INSIDE STANFORD MEDICINE

Volume 11, No. 10 ■ May 20, 2019 ■ Published by the Office of Communication & Public Affairs

A pair of neurosurgeons turned to basic science in an effort to fight the most common childhood brain cancer.

Page 4

Pilot program uses data-driven, integrated team approach to predict, prevent disease

By Amy Jeter Hansen

A Stanford Medicine pilot program combining cutting-edge tools of biomedicine with a collaborative, team-based method, offers a new approach to personalized health care that captures the promise of Precision Health: to predict, prevent and treat disease based on the individual patient.

Through the Humanwide project, primary care teams at Stanford Medicine's Primary Care 2.0 clinic in Santa Clara, California, merged high-tech and high-touch interventions to provide a diverse group of 50 patients with care that treated the whole person based on his or her unique factors, from genetics to lifestyle. Over the course of a year, the program succeeded in identifying previously undiagnosed conditions and future health risks, setting patients on a path to avert serious medical problems, such as cancer and heart disease.

"Our vision of Precision Health is to predict, prevent and cure — precisely," said Lloyd Minor, MD, dean of the Stanford University School of Medicine. "With Humanwide, we have begun to realize that vision in a clinical setting. The information gathered in this pilot suggests approaches to primary care that may ultimately benefit thousands of people."

A paper published May 13 in *Annals of Family Medicine* outlines initial learnings from Humanwide. The authors are Megan Mahoney, MD, Stanford Medicine's chief of general primary care, and Steven Asch, MD, vice-chief of primary care and population health.

Mahoney, the lead investigator, said the Humanwide design shifts the focus of primary care to detecting disease earlier, strengthening the relationship between the patient and care team and deploying the latest health technology.

"With Humanwide, we're See HUMANWIDE, page 7

STEVE FISCH

Megan Mahoney examines Debbie Spaizman, who participated in the Humanwide pilot project, at a Stanford Health Care clinic.

Immune cells crucial to causing osteoarthritis, team of scientists finds

By Bruce Goldman

Scientists at the School of Medicine have definitively linked mast cells, a class of cells belonging to the immune system, to the development of osteoarthritis, one of the world's most common causes of pain and immobility.

In a study published online May 14 in *eLife*, the scientists demonstrated for the first time that banishing mast cells — or blocking signals from the most common stimulus activating them in real life, or disabling a cartilage-degrading enzyme they release when activated — all protected mice from developing osteoarthritis induced by an experimental procedure. The results were supported by findings See OSTEOARTHRITIS, page 6

TEFI/SHUTTERSTOCK.COM

Osteoarthritis, the most frequently occurring variety of arthritis, is characterized by cartilage breakdown and inflammation in joints.

Blocking protein curbs memory loss in mice

By Bruce Goldman

NORBERT VON DER GROEBEN

Tony Wyss-Coray is the senior author of a study that found older mice performed better on memory tests when a protein on blood-vessel walls in their brains was disabled.

Mice aren't people, but like us they become forgetful in old age. In a study published online May 13 in *Nature Medicine*, old mice suffered far fewer senior moments during a battery of memory tests when School of Medicine investigators disabled a single molecule dotting the mice's cerebral blood vessels. For example, they breezed through a maze with an ease characteristic of young adult mice.

The molecule appears on the surfaces of a small percentage of endothelial cells, the main building blocks of blood vessels throughout the body. Blocking this molecule's capacity to do its main job — it selectively latches onto immune cells circulating in the bloodstream — not only improved old mice's cognitive performance but countered two physiological hallmarks of the aging brain: It restored to a more youthful level the ability of the old mice's brains to create new nerve cells, and it subdued the inflammatory mood of the brain's resident immune cells, called microglia.

Scientists have shown that old mice's blood is bad for young mice's brains. There's a strong suspicion in the scientific community that something in older people's blood similarly induces declines in brain physiology and cognitive skills. Just what that something is remains to be revealed. But, the new study suggests, there might be a practical way to block its path where the rubber meets the road: at the blood-brain barrier, which tightly regulates the passage of most cells and substances through the walls of blood vessels that pervade the human brain.

"We may have found an important mechanism through which the blood communicates deleterious signals to the brain," said the study's senior author, Tony Wyss-Coray, PhD, professor of neurology and neurological sciences, co-director of the Stanford Alzheimer's Disease Research Center and a senior research career scientist at the Veterans Affairs Palo Alto Health Care System. The lead author of the study is Hanadie Yousef, PhD, a former postdoctoral scholar in the Wyss-Coray lab.

