says. For example, in one program called apoptosis, the death sequence switches on when DNA is damaged beyond repair. Bayır and Kagan have made significant contributions to the literature on apoptosis.

Then, a few years ago, the pair decided to take on another kind of cell death called ferroptosis.

It was first described in 2012 by Columbia University's Brent Stockwell, who's now a collaborator with the Pitt duo. Stockwell had been looking for new drug candidates that could hit the cancer cells that apoptotic drugs missed, and came upon something never seen before: When he chemically depleted tumor cells of an antioxidant called GSH, a chain reaction ignited. Somehow, this reaction seemed to be using an enzyme called LOX to attack—and kill—the cancer cells.



Within the massive field of programmed cell death, ferroptosis is exploding. Of the 350-plus papers on the topic, 270 were published by labs around the world just in the past couple of years. Pitt researchers are among those leading such efforts, having developed a new technology for the study of ferroptosis—Pitt is one of the few places in the world with this capability.

In a series of papers, Bayır and Kagan have collaborated with Pitt's Sally Wenzel and others (seven labs in all, mostly from Pitt) to better understand "the reason" for ferroptosis—exactly what biomolecular line is crossed, how that signal is communicated within and between cells, which molecules pull the trigger, and how. Combining clinical observations from multiple fields of medicine, along with biochemistry, molecular biology, structural biology, and computational biology, they've uncovered new insights with potential relevance to a number of diseases. They hope to find ways to stem ferroptosis when it contributes to the degradation of tissue, as it does in brain trauma, asthma, kidney disease, and more—and, in the case of cancer, to better urge ferroptosis into action.

"LOX contains iron—*ferro* means iron," says Bayır.

"And we live in Pittsburgh, the Iron City," adds Kagan. "It would be a shame for us not to understand this process."

Imagine a body has one of those smash-into-a-truck kind of impacts. In the aftermath, immune cells activate and rush in to help, and the brain is bombarded. Soon, what were once healthy neighboring cells start going down, too—collateral damage.

The road to hell is paved with good intentions, as Kagan likes to say.

Bayır explains that, in order to both treat and monitor this flood of cell-slaughtering inflammation, doctors surgically place a catheter in the brain to siphon excess cerebrospinal fluid out. As a fellow, Bayır saw this fluid, which was just going straight to the garbage, for its worth and began examining it more closely.

"I was doing measurements for a long time," she recalls. "Dr. Kagan taught me that there's a difference between measurements and research. It's very important to understand the difference, and it takes time,

especially for MDs in training."

Back then, Kagan adds, Bayır was doing the double duty of a rising physician-scientist. "She came [into the lab] from the pediatric ICU in her blue scrubs," he tells me. He recalls when Pat Kochanek, who's now professor and vice chair of critical care medicine, first introduced her as a new fellow who wanted to study redox—a chemical reaction in which one substance is oxidized and another is reduced. Redox had long been among Kagan's areas of expertise.

Bayır began to notice a pattern: Within the first week after a patient's injury, levels of GSH progressively decreased—a sign that oxidation was on the rise. (Recall that GSH is the same antioxidant that would later figure into the discovery of ferroptosis.) But the tools at the team's disposal couldn't explain what was being oxidized or what exactly that signaling process was.

So, for the next 10 years, Bayır and Kagan worked to develop a new technology that could reveal what was happening. "It's really kind of like looking for a needle in a haystack. That's what we had to overcome," in addition to waiting for mass spectrometry technology to catch up to them, says Kagan.

Using a technique they named mass spectrometry–based redox lipidomics, they identified the products of ferroptosis and sussed out which molecules initiated the signal that causes cells to die. They published their findings in two papers in *Nature Chemical Biology* (both e-published in November 2016).

Normally, LOX oxidizes free fatty acids, like what's in corn oil. But in ferroptosis the enzyme suddenly betrays its usual mission and oxidizes a specific subgroup of cell-membrane molecules called PEs instead.

"We'd been fantasizing a lot about this," recalls Kagan. They mentioned it to Wenzel, professor of medicine at Pitt and director of the Asthma Institute at UPMC. And she told them that actually, oxygenated PE may play a role in asthma. They quickly figured out that their areas of study shared some interesting molecules in common.

"And so, in our parallel universes, we eventually found each other," Wenzel explains in a separate interview.

Wenzel, a translational researcher, is an international leader in asthma; the condition is another kind of hell that begins in good intentions.

