INSIDE STANFORD MEDICINE

Volume 9, No. 2 ■ January 23, 2017 ■ Published by the Office of Communication & Public Affairs

New research shows that wearable biosensors can indicate disease.

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# Source of opioids' side effects identified

By Tracie White

A commercially available drug may help drastically reduce two side effects of opioid painkillers — a growing tolerance to them and a paradoxical increased sensitivity to pain — without affecting the drugs' ability to reduce pain, according to a study by researchers at the School of Medicine.

These two side effects often mean patients require higher doses of opioids to maintain pain relief, increasing the risk of addiction and respiratory failure. Opioid overdoses were responsible for more than 20,000 deaths nationwide in 2015, according to the American Society of Addiction Medicine, and are now the leading cause of accidental death in the United States.

"In some patients, you don't have much margin between how much painkiller you can give and their ability to breathe normally, or the occurrence of other significant side effects," said Gregory Scherrer, PhD, PharmD, assistant professor of anesthesiology, perioperative and pain medicine and of neurosurgery. "Our goal was to understand how opioids cause their side effects, to find ways to separate these detrimental side effects from pain relief properties and to make these painkillers safer."

Scherrer is the senior author of the study, which was published online Jan. 16 in *Nature Medicine*. The lead authors are postdoctoral scholars Gregory Corder, PhD, and Dong Wang, PhD; anesthesiology instructor Vivianne Tawfik, MD, PhD; and graduate student Elizabeth Sypek.

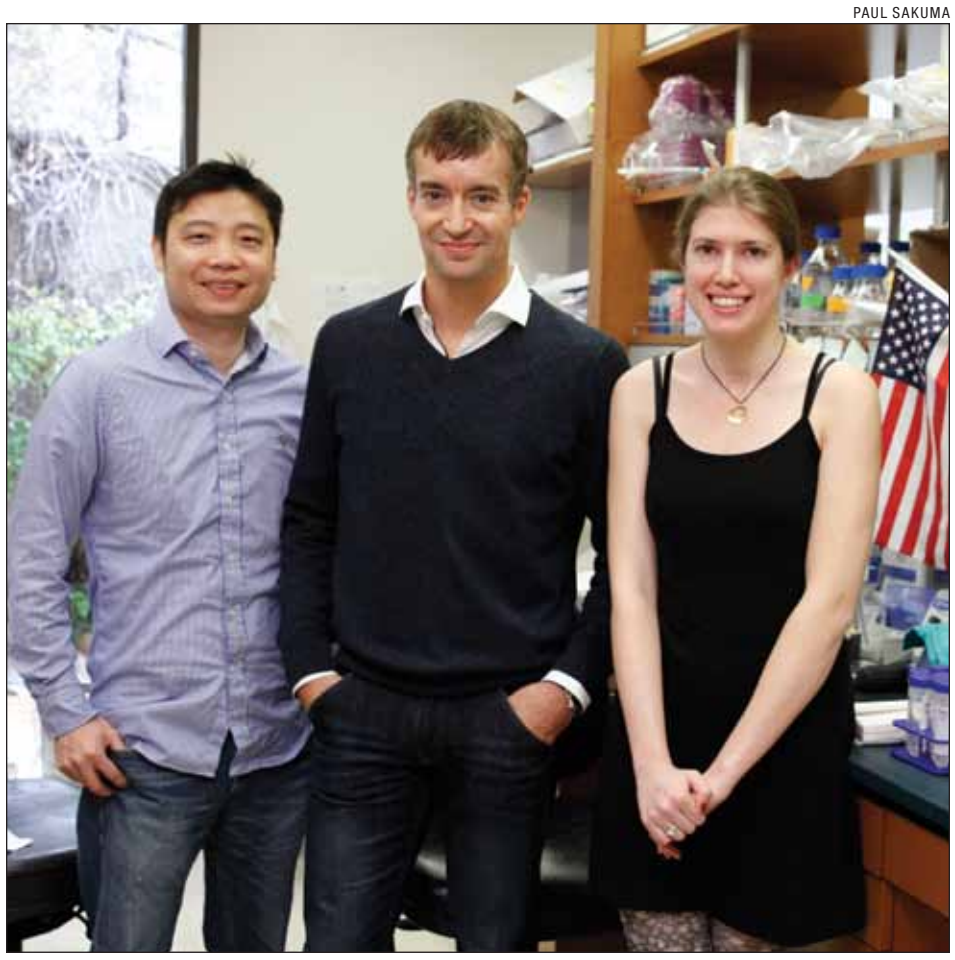
Working in mouse models, Scherrer and his colleagues found that tolerance and increased sensitivity to pain may be specifically caused by opioids' effect on peripheral pain neurons in the body, not those in the spinal cord and brain. They also established that contrary to the prevailing view in the field, microglia — non-neuronal cells found in the spinal cord and brain — are not initiating opioids' side effects because they lack the gene that forms the receptors necessary to cause them.

By using a drug that blocks only the effects of opioids on the periphery, they were able to eliminate the two side effects without reducing pain relief.

"We demonstrate that these two side effects can be drastically reduced with co-administration of an already used compound, methylnaltrexone bromide, currently used to combat constipation, which is another unwanted side effect of opioids, while still maintaining pain relief," Scherrer said.

Methylnaltrexone bromide is approved by the Food and Drug Administration for the treatment of opioid-induced constipation.

PAUL SAKUMA



Dong Wang, Gregory Scherrer and Elizabeth Sypek are co-authors of a study that found two side effects of opioids — growing tolerance to the drugs and increased sensitivity to pain — may be specifically caused by the drugs' effect on peripheral pain neurons in the body.

### Clinical trials needed

Clinical trials are now needed to test whether the findings hold true in humans, with the goal of reducing the number of deaths from overdoses while improving the function of prescription opioid painkillers, Scherrer said. Growing conocern about an epidemic of opioid-painkiller abuse has drawn the attention of physicians, patients and public health authorities. According to the Centers for Disease Control and Prevention, about 100 Americans die each day from drug overdoses, and more than half of those deaths involve opioid pain relievers.

From a clinical standpoint, these pain relievers not only present safety risks, but they often prove a disappointment in how well they work to control pain, said co-author David Clark, MD, PhD, a Stanford professor of anesthesiology, perioperative and pain medicine who treats patients at the Palo Alto Veterans Affairs Health Care System.

"They don't in general provide substantial pain relief for a long period of time," said Clark. The prevalence of chronic pain in veterans is unusually high, he said. In addition to the chronic pain from such illnesses as cancer and diabetes, many veterans also face lifelong pain from battle wounds, such as traumatic brain injuries, shrapnel injuries and injuries to limbs.

"Once patients start on opioids, the drugs may work for weeks or months, but then it's common to see the pain relief dissipate, and these patients face the decision of whether or not to increase the dose," Clark said. "With increasing doses comes the increasing risk of adverse events."

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# Gay health workers face tough choices where homosexuality is a crime

By Ruthann Richter

Jason Nagata

Jason Nagata, MD, sat in a wooden pew in a Seventh Day Adventist church in Kenya, listening to the pastor thunder away about the "abomination" of homosexuality. Nagata, then a medical student, began to sweat profusely, his face dripping and his palms wet. Flanking him in the church pew were members of his Kenyan host family, who had no idea he was gay.

"It was clear all the people in the church had similar viewpoints, as they were nodding in agreement with the pastor," he said. "I think I was just trying as hard as I could not to let anything show on my face."

In a recently published commentary, Nagata writes about the challenges of being gay and doing global health work in countries where homosexuality is a crime, punishable by death or imprisonment, and where those legal constraints are often mirrored in societal attitudes. The commentary, written while he was a pediatric resi-

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# Caffeine may counter systemic inflammation

By Bruce Goldman

School of Medicine scientists have unearthed a connection between advancing age, systemic inflammation, cardiovascular disease and caffeine consumption.

Extensive analysis of blood samples, survey data and medical and family histories obtained from more than 100 human participants in a multiyear study has revealed a fundamental inflammatory mechanism associated with human aging and the chronic diseases that come with it.

The study, which was published online Jan. 16 in *Nature Medicine*, implicates this inflammatory process as a driver of cardiovascular disease and increased rates of mortality overall. Metabolites, or breakdown products, of nucleic acids — the molecules that serve as building blocks for our genes — circulating in the blood can trigger this inflammatory process, the study found.

The study also provides evidence that caffeine and its own metabolites may counter the action of these circulating nucleic-acid metabolites, possibly explaining why coffee drinkers tend to live longer than abstainers.

"More than 90 percent of all noncommunicable diseases of aging are associated with chronic inflammation," said the study's lead author, David Furman, PhD, a consulting associate professor at the Stanford Institute for Immunity, Transplantation and Infection. More than 1,000 papers have provided evidence that chronic inflammation contributes to many cancers, Alzheimer's disease and other dementias, cardiovascular disease, osteoarthritis and even depression, he said.

"It's also well known that caffeine intake is associated with longevity," Furman said. "Many studies have shown this association. We've found a possible reason for why this may be so."

Mark Davis, PhD, a professor of microbiology and immunology and the director of the Stanford Institute for Immunity, Transplantation and Infection, shares senior authorship of the study with Benjamin Faustin, PhD, a cell biologist at the University of Bordeaux in France. Davis is

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# Researcher and colleagues announce master plan for better science

By Jennie Dusheck

An international team of experts has produced a "manifesto" setting forth steps to improve the quality of scientific research.

"There is a way to perform good, reliable, credible, reproducible, trustworthy, useful science," said John Ioannidis, MD, DSc, professor of medicine and of health research and policy at the School of Medicine.