The intervention's success points to possible treatments that could someday slow, stop or perhaps even reverse that decline. Targeting a protein on blood-vessel walls may be easier than trying See MEMORY, page 6

5 QUESTIONS

an occasional feature in which an expert answers five questions on a science or policy topic

Shaili Jain on misconceptions about PTSD

Shaili Jain, MD, clinical associate professor of psychiatry and behavioral sciences at the School of Medicine, has cared for thousands of trauma survivors over nearly two decades. A specialist in treating post-traumatic stress disorder, Jain has been part of an explosion in knowledge about the condition's biology and possible treatments.

So it frustrates her when she encounters ignorance about PTSD, especially because she believes it stands in the way of people getting therapy for it. Though it's widely considered incurable, PTSD is treatable and, in some cases, can be prevented, said Jain, who is also medical director for integrated care at the Veterans Affairs Palo Alto Health Care System. Often, people with the disorder are unaware they have it, as are their friends and family, she said. PTSD is widely known for causing nightmares, flashbacks and extreme startle reactions, but the disorder has other, more subtle, symptoms: It mutes feelings of happiness, often leads sufferers to social withdrawal and can result in damaging cellular changes that pass from parent to child.

Spurred to clear up misconceptions about PTSD, Jain has written her first book, The Unspeakable Mind: Stories of Trauma and Healing From the Frontlines of PTSD Science. *It's a portrait of the condition — its history, biology, treatment and repercussions for society — written to be accessible to the general public.*

Science writer Rosanne Spector spoke with Jain about PTSD, how she came to write about it and why it matters. She also discussed the topic in a 1:2:1 podcast, which can be heard at https://soundcloud.com/stanfordmed/the-unspeakable-mind-a-conversation-with-stanford-psychiatrist-shaili-jain-2019.

1 Why did you write a book about PTSD for the general public?

JAIN: In my mind, it was just begging for explanation. If you look at the statistics, it's such a pressing public health concern. At any given moment more than 6 million people in the United States are suffering from PTSD.

PTSD can result after many traumas. Rape, family violence, being robbed at gunpoint, escaping a major fire or car accident and being a refugee are just some examples. Higher rates of PTSD are found in military personnel, but they are also found in police officers, firefighters and low-income women and teenagers who live in high-crime, inner-city areas. The term PTSD has become part of our modern vernacular, especially since 9/11, but as a PTSD specialist, I see that the condition is often misunderstood. Meanwhile, there has been a huge growth in the science of PTSD over the past 20 years. I wanted to share all of this with the general reader so that we could elevate our understanding of it and the way we talk about it.

Shali Jain

2 With so much research on the condition, what has been learned about treating people with PTSD?

JAIN: We've made really good progress in understanding the talk therapies that work for PTSD. One key ingredient is exposure. People with PTSD avoid talking about the trauma, but PTSD thrives when they don't speak out — because then the trauma takes on a life of its own in their minds. Part of undoing that is for them to talk about the trauma — talk about it again and again and again. It sounds daunting, but under the care of a skilled mental health clinician it can be done.

Let's take an example of somebody who survived an explosion in a busy marketplace. Part of the reason they don't want to talk about the explosion is the minute their mind starts to go there, they can feel their heart racing. They can feel their blood pressure going up. They can feel the panic setting in. They start to relive that day.

The idea behind the therapy is that by careful telling and retelling of that event, or through exercises, such as exposing yourself to similar situations, like a busy marketplace, you're habituating your body to that physiological response. Over time, it goes down.

Medications also help many people with PTSD. The best evidence that we have is for SSRIs or SNRIs. They're commonly known as antidepressants, but they are also effective for PTSD. About 60 percent of sufferers will have a good response, so for now medications alone are not the answer for everyone.

3 How did PTSD come to be recognized by the psychiatry establishment and why do you think it took so long?

JAIN: PTSD has been documented since ancient times, but it's been this elusive, slippery diagnosis. I think part of the reason it went unrecognized for so long is a resistance to acknowledge that a social reality can actually change someone's biology.

Every time there's a war, there's an interest in PTSD. During World War I, shell shock was the term that was used. After the war, the interest waned again. Then in the 1970s, there was a lot of advocacy for Vietnam-era veterans and, because of women's rights groups efforts, a lot of advocacy for women who were in violent relationships or were the survivors of sexual assault. All this advocacy led to the realization that, "Hey, this is something. We need to give it a name. We need to recognize it." It pushed it over the edge. It was accepted into the 1980 version of the psychiatric bible of diagnosis, the *Diagnostic and Statistical Manual of Mental Disorders*.