Say a body comes into contact with pollen. That body will do its best to stop the allergen in its tracks by drowning it in mucus, and by restricting airways so that the pollen can't get through. But unfortunately, then, neither can air.

In 2011, *PNAS* had published Wenzel's findings on the pathways for mucus production in people with severe asthma. And wouldn't you know: One of the key molecules was LOX.

The Pitt Med colleagues found evidence that in severe asthma, LOX was abandoning its script, too; instead of keeping cellular proliferation in check, like it's supposed to, LOX sticks to a protein called PEBP1.

It's much more complicated than this, but in short: When cellular GSH levels are low and the enzyme that uses GSH is inactive, this complex comes together, which changes LOX's behavior. And then: death (for cells, at least).

The team hypothesizes that, with these complexes flooding cells along airways, hell breaks loose. Epithelial barriers deteriorate, and tissues become sitting ducks for viruses, pollutants, and all manner of toxins. And probably, immune pathways spur into action, as well. Many of these ideas were first tested by Ivet Bahar, professor and chair of computational and systems biology, and her team using computational modeling.

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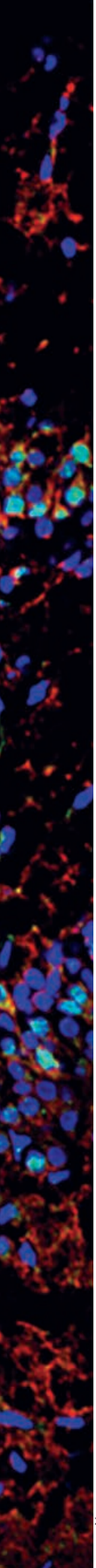
Wenzel has examined samples from people who've died of asthma attacks, and found LOX/PEBP1 in their airways. She's found it in living patients with severe asthma, as well, and also correlated its levels to those of a biomarker of disease severity, exhaled nitric oxide. The biomarker and LOX/PEBP1 had "one of the strongest relationships I could possibly imagine," she says.

The same complex showed up when Bayır examined cells from a model of traumatic brain injury, as well as when the team examined models of kidney failure, which is also believed to be a result of ferroptosis. Striking images of the complex, captured by Claudette St. Croix, a PhD associate professor of cell biology, ran in the team's paper in *Cell* in October 2017.

Wenzel cautions that there's work to do. Still to come is confirmation of the significance of what they're finding—but the data are thus far very encouraging. If the hypothesis holds together, it could mean a lot for patients with severe asthma. As of now, the only therapies available for serious asthma attacks are albuterol inhalers and oral steroids. She adds that asthma tends to run in families—and her preliminary data suggest some people may have susceptibility to this destructive complex written into their genetics.

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The team is now searching for molecules that can target the LOX/PEBP1 complex. The LOX inhibitors currently on the market can't



do the job, so new ones must be developed from scratch. A laboratory at the University of California, Santa Cruz, is collaborating, and other potential partners have expressed interest as their investigations gain steam in this wholly new area of drug discovery.

In acute radiation syndrome, at first, a body finds itself in gastrointestinal misery. Then a calm sets in—for a time. The latent phase passes, followed by what's known as manifest illness; and depending on the level of exposure, that "manifestation" can mean utter "devastation" for cells throughout the body, potentially to fatal effect.

Some of this damage, particularly in the gut, appears to be caused by ferroptosis. Bayır explains that she and her colleagues believe this intestinal damage triggers a cascade of health complications that lead to sepsis, a deadly syndrome. "If we can stop that process and get the body to repair, rather than systematically destroy, those cells, we might save the victims of devastating dirty bomb attacks," she says.

Pitt is one of four centers funded by the National Institutes of Health to prepare for such events. The Pitt Center for Medical Countermeasures Against Radiation is directed by Joel Greenberger, professor and chair of radiation oncology. Within the center, Bayır leads a project aimed specifically at stockpiling therapies to stem ferroptosis in the fast-dividing cells of the gut and bone marrow in the event of a nuclear attack.

The good news is that in this particular cell-death program, time is on a body's side. Ferroptosis isn't the initial crash and smash of electrons, but rather a reaction to it. Relevant therapies could be administered a few days after an attack and still save lives. The team is excited about their progress thus far.