"We have ways to improve compared with what we're doing currently, and there are lots of scientists and other stakeholders who are interested in doing this," said Ioannidis, who is senior author of the article, which was published Jan. 10 in the inaugural issue of *Nature Human Behavior*. The lead author is Marcus Munafò, PhD, professor of biological psychology at the University of Bristol in England.

### What's holding science back?

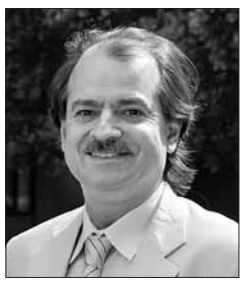
Each year, the U.S. government spends nearly $70 billion on nondefense research and development, including a budget of more than $30 billion for the National Institutes of Health. Yet research on how science is conducted — so-called meta-research — has made clear that a substantial number of published scientific papers fail to move science forward. One analysis, wrote the authors, estimated that as much as 85 percent of the biomedical research effort is wasted.

One reason for this is that scientists often find patterns in noisy data, the way we see whales or faces in the shapes of clouds. This effect is more likely when researchers apply hundreds or even thousands of different analyses to the same data set until statistically significant effects appear.

The manifesto suggests it's not just scientists themselves who are responsible for improving the quality of science, but also other stakeholders, including research institutions, scientific journals, funders and regulatory agencies. All, said Ioannidis, have important roles to play.

"It's a multiplicative effect," he said, "so you have all of these players working together in the same direction." If any one of the stakeholders doesn't participate in creating incentives for transparency and reproducibility, he said, it makes it harder for everyone else to improve.

"Most of the changes that we propose in the manifesto are interrelated, and the stakeholders are connected as if by rubber bands. If you have one of them move, he or she may pull the others. At the same time, he or she may be restricted because others don't move," said Ioannidis, who is also co-director of the Meta-Research Innovation Center at Stanford.



John Ioannidis

### Manifesto

The eight-page paper describing ways to improve science includes four major categories: methods, reporting and dissemination, reproducibility, and evaluation and incentives.

Methods could be improved, the authors reported, by designing studies to minimize bias — by blinding patients, doctors and other participants, and by registering the study design, outcome measures and analysis plan before the research begins — to prevent subsequent deviations from the study design, regardless of intriguing, serendipitous results.

The authors also state that reporting and dissemination might be improved by eliminating "the file drawer problem," the tendency of researchers to publish results that are novel, statistically significant or supportive of a particular hypothesis, while not publishing other valid but less interesting results. "The consequence," wrote the authors, "is that the published literature indicates stronger evidence for findings than exists in reality."

The file drawer effect is fueled, though, from the behavior of universities, journals, reviewers and funding agencies — not just that of individual scientists, the authors write. One way funders and journals can help is by requiring all researchers to meet certain standards. For example, the Cure Huntington Disease Initiative has created an independent standing committee to evaluate proposals and provide disinterested advice to grantees on experimental design and statistical analysis. This committee doesn't just set standards; it actually helps researchers meet those standards.

The ultimate goal is to get to the truth, Ioannidis said. "When we are doing science, we are trying to arrive at the truth. In many disciplines, we want that truth to translate into something that works. But if it's not true, it's not going to speed up computer software, it's not going to save lives and it's not going to improve quality of life."

He said the goal of the manifesto is to increase the speed at which researchers get closer to the truth. "All these measures are intended to expedite the process of validation — the circle of generating, testing and validating or refuting hypotheses in the scientific machine."

Researchers from the University of Virginia; the University of Oxford; the University of Bath; Cardiff University; the National Centre for the Replacement, Refinement and Reduction of Animals in Research; the Wharton School; and the Cure Huntington Disease Initiative Foundation also co-authored the study.

Funding was provided by the British Heart Foundation; the Cancer Research U.K.; the Economic and Social Research Council; the Medical Research Council; the National Institute for Health Research, under the auspices of the U.K. Clinical Research Collaboration; a Wellcome Trust Principal Research Fellowship; an unrestricted gift from S. O'Donnell and B. O'Donnell to the Stanford Prevention Research Center; and a grant by the Laura and John Arnold Foundation to the Meta-Research Innovation Center at Stanford. ISM

# Diabetes impairs activity of bone stem cell, inhibiting fracture repair

By Krista Conger

Bone fractures in diabetic mice heal better in the presence of a protein that stimulates the activity of skeletal stem cells, according to a study by researchers at the School of Medicine.

The protein counteracts a decrease in stem cell activity that the researchers observed both in mouse models of diabetes and in bone samples from diabetic patients who had undergone joint replacements. The researchers hope the discovery will lead to ways to help people with diabetes heal more efficiently from broken bones.

Michael Longaker

"We've uncovered the reason why some patients with diabetes don't heal well from fractures, and we've come up with a solution that can be locally applied during surgery to repair the break," said Michael Longaker, MD, co-director of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. "Diabetes is rampant worldwide, and any improvement in the ability of affected people to heal from fractures could have an enormously positive effect on their quality of life."

The study was published Jan. 11 in *Science Translational Medicine.* Longaker, a professor of plastic and reconstructive surgery, shares senior authorship of the study with Charles Chan, PhD, an instructor at the stem cell institute. Postdoctoral scholar Ruth Tevlin, MD, is the lead author.

### Healing difficulties

Diabetes mellitus is a metabolic disease characterized by the inability to either produce or to respond appropriately to insulin. It affects hundreds of millions of people worldwide and is increasing in prevalence. In addition to causing dangerous swings in blood sugar levels after meals, the condition leads to many other debilitating symptoms, including an impaired ability to heal soft tissue injuries and skeletal fractures. The precise molecular reason behind this impaired bone healing has been unknown, however.

Longaker, Chan and Tevlin built on previous research in which they and colleagues in the laboratory of co-author Irving Weissman, MD, professor of pathology and of developmental biology, identified and described a population of cells in the bones of mice that serve as skeletal stem cells, or SSCs. These adult stem cells can become all components of the skeletal system, including bone, cartilage and a part of the bone marrow known as the stroma. They subsequently showed that fracture healing in mice was severely impaired when these stem cells were depleted. That finding got them thinking.

"We wanted to apply what we knew about skeletal stem cells to the problem of impaired bone healing in people with diabetes," said Chan. "Does the disease affect fracture healing by somehow modulating the activity of these stem cells?"

The researchers used a mouse model of Type 2 diabetes, in which the disease arises when the animals are about 4 weeks old. Prior to the development of the disease, the prediabetic mice were able to heal leg bone fractures as effectively as wild-type mice, the researchers found. In contrast, after the disease had manifested itself, the repaired bone was significantly weaker and less dense than the bone in the control animals. When they compared the numbers of SSCs in the healing bone seven days after fracture, they found that the diabetic mice had significantly lower numbers of these cells than did the control animals.

### Signaling problem

A series of experiments ruled out a systemic reason for this reduction in stem cell numbers, and also confirmed that the cells themselves were fully functional. That left only a potential problem with the signals the cells were receiving from the surrounding environment, or niche. When Tevlin and her colleagues analyzed that environment, they found that the diabetic animals produced significantly lower levels of a family of signaling proteins, called hedgehog, that is known to play a critical role in many biological processes, including embryonic development and tissue regeneration.

The researchers collaborated with co-author Philip Beachy, PhD, professor of biochemistry and of developmental biology, to test whether artificially blocking the hedgehog signaling pathway could impair bone healing in nondiabetic mice. They found that control mice exposed to a molecule that blocked the pathway regrew bone that was weaker and more brittle — just like the diabetic animals.

"Next we had to test whether adding the hedgehog signaling proteins back into the local environment in diabetic animals restored their ability to heal fractures," said Longaker. The researchers collaborated with co-authors Fan Yang, PhD, assistant professor of bioengineering and orthopaedic surgery,

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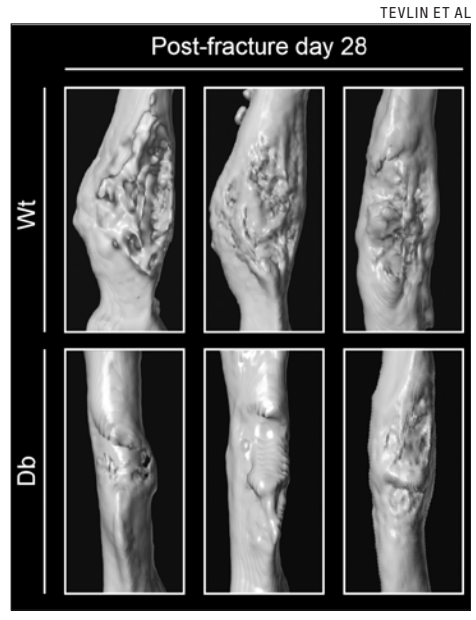


The bones of diabetic mice are more fragile than those of control animals when they heal after a fracture. Bones of the control mice (top) form larger calluses during healing, making the repairs stronger. In contrast, the bones of the diabetic mice (bottom) have smaller calluses, making the repairs more brittle.

INSIDE STANFORD MEDICINE

*Inside Stanford Medicine* is published monthly in July and December and semi-monthly the rest of the year.

*is produced by*
Office of Communication & Public Affairs
Stanford University
School of Medicine
3172 Porter Drive
Palo Alto, CA 94304
Mail code 5471
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**http://med.stanford.edu/news/**

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STANFORD MEDICINE

# Study: Toxic brain cells may drive many neurodegenerative disorders

**By Bruce Goldman**

While most of us haven't heard of astrocytes, these cells are four times as plentiful in the human brain as nerve cells. Now, a team led by researchers at the School of Medicine has found that astrocytes, which perform many indispensable functions in the brain, can take on a villainous character, destroying nerve cells and likely driving many neurodegenerative diseases.

A study describing the findings was published online Jan. 18 in *Nature*.