Even after that, some within the field were still skeptical. And though by now an amazing amount of studies have been done to validate PTSD, there's still some denial of its existence. To me it sounds bizarre, but it's the nature of the illness. The same way individuals can deny trauma, I think whole societies can deny it as well.

4 You've said that PTSD can be inherited. How is that possible?

JAIN: Although, by definition, PTSD is linked to an external traumatic event, research has shown that the condition is highly heritable. There is evidence to suggest that epigenetic changes can occur in a man's sperm or a woman's eggs because of psychological trauma. These changes are then transmitted to their future children and leave them vulnerable because of altered neurons, neuroanatomy and genes. So the children of traumatized parents are at risk, even though they may never have been exposed to a traumatic event themselves.

5 Aside from preventing violence, is there any way to prevent PTSD?

JAIN: Yes. We can perform targeted interventions in what is known as "the golden hours" — that window between trauma exposure and the onset of PTSD — where we have a chance to set people on a pathway toward recovery. For example, one study of motor-vehicle crash survivors found that playing the computer game Tetris for 20 minutes while still in the emergency department lessened intrusive memories about the accident the following week. Other studies have shown that treatment with the stress hormone cortisol can help.

We can also make PTSD care more accessible, acceptable and available to sufferers so it does not become a chronic problem. **ISM**

Foreign aid for public health bolsters America's 'soft power'

By Beth Duff-Brown

U.S. government aid for treating children and adults with HIV and malaria in developing countries has done more than expand access to lifesaving interventions: It has changed how people around the world view the United States, according to a new study by researchers at the School of Medicine.

Compared with other types of foreign aid, investing in health is uniquely associated with a better opinion of the United States, improving its "soft power" and standing in the world, the study said.

Favorability ratings of the United States increased in proportion to health aid from 2002 to 2016 and rose sharply after the implementation of the President's Emergency Plan for AIDS Relief in 2003 and the launch of the President's Malaria Initiative in 2005, the researchers reported.

Their findings were published online May 16 in the *American Journal of Public Health*. The lead author is postdoctoral scholar Aleksandra Jakubowski, PhD, MPH. The senior author is Eran Bendavid, MD, professor of medicine.

"Using data on aid and opinions of the United States, we found that investments in health offer a unique opportunity to promote the perceptions of the United States abroad, in addition to disease burden relief," the authors wrote. "Our study provides new evidence to support the notion that health diplomacy is a net win for the United States and recipient countries alike."

The Trump administration, however, has proposed a 23% cut in foreign aid in its 2020 budget, including large reductions to programs that fight AIDS and malaria overseas.

The Stanford researchers believe their study is the first to add heft to the argument that U.S. health aid boosts the "soft power" that wins the hearts and minds of foreign friends and foes.

> "Our study shows that investing in health aid improves our nation's standing abroad."

"Our study shows that investing in health aid improves our nation's standing abroad, which could have important downstream diplomatic benefits to the United States," Jakubowski said. "Investments in health aid help the United States accumulate soft power. Allowing the U.S. reputation to falter would be contrary to our own interests."

A policy debate

Many politicians and economists consider spending U.S. tax dollars on foreign aid as an ineffective, and possibly harmful, enterprise that goes unappreciated and leads to accusations of American meddling in other countries' national affairs.

The U.S. government, for the past 15 years, has contributed more foreign health aid than any other country, significantly reducing disease burden, increasing life expectancy and improving employment in recipient countries, the authors wrote. Still, this generosity has historically constituted less than 1% of the U.S. gross domestic product.

"Our results suggest that the dollars invested in health aid offer good value for money," the researchers wrote. "That is, the relatively low investment in health aid (in terms of GDP) has provided the United States with large returns in the form of improved public perceptions, which may advance the U.S. government's ability to negotiate international policies that are aligned with American priorities and preferences."

The researchers used 258 Global Attitudes Surveys, based on interviews with more than 260,000 respondents, conducted by the Pew Research Center in 45 low- to middle-income countries between 2002 and 2016.

Their analysis focused on the health sector, which includes several large programs for infectious disease control, but also support for nutrition, child health and reproductive health programs. They compared health aid to other major areas of U.S. investment: governance, infrastructure, humanitarian aid and military aid. They also constructed a database of news stories that mentioned the President's Emergency Plan for AIDS Relief or the President's Malaria Initiative by crawling through the online archives of the top three **See AID, page 3**

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
(650) 723-6911
http://med.stanford.edu/news/

Susan Ipaktchian
Director of print & Web communications
John Sanford
Editor
Robin Weiss
Graphic designer

Send letters, comments and story ideas to John Sanford at 723-8309 or at jsanford@stanford.edu. Please also contact him to receive an e-mail version of *Inside Stanford Medicine*.