Two weeks before their big *Cell* paper came out, that same journal published a review paper on ferroptosis that Bayır and Kagan wrote, along with 16 other attendees of an invitation-only meeting called the Cold Spring Harbor Symposium. The paper outlines what is known about this cell death pathway, tools for its study, and areas of promise for the future: Alzheimer's, Huntington's, Parkinson's, cancer, stroke, intracerebral hemorrhage, traumatic brain injury, ischemia-reperfusion injury, kidney degeneration, and even heat stress in plants. Pitt has projects along several of these lines—many irons in the ferroptosis fire. (A follow-up meeting is planned for November.)

One of the main challenges in studying ferroptosis is that there is no direct biomarker for it. Bayır and Kagan's redox lipidomics technology yields the closest thing to it—but the technique is slow going. "It's not high throughput. We cannot today immediately implement it into clinics," says Kagan. "But it will be done."

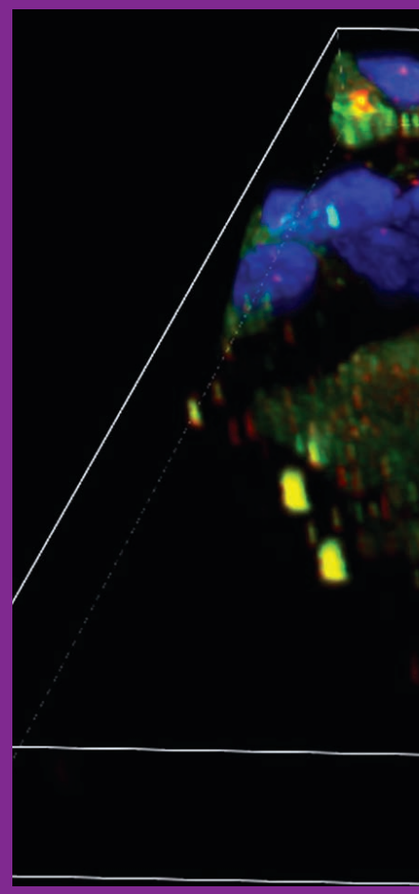
Institutions around the world are lining up to collaborate with the Pitt group and their unique technological capability. Meanwhile, Bayır and Kagan are working to break up their own monopoly. A new international laboratory for navigational redox lipidomics, to be based in Moscow, is in the works. "Pitt will of course play a major role," says Kagan, who will act as a consulting director from afar.

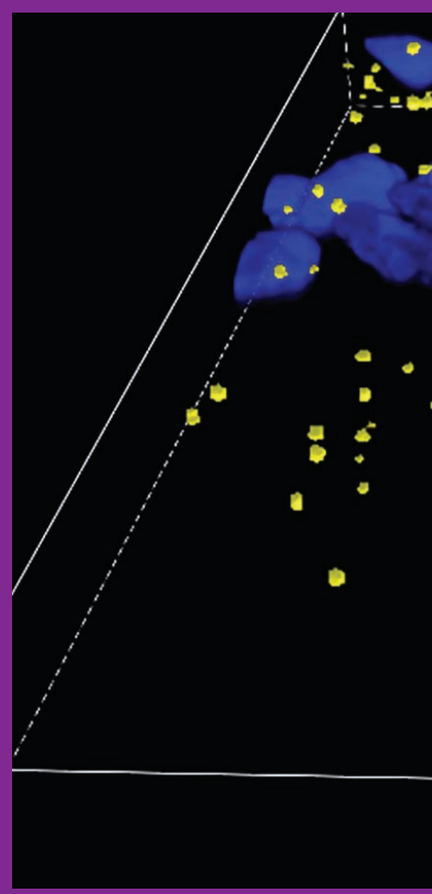
Why Moscow? For a lot of reasons, he says. Lomonosov Moscow State University, his alma mater, is the scholarly home of his longtime collaborator and friend Yury Vladimirov, who is developing another possible new way to detect ferroptosis. In fact, several other labs in that city are piecing together new tools for this fast-dividing field of study, Kagan notes. He adds that once the new facility opens, so will additional opportunities for mentoring, which has long been a passion of his. Many rising Russian scientists have come to Pitt to work in his lab.