"We've learned astrocytes aren't always the good guys," said the study's senior author, Ben Barres, MD, PhD, professor of neurobiology, of developmental biology and of neurology and neurological sciences. "An aberrant version of them turns up in suspicious abundance in all the wrong places in brain-tissue samples from patients with brain injuries and major neurological disorders from Alzheimer's and Parkinson's to multiple sclerosis. The implications for treating these diseases are profound."

Barres, who has spent three decades focusing on brain cells that aren't nerve cells, called the findings "the most important discovery my lab has ever made." Stanford postdoctoral scholar Shane Liddelow, PhD, is the study's lead author.

Up to now, the pharmaceutical industry has mostly targeted nerve cells, also known as neurons, Barres said. But a broad range of brain disorders may be treatable by blocking astrocytes' metamorphosis into toxic cells, or by pharmaceutically countering the neuron-killing toxin those harmful cells almost certainly secrete.

### Role of astrocytes

Once thought of as mere packing peanuts whose job it was to keep neurons from jiggling when we jog, astrocytes are now understood to provide critical hands-on support and guidance to neurons, enhancing their survival and shaping the shared connections between them that define the brain's labyrinthine circuitry. It's also known that traumatic brain injury, stroke, infection and disease can transform benign "resting astrocytes" into "reactive astrocytes" with altered features and behaviors. But until recently, whether reactive astrocytes were up to good or evil was an open question.

In 2012, Barres and his colleagues resolved that ambiguity when they identified two distinct types of reactive astrocytes, which they called A1 and A2. In the presence of LPS, a component found in the cell walls of bacteria, they observed that resting astrocytes somehow wind up getting transformed into A1s, which are primed to produce large volumes of pro-inflammatory substances. A2s, on the other hand, are induced by oxygen deprivation in the brain, which occurs during strokes. A2s produce substances supporting neuron growth, health and survival near the stroke site.

This raised two questions: How are A1s generated? And once they're generated, what do they do? The new study answers both questions.

### Pro-inflammatory factors

In addressing the first question, the study showed that the brain's immune cells, microglia, which become activated by LPS exposure as well as in most brain injuries and diseases, begin spewing out pro-inflammatory factors that change astrocytes' behavior.

In a series of experiments using laboratory mice, the scientists identified three pro-inflammatory factors whose production was ramped up after LPS exposure: TNF-alpha, IL-1-alpha and C1q. In the brain, all three of these substances are secreted exclusively by microglia. Each, by itself, had a partial A1-inducing effect on resting astrocytes. Combined, they propelled resting astrocytes into a full-fledged A1 state.

Next, the researchers confirmed that A1s jettison the nurturing qualities they'd had as resting astrocytes, which Barres' group has shown are essential to the formation and functioning of synapses, and instead became toxic to neurons.

In vertebrates, nerve cells called retinal ganglion cells send information from the retina to vision-processing centers in the brain. RGCs can thrive in culture, but only if accompanied by astrocytes. The scientists cultured rodent RGCs with either resting or A1 astrocytes and counted the resulting synapse numbers. RGCs cultured in combination with A1s produced only half as many synapses as RGCs grown with resting astrocytes, and those that formed didn't work very well.

Further experiments showed that A1s lose resting astrocytes' capacity to prune synapses that are no longer needed or no longer functional whose continued existence undermines efficient brain function.

Indeed, when the researchers cultured healthy RGCs with increasingly stronger concentrations of the broth in which A1s had been bathing, almost all the RGCs eventually died. This and other experiments showed that A1s secrete a powerful, neuron-killing toxin.

The same treatment killed many other types of neurons, including both the spinal motor neurons that die in amyotrophic lateral sclerosis and the human dopaminergic neurons whose mysterious loss is the cause of Parkinson's disease. A1 bathwater also impaired the development of yet another class of non-neuron brain cells called oligodendrocytes — essentially fat-filled flapjacks that wrap themselves around nerve fibers, providing electrical insulation that speeds long-distance signal propagation. Autoimmune destruction of oligodendrocytes and their fatty contents gives rise to multiple sclerosis.

### Staving off A1 formation

In another experiment, the researchers severed rodents' optic nerves — an act ordinarily lethal to RGCs, whose outgoing fibers, called axons, constitute the optic nerve. In the central nervous system, severing axons causes the entire neuron to die quickly, but why they die has been a mystery. The investigators determined the cause: A1s. They observed that those reactive astrocytes formed quickly after axons were severed, but that neutralizing TNF-alpha, IL-1-alpha and C1q with antibodies to these three substances prevented A1 formation and RGC death in the animals.

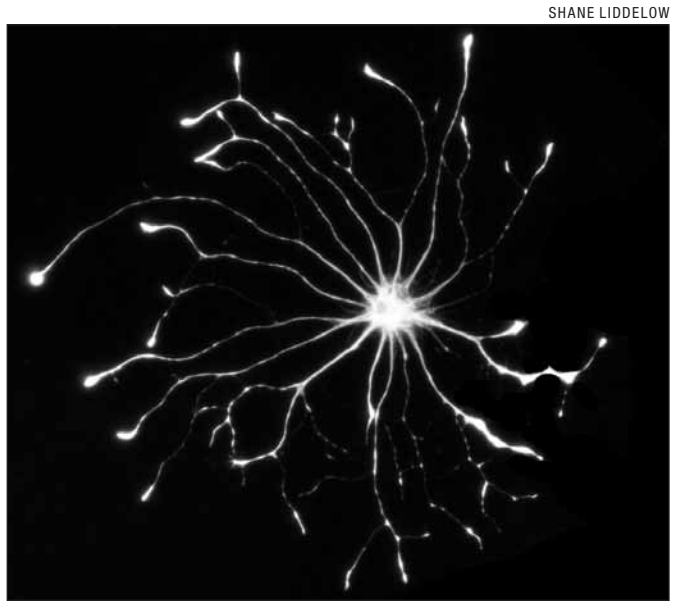
Finally, the researchers analyzed samples of human brain tissue from patients with Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis. In every case, they observed large numbers of A1s preferentially clustering where the disease was most active. For example, in the samples from Alzheimer's patients, nearly 60 percent of the astrocytes present in the prefrontal cortex, a region where the disease takes a great toll, were of the A1 variety. Because A1s are highly toxic to both neurons and oligodendrocytes, these findings strongly imply that A1 formation is helping to drive neurodegeneration in these diseases.

An effort to identify the neurotoxin secreted by A1 astrocytes is underway, Barres said. "We're very excited by the discovery of neurotoxic reactive astrocytes," he said, "because our findings imply that acute injuries of the retina, brain and spinal cord and neurodegenerative diseases may all be much more highly treatable than has been thought."

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Barres is the co-founder of a biotechnology company, Annexon Biosciences, which has produced, and filed for a patent for, an inhibitory antibody to C1q. Drugs to block TNF-alpha and IL1-alpha already exist.

SHANE LIDDELOW



**Star-shaped cells known as astrocytes are essential to the healthy function of neurons in the brain, but a new study shows that an aberrant version of astrocytes may contribute to many neurodegenerative diseases.**

Other Stanford co-authors are graduate student Kevin Guttenplan; postdoctoral scholars Laura Clarke, PhD, Todd Peterson, PhD, Brooke Napier, PhD, and Christopher Bohlen PhD; Frederick Bennett, MD, an instructor of psychiatry and behavioral sciences; medical student Mariko Bennett, PhD; life science research assistant Alexandra Munch; former postdoctoral scholar Won-Suk Chung, PhD; and Marion Buckwalter, MD, PhD, associate professor of neurosurgery and of neurology and neurological sciences.

Researchers from the University of California-San Francisco, the Technical University of Munich, Boston Children's Hospital, Johns Hopkins University and Harvard University also co-authored the work.

The study was funded by the National Institutes of Health, the Christopher and Dana Reeve Foundation, the Novartis Institute for Biomedical Research, the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the JPB Foundation, the Cure Alzheimer's Fund, the Glenn Foundation and Vincent and Stella Coates.

Stanford's Department of Neurobiology also supported the work. ISM

## Bones

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and postdoctoral scholar Xinming Tong, PhD, to devise a biologically friendly hydrogel into which the hedgehog signaling proteins were embedded. The gel was applied directly to the fracture site. "And these animals healed just like normal mice," said Longaker, who holds the Deane P. and Louise Mitchell Professorship in the School of Medicine.

### Clues in human bone samples

Finally, the team reached out to co-author Stuart Goodman, MD, PhD, professor of orthopaedic surgery, to obtain bone samples from patients with diabetes who were undergoing joint replacement for osteoarthritis. They compared the expression of proteins important to the hedgehog signaling pathway from these samples with others obtained from non-diabetic patients. Normally this tissue would be discarded by the surgeon, but in this case it held important clues.

"What we saw in these human samples completely echoed what we saw in the mice," said Chan. "The bones from the diabetic patients displayed significantly reduced expression of these important signaling proteins."

Longaker, Chan and Tevlin believe the inhibition of the hedgehog signaling pathway arises from diabetes-associated inflammation that causes high levels of a molecule called tumor necrosis factor alpha. TNF-alpha levels are known to be elevated in patients with diabetes, and the researchers observed a corresponding increase in their mouse models of the disease. They also showed that these increased levels of TNF-alpha inhibited the expression of some hedgehog family members. Directly inhibiting all TNF-alpha activity, however, could have other dire consequences for an animal or a human patient because TNF-alpha plays many important biological roles.

"Here we've devised a feasible strategy for reversing a tissue-specific pathology — the inability to heal skeletal fractures efficiently — in a complex metabolic disease like diabetes, through the local application of a compound to stimulate the activity of adult stem cells," said Longaker said. "We anticipate that hedgehog-mediated molecular therapies that directly target stem cells in human patients could be therapeutic."