STANFORD MEDICINE

Researchers show how big data can be used for personal health

By Hanae Armitage

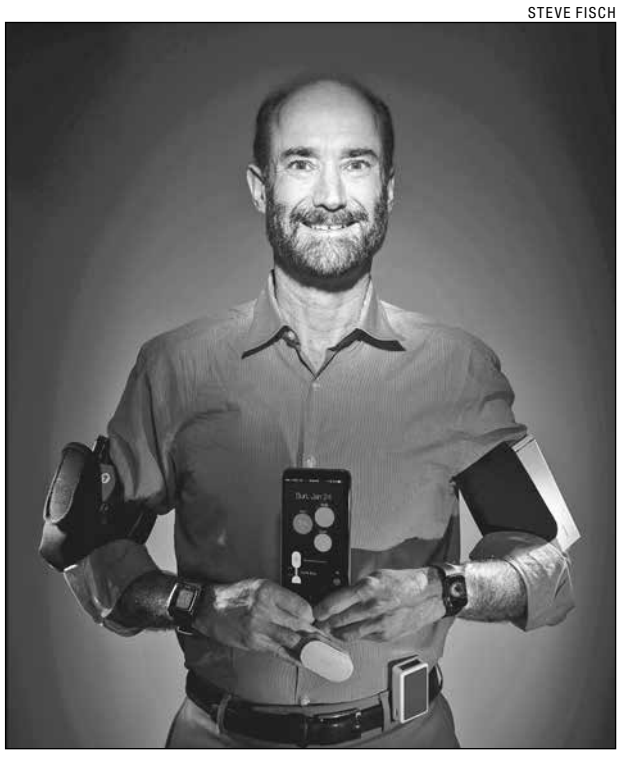
Scientists at the School of Medicine and their collaborators followed a cohort of more than 100 people over several years, tracking the biology of what makes them them.

Now, after collecting extensive data on the group's genetic and molecular makeup, the researchers are piecing together a new understanding of what it means to be healthy and how deviations from an individual's norm can flag early signs of disease.

The results point to a need for a paradigm shift, said Michael Snyder, PhD, professor and chair of genetics.

"I would argue that the way medicine is practiced is deeply flawed and could be significantly improved through longitudinal monitoring of one's personal health baseline," said Snyder, who holds the Stanford W. Ascherman, MD, FACS, Professorship in Genetics. "We generally study people when they're sick, rarely when they're healthy, and it means we don't really know what 'healthy' looks like at an individual biochemical level."

STEVE FISCH



Michael Snyder and a team of researchers tracked the biology of more than 100 people over several years to piece together a new understanding of what it means to be healthy and how deviations from an individual's norm can flag early signs of disease.

Over the course of the study, the researchers uncovered more than 67 clinically actionable health discoveries that ranged from high blood pressure, arrhythmias, cardiomyopathy and early stage cancer detection, among others. The study intertwined data from wearable technologies, genome sequencing and microbial and molecular profiling to establish a baseline of sorts for each participant. Every person's broad swath of data painted a picture of their biological baseline, and as scientists tracked how that picture changed, they also kept tabs on any abnormalities that could signal the development of disease.

"What this paper really shows is that if doctors and scientists do more advanced profiling reasonably frequently, they'll discover clinically actionable information for patient health at a broader scale than has ever been shown before," Snyder said.

A paper describing the research findings was published May 8 in *Nature Medicine*. Snyder and Francois Haddad, MD, clinical associate professor of medicine, are co-senior authors. Sophia Miryam Rose, MD, PhD, instructor of neurosurgery, and Kévin Contrepois, PhD, scientific director at the Stanford Metabolic Health Center, share lead authorship.

A range of change

In most clinical research, the cohort of participants has an underlying unifier — a shared disease or biological peculiarity of some kind. In this study, the grounding factor was the long-term collection of big data, although many of the participants had an increased risk for prediabetes and diabetes, too. The open nature of the cohort was purposeful, Snyder and Haddad said. The idea was to monitor ordinary, relatively healthy people; get a good idea of their biological norms, such as their heart rate, blood pressure, immune molecules and gene expression; and then watch for changes that might hint at a shift in health.

> "We were able to catch a lot of these things before they were even symptomatic."