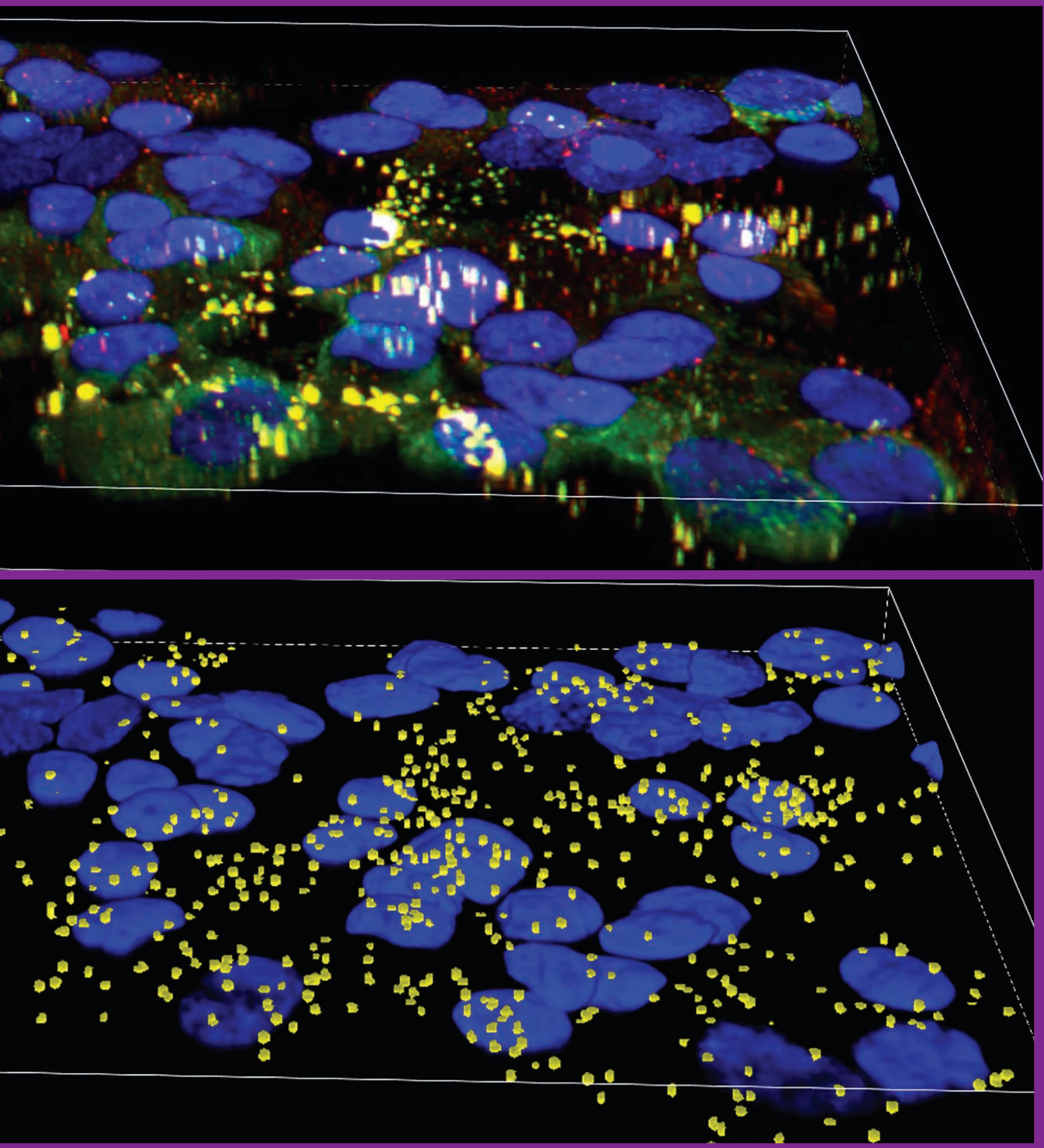
Here in Pittsburgh, a soon-to-be-launched Neuroscience Institute at Children's Hospital, directed by Bayır, will be central to this new partnership. In time, the Pitt team's bicontinental efforts could grow to a global ferroptosis force to be reckoned with.

"There will be people recruited not only from Russia, but also Europe, Germany, and Portugal," Kagan says. "It will be very international and interdisciplinary. That's what science is all about."

When he presents at conferences, Kagan uses a slide with pictures of his happy American home of 26 years. Pittsburgh, he likes to mention, is also home to 446 bridges. And now the Iron City is building yet another new byway to the broader world. ■







In the epithelial cells (nuclei in blue) within the airways of patients with severe asthma, Wenzel found the LOX/PEBP1 complex (yellow and green). She then compared its abundance to levels of a biomarker for asthma severity—exhaled nitric oxide—in the patients and found "one of the strongest relationships I could possibly imagine," she says.

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