More research is necessary before trying this approach in humans, but the researchers are hopeful that local application of hedgehog proteins will be shown to be both safe and effective. Their findings further validate the idea that tissue-specific stem cells are likely to play vital roles in tissue regeneration and response to injury.

"This research represents a significant step forward toward realizing the promise of Proposition 71, which established the California Institute for Regenerative Medicine," said Chan. "We've looked to stem cells to learn why people with diabetes don't heal bone fractures properly, and come up with an approach that we are excited to try in the clinic."

The paper provides a complete list of the Stanford co-authors of the study.

The research was supported by the National Institutes of Health, the Thomas and Stacey Siebel Foundation, the Prostate Cancer Foundation, the National Institute on Aging, the California Institute for Regenerative Medicine, the Oak Foundation, the Hagey Laboratory for Pediatric Regenerative Medicine, the Gunn/Olivier Research Fund, the National Science Foundation, the Stanford University Transplant and Tissue Engineering Center, the Plastic Surgery Foundation/Plastic Surgery Research Council and the American Society of Maxillofacial Surgeons.

Stanford's Department of Surgery also supported the work. ISM

# Wearable sensors can tell when you are getting sick, study shows

**By Jennie Dusheck**

Wearable sensors that monitor heart rate, activity, skin temperature and other variables can reveal a lot about what is going on inside a person, including the onset of infection, inflammation and even insulin resistance, according to a study by researchers at the School of Medicine.

An important component of the ongoing study is to establish a range of normal, or baseline, values for each person in the study and when they are ill. "We want to study people at an individual level," said Michael Snyder, PhD, professor and chair of genetics.

Snyder is the senior author of the study, which was published online Jan. 12 in *PLOS Biology*. Postdoctoral scholars Xiao Li, PhD, and Jessilyn Dunn, PhD, and software engineer Denis Salins share lead authorship.

Altogether, the team collected nearly 2 billion measurements from 60 people, including continuous data from each participant's wearable biosensor devices and periodic data from laboratory tests of their blood chemistry, gene expression and other measures. Participants wore between one and seven commercially available activity monitors and other monitors that collected more than 250,000 measurements a day. The team collected data on weight; heart rate; oxygen in the blood; skin temperature; activity, including sleep, steps, walking, biking and running; calories expended; acceleration; and even exposure to gamma rays and X-rays.

"I was very impressed with all the data that was collected," said Eric Topol, MD, professor of genomics at the Scripps Research Institute, who was not involved in the study. "There's a lot here — a lot of sensors and a lot of different data on each person."

The study demonstrated that, given a baseline range of values for each person, it is possible to monitor deviations from normal and associate those deviations with environmental conditions, illness or other factors that affect health. Distinctive patterns of deviation from normal seem to correlate with particular health problems. Algorithms designed to pick up on these patterns of change could potentially contribute to clinical diagnostics and research.

The work is an example of Stanford Medicine's focus on precision health, whose goal is to anticipate and prevent disease in the healthy and to precisely diagnose and treat disease in the ill.

### An unexpected diagnosis

On a long flight to Norway for a family vacation last year, Snyder noticed changes in his heart rate and blood oxygen levels. As one of the 60 participants in the digital health study, he was wearing seven biosensors. From previous trips, Snyder knew that his oxygen levels normally dropped during airplane flights and that his heart rate increased at the beginning of a flight — as occurred in other participants. But the values typically returned to normal over the course of a long flight and after landing. This time, his numbers didn't return to baseline. Something was up, and Snyder wasn't completely surprised when he went on to develop a fever and other signs of illness.

Two weeks earlier, he'd been helping his brother build a fence in rural Massachusetts, so his biggest concern was that he might have been bitten by a tick and infected with Lyme disease. In Norway, Snyder persuaded a doctor to give him a prescription for doxycycline, an antibiotic known to combat Lyme disease. Subsequent tests confirmed that Snyder had indeed been infected with the Lyme microorganism.

Snyder was impressed that the wearable biosensors picked up the infection before he even knew he was sick. "Wearables helped make the initial diagnosis," he said. Subsequent data analysis confirmed his suspicion that the deviations from normal heart rate and oxygen levels on the flight to Norway had indeed been quite abnormal.

"The fact that you can pick up infections by monitoring before they happen is very provocative," said Topol.

### More discoveries

For Snyder, the Lyme diagnosis is just the tip of the iceberg — part of very early work to begin querying massive data sets of health information. The results of the current study raise the possibility of identifying inflammatory disease in individuals who may not even know they are getting sick. For example, in several participants, higher-than-normal readings for heart rate and skin temperature correlated with increased levels of C-reactive protein in blood tests. C-reactive protein is an immune system marker for inflammation and often indicative of infection, autoimmune diseases, developing cardiovascular disease or even cancer. Snyder's own data revealed four separate bouts of illness and inflammation, including the Lyme disease infection and another that he was unaware of until he saw his sensor data and an increased level of C-reactive protein.

The wearable devices could also help distinguish participants with insulin resistance, a precursor for Type 2 diabetes. Of 20 participants who received glucose tests, 12 were insulin resistant. The team designed and tested an algorithm combining participants' daily steps, daytime heart rate and the difference between daytime and nighttime heart rate. The algorithm was able to process the data from just these few simple measures to predict which individuals in the study were likely to be insulin-resistant.

The study also revealed that declines in blood-oxygen levels during airplane flights were correlated with fatigue. Fortunately, the study showed that people tend to adapt on long flights; oxygen levels in their blood go back up, and they generally feel less fatigued as the hours go by.

"The desaturation of oxygen in flight was not something I anticipated," said Topol. "Whenever you walk up and down the aisle of a plane, everyone is sleeping, and I guess there may be another reason for that besides that they partied too hard the night before. That was really interesting, and I thought it was great that the authors did that."

Topol noted that one of the biosensors used in the study doesn't work very well and that another has been recalled. "A few are not going to hold up," he said. "Either they are not going to be available or they are going to be proven to not be very accurate. But what is good about what the authors did here is that they weren't just relying on one device. They did everything they could with the kind of sensors that are available today to get data that was meaningful."

STEVE FISCH

Wearing several biosensors, Michael Snyder noticed changes in his heart rate and oxygen levels during a flight last year. When he later developed a fever, he suspected he had been infected with Lyme disease. Subsequent tests confirmed his suspicion.

### The future of wearable devices

During a visit to the doctor, patients normally have their blood pressure and body temperature measured, but such data is typically collected only every year or two and often ignored unless the results are outside of normal range for entire populations. But biomedical researchers envisage a future in which human health is monitored continuously.

"We have more sensors on our cars than we have on human beings," said Snyder. In the future, he said, he expects the situation will be reversed and people will have more sensors than cars do. Already, consumers have purchased millions of wearable devices, including more than 50 million smart watches and 20 million other fitness monitors. Most monitors are used to track activity, but they could easily be adjusted to more directly track health measures, Snyder said.

With a precision health approach, every person could know his or her normal baseline for dozens of measures. Automatic data analysis could spot patterns of outlier data points and flag the onset of ill health, providing an opportunity for intervention, prevention or cure.

Other Stanford-affiliated co-authors of the study are researcher Gao Zhou; postdoctoral scholars Wenyu Zhou, PhD, and Sophia Miryam Schüssler-Fiorenza Rose, MD, PhD; research dietician Dalia Perelman; undergraduate summer intern Ryan Runge; genetic counselor Shannon Rego; high school student Ria Sonecha; Somalee Datta, PhD, director of the Genetics Bioinformatics Service Center; and Tracey McLaughlin, MD, associate professor of medicine.

Researcher Elizabeth Colbert, of the Veterans Affairs Palo Alto Health Care System, is also a co-author.

This research was funded by the National Institutes of Health, the National Institute of Diabetes and Digestive and Kidney Diseases and a gift from Bert and Candace Forbes.

Stanford's Department of Genetics also supported the work. **ISM**

# Technique reveals movements of immune cells in humans

**By Jennie Dusheck**

A study led by researchers at the School of Medicine has for the first time demonstrated a way to visualize and monitor the behavior of immune cells used to treat cancer patients.

The new technique allows researchers to see where immunotherapy cells go as they hunt down tumors in the human body. The imaging technique also reveals whether the immune cells, called T cells, have found a tumor; how many T cells have arrived at the tumor; and whether the T cells are alive.

The ability to see whether T cells are attacking tumors is useful both for clinicians trying to learn if a treatment is working in an individual cancer patient and also for researchers trying to understand why immunotherapy doesn't always work.

A paper describing the work was published online Jan. 18 in *Science Translational Medicine*. The senior author is Sanjiv "Sam" Gambhir, MD, PhD, professor and chair of radiology at Stanford. Lead authorship of the study is shared by former Stanford postdoctoral scholars Khun Keu, MD; Timothy Witney, PhD; and Shahriar Yaghoubi, PhD.

"We can now watch anywhere in your body where those T cells may be," said Gambhir, who holds the Virginia and D.K. Ludwig Professorship in Cancer Research. "This is the first demonstration in humans of actually noninvasively imaging the immune system in action with reporter gene technology. It's never been done before in a living human, and without the need to remove any tissue."

The work was done in patients with a type of deadly brain cancer called glioblastoma, but the groundbreaking technique could be used to track immune cells targeting any kind of cancer, Gambhir said.

### The limitations of immunotherapy

In one form of standard immunotherapy, a medical team harvests T cells from a cancer patient's blood and genetically engineers them to do a better job of hunting down and killing the patient's cancer cells. Such immunotherapy some-

**See IMMUNOTHERAPY, page 5**

# Digital health center aims to connect faculty with technology companies

**By Tracie White**

The School of Medicine has launched a center to support collaborations between Stanford faculty and Silicon Valley technology companies to develop, test and implement new digital health tools.