Between blood draws, stool samples and wearables, such as smartwatches and glucose monitors, the scientists gathered all kinds of data to piece together a high-resolution understanding of each participant's biology.

Some participants were tracked for as long as eight years. The average was about three years. Of the 109 participants, the majority of the group had a clinically actionable outcome. In other words, some readout, be it from a blood test or smartwatch, flagged a potential health problem that was treatable or manageable. None of these issues had been detected before, Snyder said.

"We caught a lot of health issues because we noticed their delta, or their change from baseline," he said. "For instance, we caught nine people with diabetes as it was developing by continuously monitoring their glucose and insulin levels."

Through genetic sequencing, the team identified 13 disease-related findings, two of which were associated with serious heart defects. "The person was quite young, and we found that they had a mutation in a gene that puts you at risk for cardiomyopathy, which causes problems for the heart muscle," Snyder said. "And sure enough, subsequent cardiac testing proved this person had heart disease."

From data to detection

One of the more serious discoveries came from a participant who was found to have lymphoma after researchers noticed the person had an enlarged spleen, an organ to which lymphoma often spreads, and molecular data consistent with lymphoma. The participant went on to receive therapy to successfully treat the disease.

The list goes on: 18 people discovered they had high blood pressure; two people had precancers. Multiple people found out they had low hemoglobin, and six participants had arterial plaques.

A lot of these findings would have been missed with more typical health screens that are the norm in medicine today, Snyder said. "We were able to catch a lot of these things before they were even symptomatic," he said. "And in most cases, it either led to folks being followed more carefully or to a medical intervention. For example, in the case of the individuals with plaques in their arteries, it led to an increase in their statins. So these findings really did change health management for many people."

Even with something as simple as a continuous heart rate monitor, the scientists helped diagnose sleep apnea in one participant; in others, the heart rate changes were linked to infections.

"As an infectious state sets in, heart rate often increases, so if we know a person's baseline heart rate, an elevated heart rate that should otherwise be normal can tell us that an infection is brewing," Haddad said.

With those kinds of takeaways, there's a strong case for expanding the cohort in the future, Snyder said, and that's the researchers' next step.

Outside of participant health, the researchers think they may have even discovered new biomarkers for certain diseases and possibly new molecular markers for cardiovascular disease risk, among other things. For now, these are preliminary findings; the researchers will have to conduct follow-up studies to confirm their suspicions.

"I can't tell you exactly what we're going to find if we follow a group of 100 people over time with advanced technologies, but I can tell you we will often find things that are important for their health," Snyder said. "Right now, we're pretty much in the dark until we profile people, but this approach could provide us a much better view of people's norms, what it means to be healthy and what it means when people deviate from that. Ultimately, we want to shift the practice of medicine from treating people when they are ill to a focus on keeping them healthy by predicting disease risk and catching disease before it is symptomatic."

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.

Snyder is a member of Stanford Bio-X, the Stanford Cardiovascular Institute, the Stanford Cancer Institute, the Stanford Maternal & Child Health Research Institute and the Wu Tsai Neurosciences Institute at Stanford.

Other Stanford co-authors of the study are Kegan Moneghetti, MD, instructor of medicine; research scientist Wenyu Zhou, PhD; genetic counselors Orit Dagan-Rosenfeld, PhD, and Shannon Rego; postdoctoral scholars Tejaswini Mishra, PhD, Ariel Ganz, PhD, Jessilyn Dunn, PhD, Daniel Hornburg, PhD, Sara Ahadi, PhD, and M. Reza Sailani, PhD; health educator Dalia Perelman; genetics research coordinator Melanie Ashland; clinical exercise physiologist Jeffrey Christle, PhD; life science researcher Monika Avina; former Stanford research coordinator Pats Limcaoco; graduate student Camilo Ruiz; Marilyn Tan, MD, clinical assistant professor of medicine and Tracey McLaughlin, MD, professor of medicine.

Researchers from the Veteran Affairs Palo Alto Health Care System, St Vincent's Hospital, University of Melbourne, University of California-Berkeley, Jackson Laboratory for Genomic Medicine, UC-San Francisco and UCLA contributed also to this research.

The study was funded by the National Institutes of Health and the Swiss National Science Foundation.

Stanford's departments of Genetics and of Cardiovascular Medicine also supported the work. **ISM**

Aid

continued from page 2

newspapers by circulation in each of the 45 countries.

They found that the probability of populations holding a very favorable opinion of the United States was 19 percentage points higher in the countries where and years when U.S. donations for health care were highest, compared with countries where and years when health aid donations were lowest. Using another metric, the researchers found that every additional $100 million in health aid was associated with a nearly 6 percentage-point increase in the probability of respondents indicating they had a "very favorable" opinion of the United States.

In contrast, the researchers found, aid for governance, infrastructure, humanitarian and military purposes was not associated with a better opinion of the United States.

Bendavid, an infectious diseases physician and core faculty member of Stanford Health Policy, said that when he set out to conduct this research, he believed it would result "in a resounding thud" — that the "soft power" of health aid would have no impact on public opinion.

"For me, the notion that this program — hatched and headquartered in D.C. — would have impacts among millions in Nairobi and Dakar, seemed farfetched," Bendavid said. "I was incredulous until all the pieces were in place."

The 'America First' agenda

The Trump administration's "America First" agenda is calling for significant cuts to global health aid, particularly to the highly successful AIDS relief program, which was established by President George W. Bush. The administration's budget, released in March, proposed a $860 million cut to the program; the President's Malaria Initiative is facing a $331 million reduction in federal funding. That's a decline of 18% and 44%, respectively.

Eran Bendavid

The U.S. contribution to the Global Fund to Fight AIDS, Tuberculosis and Malaria would also decline by 17%, or $225 million, according to the Kaiser Family Foundation.

Yet beyond the reputational damage to the United States, such cuts could be a major setback to improving health outcomes in developing countries, the researchers said. After all, HIV knows no borders, and having more resilient health care systems is instrumental when facing public health crises, such as the Ebola outbreak in the Democratic Republic of Congo, Jakubowski said.

"The most direct impact of cutting the United States' health aid allocations is the potential to undermine or reverse the progress that has been enabled by U.S. aid in curbing mortality and the spread of disease," Bendavid said. "However, this study suggests there are also repercussions to the United States: the relationships the U.S. has built with recipient nations could also be undermined."

Other Stanford co-authors are Steven Asch, MD, MPH, professor of medicine, and former graduate student Don Mai.

Stanford's Department of Medicine supported the work. **ISM**

Neurosurgeons turn to basic science in fight against childhoo

By Krista Conger

Teresa Purzner, MD, PhD, is a hands-on, all-in physician.

As a neurosurgical resident at the University of Toronto in 2009, she rotated through a variety of specialties, including one in pediatric neurosurgery. During the three months of her pediatric rotation, she dealt with desperately ill children and their parents on a daily basis. Often, she participated in conversations that involved delivering bad news — telling parents, for example, that their child, suffering from a deadly brain tumor called a medulloblastoma, might have a chance of being cured, but the chemo and radiation treatment was likely to cause permanent cognitive and neurological damage.

"It is a devastating conversation," she recalled. "You're basically delivering, and living in, every parent's worst nightmare. One mother who lost her son shortly after diagnosis told me that she was thankful not to have had to put him through the treatment we were recommending."

When it all got to be too much, she and her husband, Jamie Purzner, MD, also a neurosurgical trainee, just walked away from their residencies. Frustrated with the challenges of treating children with the tumors, they put their clinical careers on hold to tackle the root of the problem: the cells in the brain that run wild during the tumor's development.

"A few brief conversations with the experts around us really sealed the deal," Teresa Purzner said. "It was clear that medulloblastoma was a tangible and interesting problem, and that amazing strides had already been made in understanding the link between developmental biology and medulloblastoma development."

ETHAN HILL

Teresa and Jamie Purzner came to Stanford to study medulloblastoma and search for a way to better treat the brain cancer.

The time was ripe, they felt, to bridge the gap between this new, conceptual understanding of the disease and the desperate need they'd witnessed in the clinic. But they needed to find the right place to do the necessary research.

The pair considered hundreds of laboratories in the United States and Canada. But rather than seeking out labs and investigators experienced in translating existing research results into clinical applications — a bench-to-bedside approach — they focused on laboratories drilling into the nuts and bolts of biological processes.

Trading scrubs for lab coats

"Basic science is where fundamental discoveries occur," Teresa Purzner said, "and basic science can tell you whether a specific potential treatment is likely to be successful. But basic scientists often underestimate their value. They are at least as well-positioned as clinicians to figure out what the best target is from a biological perspective."

The couple shed their scrubs for lab coats in 2012. During the next six years, they worked as graduate students in the Stanford School of Medicine's Department of Developmental Biology to understand, at the most basic level, what causes the brain tumors.