The Center for Digital Health aims to advance the field of digital health by promoting these partnerships, performing clinical research and educating the next generation of physicians and digital health care leaders.

"Digital health is a space where Stanford should be leading the way," said Sumbul Desai, MD, clinical associate professor of medicine and executive director of the center. "The new center will be focused on leveraging our resources and encouraging collaborations that will lead to better health care through digital technology."

Lloyd Minor, MD, dean of the School of Medicine, said the center will further advance Stanford's mission to improve patient care through precision health. "With our biomedical expertise and location in Silicon Valley, Stanford Medicine is uniquely positioned to be a leader in the field of digital health," Minor said. "The new Center for Digital Health fits well within the framework of the biomedical revolution in precision health at Stanford by using the most advanced digital technologies and tools to develop care that is tailored to individual patients."

### Connecting and enabling faculty

"The goal of the center is to encourage collaborations that will help create the next generation of health care," said Lauren Cheung, MD, MBA, clinical assistant professor of medicine and senior director of strategy and operations for the center.

The center grew out of a need to provide support and guidance to faculty who were repeatedly being contacted by both startups and established technology companies with offers to collaborate, Desai said.

Lauren Cheung

Sumbul Desai

Mintu Turakhia

"We wanted to leverage that interest and generate more opportunities for the faculty by providing the infrastructure and resources needed to encourage these relationships," Desai said. "We can help connect interested faculty with industry, or vice versa. Say, for example, there's a faculty member interested in pulmonary digital health research. We may know a company with the same interest. We can help connect them."

### Doing research quickly, inexpensively

"There are hundreds upon hundreds of digital health startups now, and it is very difficult for patients, doctors, hospitals, insurers, regulators and investors to know which solutions will work and which will stick," said Mintu Turakhia, MD, assistant professor of cardiovascular medicine and senior director of research and innovation at the center. "High-quality evidence is needed to make informed decisions. We generate this evidence quickly and cheaply, targeting the real-world outcomes that matter for all of these stakeholders."

Turakhia leads efforts to advance research in digital health at Stanford that ranges from technology assessments and implementation studies to multicenter trials. He is the principal investigator for five digital health trials. The largest of these is a 25-site, 400-patient randomized trial to test digital interventions combined with health coaches to determine whether they improve medication adherence for people with atrial fibrillation, which affects 4 million U.S. adults.

"Even after generating rigorous evidence, there can be a long, complicated path to implementation with many unanswered questions," Turakhia said. Such questions include: What is the best way to incorporate new digital tools into the practice of health care? Will new advances actually improve patient care? Are they are worth the costs?

"Currently there is very little evidence to support how best to incorporate digital tools into practice," Cheung said. "Stanford faculty have the expertise to help with the design and implementation of new digital health tools."

### Training and education

The center will provide training to physicians in digital health medicine at Stanford through fellowships, internship opportunities, conferences and traditional classroom material, Desai said. In addition, the center will offer educational programs to industry members.

The center is also accepting proposals for health care research projects focused on innovative uses for Apple Watches. In addition to providing up to 1,000 of the watches, the center will award $10,000 to the winning project for one year, starting in April.

Learn more about the center at http://med.stanford.edu/cdh.html. **ISM**

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# Immunotherapy

**continued from page 4**

times works, but most often does not. But it's hard for clinicians to tell when it's not working, and challenging to know why it's not.

"That's the problem," Gambhir said. "How do you know whether the T cells are doing their job or not? There's no way to tell."

Sanjiv Gambhir

Right now, the only way to find out if the T cells are attacking the cancer is to wait to see if the tumors shrink, but that can take months. And even when a treatment is working, some tumors may appear to get bigger for a while — a result of inflammation. So a temporarily enlarged tumor doesn't necessarily mean treatment has failed. Even if clinicians are sure treatment has failed, they don't know why. Did the T cells not reach the tumor? Or did the T cells get to the tumor but fail in their attack?

"We are shooting blind," said Gambhir, who is also the director of the Canary Center at Stanford for Cancer Early Detection. "There are no real tools to see if treatment is working."

It can take a medical team several months to determine if immunotherapy is working. If it hasn't, the cancer may have spread or become more resistant in the meantime, greatly increasing the likelihood the patient will die.

### Releasing the hounds

Ten years ago, Gambhir and his lab began looking for ways to find out what the immune cells do once they are released back into the patient's bloodstream to hunt down cancer cells.

The researchers first engineered T cells to better recognize the patient's cancer cells. Later, they added a "reporter gene" to the T cells. This gene made a protein they could see with a positron emission tomography scan.

The tagged T cells are a little like bloodhounds that bay loudly as they chase down their prey. The baying tells the dogs' human handlers where both the bloodhounds and the prey are. In the same way, the researchers could tell when T cells were near their prey — a tumor — because they could see the protein products of the reporter genes clustering there.

PET scans showing the T cells' locations tell researchers how many T cells have reached a tumor — whether it's 6 million cells or 50 million — and whether the cells are alive.

"And you can come back and redo the imaging after a few days, weeks or months," said Gambhir. Repetition of the scan provides a timeline of T cell behavior.

One thing the new technique cannot do is tell researchers whether the T cells are actually attached to tumor cells. But that's coming, Gambhir said.

"Right now, the reporter gene is always on," he said. "But we could change the reporter gene so it only comes on after it latches onto the tumor cell and kills it." That approach works in mice, but isn't quite ready for human trials, he said.

### Surprises

The new T cell imaging technology can also reveal, indirectly, where other unsuspected tumors are. "In one patient," said Gambhir, "the T cells went to the tumor in the brain, as expected. But some of the T cells wandered away to another area of the brain." Even though the second tumor had been invisible to standard imaging, the "bright" T cells in the PET scan revealed its presence.

But the biggest surprise, said Gambhir, is that the technique worked at all. "Some people are going to say, 'This is not possible; how did they get this to work?'"

Part of it is chance and part of it is a lot of his team's prior research efforts, he said, estimating that his lab has produced 50 papers over the last 15 years in the quest to make the T cell imaging technology work, first in animals, and, now, finally, in humans.

Glioblastoma is a particularly intractable cancer for which immunotherapies have a long way to go, said Gambhir. In all of the cases in the study, the team was able to visualize the T cells. "But in every single case, the patient still died," Gambhir said. "So the question is, what is going wrong? Is it that the T cells just are not surviving long at the tumor site? Is the tumor too aggressive? Do the T cells kill some of the tumor cells but the rest go on?"

**"Some people are going to say, 'This is not possible; how did they get this to work?'"**

For glioblastoma patients, said Gambhir, the new technique will allow researchers to see immunotherapies in action and thus be better able to understand, and hopefully fix, things that go wrong with them.

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

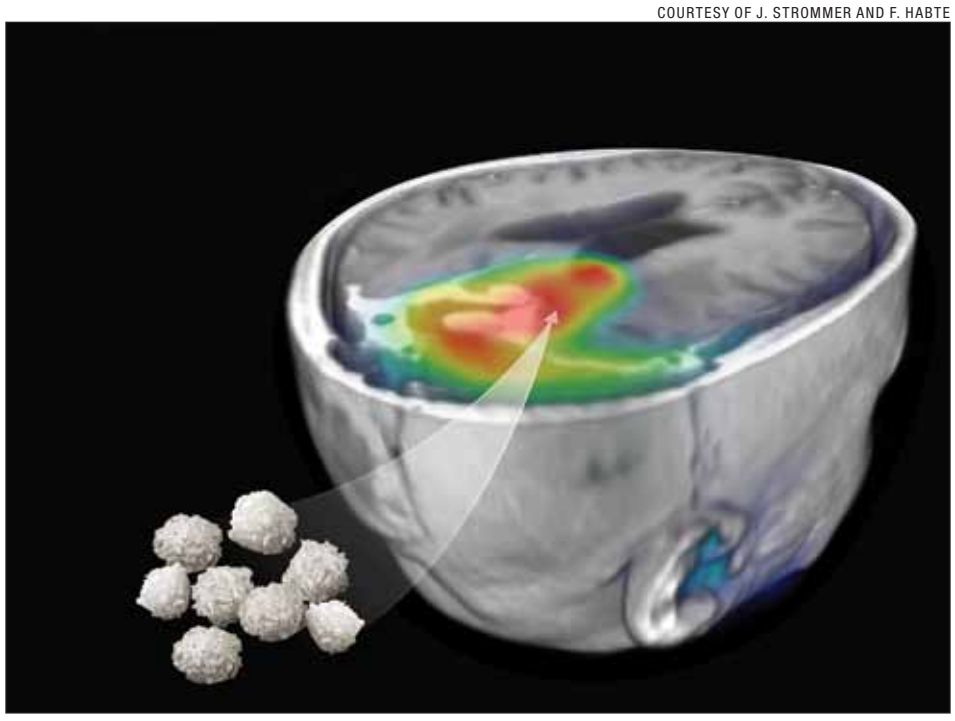
Other Stanford co-authors of the paper are statistician Jarrett Rosenberg, PhD, and the Canary Center's director of preclinical imaging core Frezghi Habte, PhD.

Researchers from the Hôpital de la Cité-de-la-Santé de Laval in Canada; University College in London; CellSight Technologies; City of Hope; University of California-Los Angeles; Sangamo BioSciences Inc.; and Seattle Children's Research Institute also co-authored the study.

The study was funded by the National Cancer Institute, the Ben & Catherine Ivy Foundation, Sangamo Biosciences, the Wellcome Trust and the Royal Society.