Their unconventional career rewind has been uncommonly successful. The pair identified a potential new drug treatment for the disease, and with the support of Stanford SPARK — a program launched in 2006 to advance promising discoveries from the lab to the clinic — Teresa Purzner went on to test it in mice and to coordinate the launch of a phase-1 clinical trial that recently began enrolling patients. The two published their findings in *Science Signaling* in September 2018.

"This was completely unbiased discovery science," said developmental biologist Margaret Fuller, PhD, who advised the Purzners during the latter part of their graduate work. "Teresa and Jamie used an unbiased screen to identify a new component of a well-known developmental pathway, identified where in the pathway it functions and then showed that blocking this step can kill medulloblastoma cells implanted into mice. It's a remarkable achievement."

Along the way, the pair faced many challenges, including the myriad difficulties of escorting a basic science finding through preclinical studies in animals to testing in humans. Teresa Purzner took charge of marshaling support from funding agencies, national research consortiums and drug companies often wary of the fraught arena of clinical trials that enroll terminally ill children. The couple also started a family; their three children were born during their graduate school careers.

Not bad for some seemingly misplaced neurosurgeons.

"There are 101 valid reasons to not do what we did, and 100 more reasons why we should have failed once we decided to do it," Teresa Purzner said. "But we benefited from an amazing cast of collaborators at Stanford and elsewhere who spent hundreds and hundreds of hours helping to overcome many hurdles in the path to this trial. We were all very dedicated to doing everything possible to help these kids."

Matthew Scott, PhD, now professor emeritus at Stanford, was a developmental biologist in January 2012 when he received an email from Jamie Purzner inquiring about research positions. Scott was taken aback. "It's not often that neurosurgeons want to come train in my lab as graduate students," he said. "I thought, 'These people are doctors; they don't really want to do research full time.' It was totally unbelievable, and unprecedented, for people with their training and skill."

> **"It's really a great example of the application of basic science."**

Scott, who is married to Fuller, is known for his 1984 discovery in fruit flies of a short DNA sequence called a homeobox. Homeobox genes coordinate the activities of sets of other genes, acting within cells or groups of cells to control development. Proteins made from homeobox genes bind to specific DNA sequences throughout the genome to control genes used during early embryonic development to determine body patterning — ensuring that the wings, legs and abdominal sections fall neatly into place to generate the tiny flies drawn to the overripe fruit on your kitchen counter.

At first blush, none of this seems like something that would have especially interested pediatric neurosurgeons intent on discovering a new cancer treatment. But Scott also was known for identifying and studying signaling systems that allow groups of cells to communicate with one another during development — an area with more obvious relevance to the couple's interest. Mutations in some of these pathways, Scott found, are linked to the development of some types of cancers, including medulloblastoma.

In the early 1990s, the Scott lab began working on an important system called hedgehog signaling — first in fruit flies and later in mammals. It's named hedgehog because a mutation in the gene for a key protein in the signaling system results in fruit fly embryos that are spiny, like hedgehogs.

The hedgehog protein is produced and secreted by particular cells in the fruit fly embryo. When it binds to a receptor protein called patched on the surface of a cell, a cascade of activity is triggered that begins with proteins on the cell surface and ends with other proteins entering the nucleus. There, they stimulate the process by which genes lead to the production of proteins that govern how cells multiply and develop. In the absence of hedgehog binding, patched keeps the pathway turned off.

In 1996, Scott's research into the pathway revealed that mutations in patched are often found in people with an inherited condition associated with frequent skin cancers and skeletal abnormalities called basal-cell nevus syndrome. When patched is missing or mutated, the hedgehog pathway is constantly active, and the cells receive ongoing signals to grow and divide. Carriers of patched mutations not only develop frequent basal-cell skin cancers, but they also often develop medulloblastomas.

The Scott lab then showed that during normal development, hedgehog signaling triggers growth of the cerebellum, the portion of the brain at the back of the head near the spinal cord. Loss of patched lets the normal growth signal happen when it should not. This explanation for the development of medulloblastomas piqued the Purzners' interest.

In the lab (and home) of Matthew Scott

But choosing to come to America to work as graduate students put the Purzners in a funding gray area. They could no longer apply for grants meant to support Canadian clinicians doing research in Canada. They also couldn't qualify for funding meant for Americans. Although they eventually secured enough money to support themselves and their growing family, including funding from Stanford Bio-X, their financial stability was far from certain when they arrived at Stanford in June of 2012 in a truck packed with boxes they loaded the day after their last neurosurgical calls ended.

"We had no American bank account or credit cards and no plan for where we were going to live," Teresa Purzner recalled. "Matt and Margaret realized this and insisted we live with them until we found a place to rent."