Stanford's Department of Radiology also supported the work. **ISM**

COURTESY OF J. STROMMER AND F. HABTE



A PET scan image of the brain of a glioblastoma cancer patient shows the journey of T cells that had been engineered to attack the patient's tumor. Researchers used a technique that enabled them to track the location, numbers and viability of the introduced T cells.

# Bodywide immune response important for fighting cancer

**By Krista Conger**

Fighting off cancer requires the concerted efforts of immune molecules throughout the body, rather than just in the tumor itself, according to a new study of laboratory mice by researchers at the School of Medicine.

The finding helps settle an ongoing dispute among clinicians as to whether systemic, or whole-body responses, are as important as a robust response by immune cells in the tumor itself. The study may help clinicians understand why some people with cancer respond favorably to cancer immunotherapy, while others experience little or no benefit. It also suggests ways that the effectiveness of ongoing therapies could be quickly and easily monitored.

> "Physicians could learn quickly whether a therapy is working."

"Immunotherapy can be remarkably effective against cancer, but we don't know why some patients respond and some don't," said Edgar Engleman, MD, professor of pathology and of medicine. "We don't understand the parameters that determine efficacy. In this study, we analyzed millions of living cells simultaneously for 40 parameters from multiple tissues throughout the body to show that you need a systemwide immune response to effectively attack and eradicate a tumor."

Engleman is the senior author of the study, which was published online Jan. 19 in *Cell*. The lead authors are Matthew Spitzer, PhD, a former Stanford graduate student who is now a postdoctoral scholar at UC-San Francisco; former Stanford postdoctoral scholar Yaron Carmi, PhD, who is now an assistant professor at Tel Aviv University; and Stanford postdoctoral scholar Nathan Reticker-Flynn, PhD.

### Two-pronged approach

The researchers compared the immune responses of a special group of laboratory mice engineered to spontaneously develop triple-negative breast cancers. These cancers are resistant to a type of immunotherapy known as checkpoint blockade. Recently, however, Engleman and his colleagues showed that they could stimulate a successful immune response and eradicate tumors in the animals with a two-pronged approach that incorporated both a tumor-binding antibody and molecules that activated a type of immune cell called a dendritic cell.

"This finding allowed us to directly compare the responses to two immunotherapies," said Engleman. "What's going on in an effective response that's not happening in the ineffective response? What we found was quite revealing and gratifying."

Spitzer, Carmi and their colleagues collaborated with co-author Garry Nolan, PhD, a professor of microbiology and immunology at Stanford who has developed a way to use a technique known as mass cytometry to monitor the physical attributes of individual cells in samples of millions or billions. This allows researchers to piece together a dynamic picture of how multiple cell populations respond in real time to changing conditions like disease or drug therapies.

Spitzer and his colleagues used the technology to monitor the rise and fall of various populations of immune cells within the tumor as well as in other tissues — including the lymph nodes, spleen, bone marrow and peripheral blood — throughout the body immediately after immunotherapy and throughout tumor rejection.

The researchers found that in animals treated with the effective, two-pronged approach, the prevalence of immune cells — including macrophages, dendritic cells and T cells — in the tumor itself increased dramatically within three days of treatment, during a period known as "priming." These cells also divided more rapidly. In contrast, the tumors of the animals receiving the ineffective therapy, checkpoint blockade, displayed no such increase in prevalence or proliferation.

### Increase in regulatory T cells

Importantly, the researchers also observed an increase in a class of T cells called regulatory T cells in the effectively treated animals during priming. The presence of these cells during tumor rejection was surprising because they have in the past been correlated with a negative prognosis for many tumors.

"Our observation of an increase in the prevalence of these cells in successfully treated tumors runs counter to conventional wisdom and points out the complex nature of immune responses that lead to successful immunotherapy," said Engleman.

STEVE FISCH

Edgar Engleman is the senior author of a study that found a systemwide immune response is needed to effectively attack a tumor.

Tumor rejection in the effectively treated animals began by day eight. However, in contrast to the priming phase, the researchers observed no differences in the rate of immune cell proliferation in tumors between the two groups of animals during this time. In fact, immune cell proliferation in the tumor ceased altogether by the rejection phase. This finding suggests that, although the initial immune response occurred primarily in the tumor, immune responses in other parts of the body are likely responsible for sustaining the immune attack.

Spitzer and his colleagues observed increases in the number and activity of immune cells in lymph nodes near the tumor during both the priming and rejection phases in the effectively treated animals. Surprisingly, the same types of immune cell increases were seen during the priming and rejection phases in the spleen as well as in lymph nodes that were located a great distance from the tumor. Moreover, the researchers were able to document similar stage-specific changes in the activity and prevalence of immune cells in the peripheral blood.

In particular, a marked increase in a type of memory CD4 T cell was seen in the blood, as well as in peripheral lymphoid organs, during the rejection phase in mice that received effective therapy. This finding is important because the rise in these CD4 T cells may prove useful as an indicator of treatment efficacy in cancer patients who receive different forms of immunotherapy, thus allowing researchers and clinicians to develop a way to accurately monitor the effectiveness of

**See IMMUNE, page 8**

## Inflammation

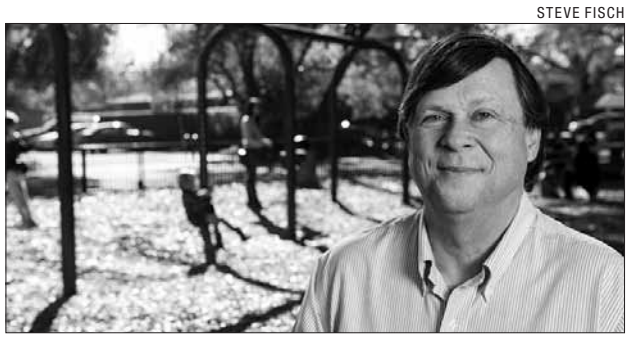
**continued from page 1**

also a Howard Hughes Medical Institute investigator.

### Caffeine link

"Our findings show that an underlying inflammatory process, which is associated with aging, is not only driving cardiovascular disease but is, in turn, driven by molecular events that we may be able to target and combat," said Davis, who holds the Burt and Marion Avery Family Professorship.

STEVE FISCH



Mark Davis is a senior author of a study that found a link between chronic inflammation and the chronic diseases that accompany aging.

Notably, this inflammatory mechanism was found to be activated only in some, but not all, of the older study participants. Those in whom it was relatively quiescent tended to drink more caffeinated beverages. Laboratory experiments revealed that the mechanism was directly countered by caffeine and associated compounds.

The investigators made this discovery using data gathered from the Stanford-Ellison cohort, a long-term program begun 10 years ago by Davis and study co-author Cornelia Dekker, MD, professor of pediatric infectious diseases, to study the immunology of aging. In that program, healthy participants ages 20-30 and another group older than 60 were monitored annually via surveys, blood draws and reviews of their medical histories.

For the new study, the researchers compared blood drawn from older versus younger study participants to see which genes tended to be more highly activated in older people. They zeroed in on two clusters of genes whose activity was associated with the production of a potent circulating inflammatory protein called IL-1-beta. The genes within each cluster appeared to work in coordination with one another.

The researchers also looked at two particular groups of older participants: One with high activation of one or both inflammatory gene clusters, and the other with one or both clusters exhibiting low activation. On reviewing these individuals' medical histories, the scientists learned that nine of the 12 subjects with high cluster activity had high blood pressure, compared with only one of the 11 subjects with low cluster activity. Follow-up studies by study co-author Francois Haddad, MD, a clinical associate professor of cardiovascular medicine, revealed that individuals in the "high" group were much more likely to have stiff arteries — a risk factor for cardiovascular complications — than those in the "low" group.

Furthermore, those in the low group were eight times as likely as those in the high group to report having at least one close family member who had lived to age 90 or older. Not only that, but participants in the high group who were older than 85 in 2008 were substantially more likely to have died by 2016 than were those in the low group. The high group's blood also showed signs of increased activity of free radicals, which can harm cells, compared with the low group's blood. The high group also had elevated concentrations of IL-1-beta, as well as of several nucleic-acid breakdown products that can be produced by free-radical action.

The researchers found that incubating a type of immune cell with two of those nucleic-acid metabolites boosted activity in one of the gene clusters, resulting in increased IL-1-beta production. When injected into mice, the substances triggered massive systemic inflammation, along with high blood pressure. In addition, immune cells infiltrated and clogged the animals' kidneys, increasing renal pressure substantially.

### How caffeine may affect longevity

Intrigued by the correlation between older participants' health, gene-cluster activation and self-reported rates of caffeine consumption, the researchers followed up and verified that blood from the group with low cluster activity was enriched for caffeine and a number of its metabolites, compared with blood from the group with high cluster activity. (Examples of these metabolites are theophylline, also found in tea, and theobromine, which abounds in chocolate.)

Incubating immune cells with caffeine and its breakdown products along with the inflammation-triggering nucleic acid metabolites substantially prevented the latter from exerting their powerful inflammatory effect on the cells.

"That something many people drink — and actually like to drink — might have a direct benefit came as a surprise to us," said Davis, who noted that the study did not prove a causal link. "We didn't give some of the mice coffee and the others decaf. What we've shown is a correlation between caffeine consumption and longevity. And we've shown more rigorously, in laboratory tests, a very plausible mechanism for why this might be so."

Other Stanford co-authors are postdoctoral scholars Junlei Chang, PhD, Christopher Bohlen, PhD, and Gabriela Fragiadakis, PhD; former graduate student Matthew Spitzer, PhD; life science research associate Edward Ganio; assistant professor of anesthesia, perioperative and pain medicine Brice Gaudilliere, MD, PhD; professor of microbiology and immunology Garry Nolan, PhD; and professor of hematology Calvin Kuo, MD, PhD.

Researchers from the Sidra Medical and Research Center in Qatar, the French National Institute of Health and Medical Research and the University of North Carolina also co-authored the study.

The study was funded by the National Institute of Allergy and Infectious Diseases and the Ellison Medical Foundation.

Stanford's Department of Microbiology and Immunology also supported the work. **ISM**

# Opioids

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"If you're coming back from the battlefield in your 20s, what does your future look like if you are taking these drugs over the next 50 or so years of life?" he added.

### Knocking out morphine receptors

At a molecular level, opioids work by attaching to specific protein-binding sites on neurons throughout the body. These sites are called mu opioid receptors. The binding of morphine and similar painkillers, including oxycodone, fentanyl and hydrocodone, obstruct the neurons' pain signals so that they don't reach brain regions for pain perception. However, mu opioid receptors are present on many types of neurons in the nerves, spinal cord and brain. Which of these populations of receptors is specifically responsible for side effects has been unclear, preventing the development of therapeutic strategies to separate side effects from pain relief.

Based upon previous research that indicated peripheral pain neurons, known as nociceptors, may be responsible for certain unwanted side effects from opioids, the researchers hypothesized that if they could block mu opioid receptors on peripheral pain neurons specifically, they could eliminate the bad effects while keeping the good effects of the drugs.

To test their hypothesis, the researchers injected morphine into both normal mice and a group of mice in which the mu opioid receptors had been knocked out in peripheral pain neurons.

"What we did was to remove morphine receptors from only one type of neuron — the pain neurons in the periphery — to test the function of the mu opioid receptors in the periphery. We wanted to determine precisely what morphine is doing on the periphery," Scherrer said. "Then we did a number of pain tests in the mice to measure the efficacy of morphine."

Researchers found that the acute pain relief from the drugs remained the same, but that when injected chronically it actually lasted much longer in the mice lacking the mu opioid receptor compared with the normal mice. The researchers concluded that the action of morphine on nociceptors was causing the growing tolerance to the drug in the normal mice.

They also concluded that the pain-relieving properties generated by opioids must occur primarily in the brain because the drugs worked without acting on the nociceptors in the altered mice.

### Existing drug

Next, researchers showed that by using the opioid receptor antagonist drug methylnaltrexone bromide, which block mu opioid receptors in the periphery, they were able to prevent the tolerance and increased sensitivity to pain caused by opioid use while keeping the pain relief.

> "The trick was to use a compound that blocks pain receptors only in the periphery."

"The trick was to use a compound that blocks pain receptors only in the periphery," Scherrer said. "Methylnaltrexone bromide is an already known compound that doesn't cross the blood brain barrier and stays on the periphery."

Results showed that with co-administration of methylnaltrexone bromide and morphine to mice, no significant difference in pain relief occurred, but the two side effects — tolerance and increased pain sensitivity — were almost completely lost, the study said.

There's an urgent need to test the findings of this mouse study in human trials, Scherrer said.

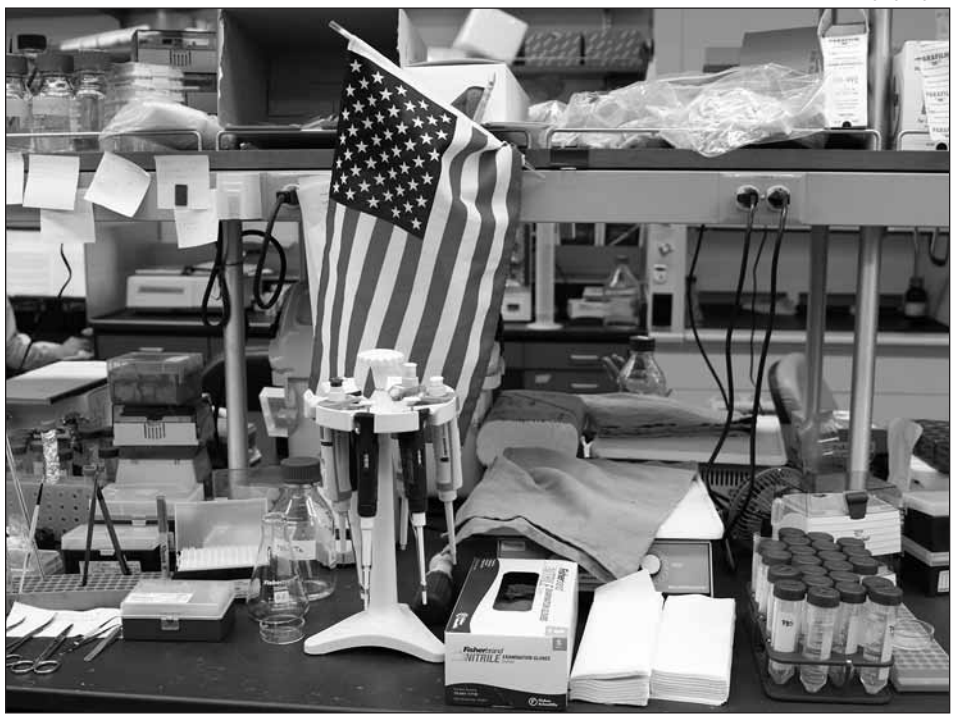
"This is the same drug which is used for reducing the side of effect of constipation caused by opioid painkillers," Scherrer said. "It's a safe drug. There is great potential for translating this to the clinic."

The future ramifications for possibly reducing the opioid dose needed to maintain pain relief go beyond the clinic, Clark said.

"The issues of drug abuse and exposure which can lead to heroin abuse are huge problems," Clark said. "By limiting the dosages of the drugs given to the patient, we also reduce the amount of these drugs leaking out into the community."

The team's work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

PAUL SAKUMA



The Scherrer lab investigates the mechanisms by which opioids work at the molecular level in order to develop more efficient and safer treatments for managing chronic pain.

The other co-authors, all from Stanford, are Sara Low, visiting student researcher; Jasmine Dickinson, graduate student; Chaudy Sotoudeh, lab manager and research assistant; Ben Barres, MD, PhD, professor of neurobiology, of developmental biology and of neurology and neurological sciences; and postdoctoral scholar Christopher Bohlen.

The research was supported by the National Institutes of Health; a Rita Allen Foundation and American Pain Society Award in Pain; the Foundation for Anesthesia Education and Research; the Department of Defense; the National Science Foundation; Stanford Bio-X; and the Damon Runyon Cancer Research Foundation.

Stanford's Department of Anesthesiology and Perioperative and Pain Medicine and the Stanford Neurosciences Institute also supported the work. ISM

# Nagata

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dent at Stanford, was published online Jan. 6 in *Global Health Promotion*.

Nagata said the challenges became painfully apparent when he went to Kenya in 2010 to do research on nutritional support and food insecurity among HIV-positive individuals there. He was based in Mfangano Island, a rural fishing and agricultural community in Lake Victoria, where HIV prevalence is high among the highly mobile population.

At the time, the Kenyan government was considering a constitutional amendment to provide protections for LGBT individuals, who under Kenyan law may be punished with up to 14 years in prison. The proposed amendment was the subject of much heated public debate and was fiercely opposed by the pastor at the church that Nagata and his host family attended, he said.

### 'A challenge I have grappled with'

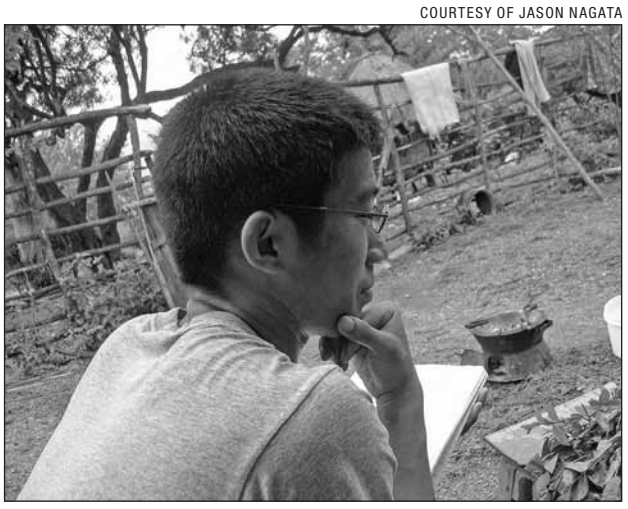
A few years earlier, Nagata had told his parents, both ordained clergy, that he was gay, and they had been supportive, he said. But he could not imagine how his host family or his Kenyan colleagues might react to this news, given the very different political climate for the LGBT community in the East African country.

"It's a challenge I have grappled with a lot," said Nagata, 30, who is now a fellow in adolescent medicine at UC-San Francisco. "I wondered to what extent it would be safe or practical to come out to colleagues whom I would be working with internationally. Because coming out is a very big deal. I was still hiding a big part of myself from others, like my host family."

Michele Barry, MD, professor of medicine and director of Stanford's Center for Innovation in Global Health, said she advises medical residents working abroad to be cautious on issues of sexual identity. The center sponsors the Johnson & Johnson Global Health Scholars program, which sends residents abroad for rotations of up to six weeks.

"I talk to the residents about really being very circumspect and suggesting that they not share their sexual orientation," she said. "I think it can be very charged

COURTESY OF JASON NAGATA



In a recently published commentary, Nagata writes about the challenges of being gay and doing global health work in countries where homosexuality is a crime.

information. I give residents an opportunity to opt out of the program if they don't feel comfortable in not being open about their sexuality."

### Value vs. risk of openness

Barry said she views the issue in the context of respecting local culture.

"It's like dressing culturally appropriately, even if you don't agree with it. It's very akin to women who go into a culture where they have to wear a hijab," she said. "They may not agree with it, but they are guests in the country, so I ask them to respect that others wear a hijab and not argue the fact. We have to be respectful of the culture."

In his commentary, Nagata weighs the value of being open about one's sexual minority status with the possible risks and dangers of exposure. He said U.S. clinician-scientists may change minds and open up the conversation on the issues by revealing their status to people who might not otherwise interact with an LGBT individual.

"People's opinions of LGBT rights and same-sex marriage might be changed if they experience solidarity with a LGBT family member or friend," he wrote.

U.S. scientists also may have the opportunity to advocate for change in their host countries by working with local activists who are experienced and understand the risks, he said. For instance, they could seize the opportunity to call attention to the health issues prevalent in the LGBT community and the difficulties these individuals face in finding doctors willing to treat them and able to provide appropriate care. Because of stigma and discrimination, LGBT individuals are more prone to anxiety, depression and suicide and suffer from higher rates of HIV and sexually transmitted diseases, he said.

He said researchers from the United States who are open and speak out on the issues might be somewhat insulated from backlash because they come from a country where LGBT rights are protected.

On the other hand, he said, they have to be cognizant of the risks they may face, including physical violence. "Not everyone can be a martyr and put their lives in danger for a cause," he writes.

### 'It's a conundrum'

Barry agreed. "I completely empathize with the urge to show solidarity, but it's not the time to endanger yourself," she said. "It's a conundrum."

In his own case, Nagata said he agonized about how to approach his Kenyan host family, whom he plans to revisit to continue his work.

"Even as some of my friends and colleagues reviewed the article, they advised me not to come out under any circumstances to my host family," he said. "Though sentiments have changed in the United States in recent years, there are still very strong sentiments [against homosexuality] in Kenya. So while I think it would be an interesting viewpoint for them to hear, I could see them saying, 'You're not welcome anymore.'"

For the moment, he has decided to confine his advocacy to working from afar by writing about the issues.

"I think my advice overall is that there is no one right answer for how to deal with this," he said. "People have to balance the potential risk of adverse consequences with their own ideals for advocacy and promotion of universal human rights.

"While it would be great to fight for a cause you believe in, there are also practical consequences to think about," he said. ISM

# Leslee Subak appointed chair of obstetrics and gynecology

By Jennie Dusheck

Leslee Subak, MD, has been appointed the chair of the Department of Obstetrics and Gynecology at the School of Medicine, effective May 1.

Subak will succeed Jonathan Berek, MD, the Laurie Kraus Lacob Professor, who has served as chair of the department since 2005.

Subak earned her medical degree at Stanford in 1991 and went on to a distinguished career as a scientist, clinician and educator at the University of California-San Francisco, where she's now a professor of obstetrics, gynecology and reproductive science, of epidemiology and biostatistics, and of urology. She is an expert in urogynecology and pelvic surgery, and the principal investigator for federally funded research projects on the epidemiology of urinary incontinence in women, evaluating treatments for urinary incontinence.

"Leslee has made indispensable contributions to the fields of obstetrics and gynecology," said Lloyd Minor, MD, dean of the School of Medicine, in a statement announcing the appointment. "Her astute leadership of major clinical and translational research programs has led to fundamental discoveries in many areas of women's health, and her own scientific investigations have significantly advanced the treatment of women suffering from urinary incontinence."

Subak's research has focused on multidisciplinary approaches to treating incontinence in women, including cooperation between basic and clinical investigators researching the female bladder, pelvic floor and urethra; and among clinicians and experts in epidemiology and biostatistics. She is an expert on the economic impacts of incontinence and has shown that weight loss can be an effective treatment for the condition. Subak is also dedicated to training the next generation of leaders in women's health, running fellowship and junior faculty K-12 training programs.

Leslee Subak

### Excited about return to Stanford

"I look forward to helping each member of the department to thrive in her or his unique career path," she said in an email. "This, in turn, nurtures excellence in all we do. As a team, I am confident that we will advance each of our academic missions, become the preeminent health care provider for women in the greater Bay Area (and beyond), and have fun doing it!"

Subak said she's excited about returning to Stanford. She met her future wife, Linda McAllister, MD, PhD, when they were medical students at Stanford. And Subak said they and their three teenagers are "huge" fans of Stanford women's soccer and basketball.

"I am looking forward to being back at a university with the wealth of undergraduate and graduate programs and broad opportunities to teach and collaborate," she said. "Stanford's culture of collaboration — starting with Dean Minor and hospital leadership and extending to the department chairs, faculty and staff — is phenomenal and a perfect fit for my approach and philosophy. I love building and participating in collaborations — across departments, schools and continents and across clinical, training and research missions. Helping to continue and advance the shared vision of the outstanding Stanford Obstetrics and Gynecology Department will be an honor."

The search committee for the chair position was led by Ron Pearl, MD, professor and chair of anesthesiology, perioperative and pain medicine; and Maria Grazia Roncarolo, MD, professor of pediatrics and of medicine.

Minor offered special thanks to Berek, the outgoing chair. "During his tenure, the department witnessed tremendous achievements, including the creation and expansion of our Women's Cancer Center, growth of our various women's health and wellness programs and recruitment of top faculty."

Subak concurred: "I am so fortunate to take the helm from Dr. Jonathan Berek, an innovative, compassionate and steady steward. I will work tirelessly to fill his large shoes and continue the trajectory of growth and excellence." ISM

## OF NOTE

reports on significant honors and awards for faculty, staff and students

Kay Chang

Korey Hood

John Leppert

S.V. Mahadevan

Elizabeth Mormino

Heather Moss

Jochen Profit

Hua Tang

Wen-Kai Weng

**KAY CHANG**, MD, was promoted to professor of otolaryngology-head and neck surgery, effective Nov. 1. He is a pediatric otologist with research interests in the prevention of ototoxicity, neonatal hearing screening, the genetics of hearing loss and pediatric cochlear implants.

**KOREY HOOD**, PhD, was appointed professor of pediatrics and of psychiatry and behavioral sciences, effective Nov. 1. His research and clinical efforts aim to improve the health and quality of life of people with diabetes and other chronic diseases.

**JOHN LEPPERT**, MD, was promoted to associate professor of urology, effective Nov. 1. His research and clinical work focuses on kidney cancer surgery, kidney cancer detection and responses to cancer therapy.

**S.V. MAHADEVAN**, MD, was promoted to professor of emergency medicine, effective Dec. 1. He is the interim chair of emergency medicine. His research and clinical interests include emergency medicine education, trauma, emergency medical services and global health.

**ELIZABETH MORMINO**, PhD, was appointed assistant professor (research) of neurology and neurological sciences, effective Jan. 1. Her research interests include brain imaging with a focus on Alzheimer's disease and aging.

**HEATHER MOSS**, MD, PhD, was appointed assistant professor of ophthalmology, effective Nov. 1. Her research aims to identify and develop markers of impaired optic nerve structure and function that can guide management of idiopathic intracranial hypertension to prevent blindness. Her clinical focus is on neuro-ophthalmology, including the diagnosis and treatment of brain, nerve and muscle disorders that affect vision.

**JOCHEN PROFIT**, MD, was promoted to associate professor of pediatrics, effective Dec. 1. His research focuses on improving the design of health-care systems to improve outcomes for sick newborns.

**HUA TANG**, PhD, was promoted to professor of genetics, effective Dec. 1. In her research, she develops statistical and computational approaches to delineate the evolutionary history of the human population and to examine the genetic architecture of complex traits and diseases in minority populations.

**WEN-KAI WENG**, MD, PhD, was promoted to associate professor of medicine, effective Dec. 1. His clinical focus is blood and marrow transplantation, and his research focuses on lymphoma, including immunotherapy and efforts to understand its pathobiology. ISM

# Immune

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ongoing immunotherapies with a simple, noninvasive blood test. This possibility was supported when the researchers analyzed immune cells in the blood of patients with melanoma who had received immunotherapy. The results showed that a similar subset of CD4 T cells was associated with a positive response.

"The idea would be to use the rise of these CD4 T cells as a biomarker to tailor treatment to each individual," said Engleman. "Physicians could learn quickly whether a therapy is working, or if it should be abandoned in favor of a new approach."

### Validating importance of systemic response

Beyond identifying a potential biomarker of effective therapy, the researchers showed that when the CD4 T cells in successfully treated mice were injected into the tumors of untreated animals, the cells stopped the tumors from growing.

The importance of the systemic immune response was validated when the researchers gave the mice a compound that inhibited the ability of immune cells to migrate from secondary lymphoid organs, such as the lymph nodes and spleen, to the tumor site. This intervention allowed sustained tumor growth even in the face of a previously effective treatment.

"In the past, researchers focused on understanding in very minute detail what is happening at the molecular level in immune cells inside the tumor," said Engleman. "But we took an approach that allowed us to zoom out and look at the immune system as a whole. This enabled us to unveil how immune cells work together throughout the body to reject a tumor, and the approach promises to be widely useful in many clinical situations."

In addition to guiding cancer therapy, the researchers also believe the technique could be useful in tracking the changes that occur during an autoimmune disease flare, or to learn more about how the body marshals its forces to fight off an infection.

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Other Stanford co-authors are former life sciences research associate Deepthi Madhireddy; graduate students Maria Martins and Tyler Prestwood; former postdoctoral scholar Pier Gherardini, PhD; former research technician Jonathan Chabon; and assistant professor of pathology Sean Bendall, PhD.

The research was supported by the National Institutes of Health, the Gates Foundation and the Department of Defense.

Nolan has a personal financial interest in, and Bendall has been a paid consultant for, Fluidigm, which manufactures the mass cytometer used in the study. Engleman is a founder and board member of Bolt Biotherapeutics, which holds the license for one of the immunotherapies used in the study.

Stanford's departments of Medicine and of Pathology also supported the work. ISM