About 350 people a year in the United States are diagnosed with medulloblastoma, which develops in the cerebellum. It is the most common brain cancer in children. Even with the best treatments, only about 70 percent will live five years or more after their initial diagnosis and the prognosis for those who experience a recurrence is dire.

Frequently, the tumors spread to other parts of the brain and central nervous system. Treatment options

od brain cancer

TIMOTHY ARCHIBALD

Teresa Purzner with developmental biologist Matthew Scott, whose lab she and Jamie Purzner worked in.

are bleak and include whole brain and spinal radiation in combination with chemotherapy for as long as a year. Children are particularly susceptible to damage from these therapies because their brains are developing.

About 25% of all medulloblastomas are caused by mutations in genes for proteins involved in the hedgehog pathway, including patched. Although drugs that inhibit the pathway can often temporarily shrink tumors in patients, the cancer cells rapidly become resistant to the treatment when the cells develop mutations that reactivate the pathway. Targeting the very last step — the moment when the proteins reach the nucleus and bind to the DNA to turn genes on — should leave the cancer cells fewer options to wiggle out of the treatment, researchers believe. But how to do that?

The Purzners focused on the granule neuron precursor cells in the brain that give rise to hedgehog-associated human medulloblastomas. In mice, GNPs rapidly multiply between day one and day seven after birth in response to hedgehog pathway signaling. Between day seven and day 14, the proliferation rate slows and the cells begin to become granule neurons. After day 14, any remaining GNPs mature into granule neurons, which are the most common type of neuron in the brain.

Occasionally, however, GNPs ignore the normal developmental signals and keep multiplying after day 14. This increases the chance that the cells will accumulate additional mutations and become cancerous. Learning why this happens might be the key to stopping the rapid increase in medulloblastoma cells, the Purzners reasoned.

A chance encounter with Joshua Elias, PhD, assistant professor of chemical and systems biology, whose laboratory was one floor above Scott's, gave the Purzners an idea of how to start. The Elias lab focuses on proteomics — the study of all aspects of proteins in a cell or tissue to learn how cells and tissues develop and function. For example, a cell often adds or removes small chemical tags, called phosphate groups, from proteins to control their function. A phosphate tag in one location on a protein may cue it to bind to a second protein, move to another part of the cell or latch onto DNA to activate certain genes, whereas a tag in a different location on the same protein could trigger another set of biological outcomes. Conversely, removal of these phosphate groups can quickly inhibit the protein's activity.

The cell's ability to toggle a protein's activity in this way allows the cell to react quickly and appropriately to changing conditions or developmental stages. For researchers, the ability to chart changes in the patterns and locations of phosphate tags across a panel of proteins over time can provide an intimate look at the workings of a cell during development or disease progression.

Combining expertise

Teresa Purzner decided to compare the pattern of phosphate tags, or protein phosphorylation, on GNP proteins isolated from the brains of newborn mice at day seven with those of GNPs isolated at day 14 and day one. Jamie Purzner, in contrast, focused on sussing out changes in which proteins are produced at different cell stages. Although Teresa Purzner's approach yielded more immediately promising results, they remained closely involved in each other's projects.

"It was pretty darn fun combining our expertise and thinking over the problems together from two perspectives," she said.

The Purzners found that the protein phosphorylation pattern of the rapidly dividing day seven GNP cells more closely resembles that of medulloblastoma cells than that of GNP cells on day one or day 14. Further detective work homed in on a phosphate-adding protein called CK2 that is likely responsible for many of the phosphate-tagging events observed in day seven cells — including some that are critical to the last steps in the hedgehog pathway.

Blocking CK2 activity in mice during days three to seven left the animals with significantly fewer granule neurons than control animals had, the Purzners found. Furthermore, a CK2 inhibitor slowed or stopped the growth of mouse medulloblastoma cells implanted in mice — even cancer cells resistant to other hedgehog pathway inhibitors.

"We'd put these angry medulloblastoma cells into the flanks of mice and see complete tumor regression when CK2 was inhibited," Teresa Purzner said. "When we transplanted the medulloblastoma cells into the cerebella of mice, we found that, although the control animals had to be euthanized within 17 days due to cancer progression, 43 percent of mice treated with a CK2 inhibitor for 30 days lived past 100 days — basically until the experiment was terminated."

"This was astonishingly effective," Scott said. "The kinase acts very late in the hedgehog pathway, so it's difficult for the cancer cells to mutate around it. It's really a great example of the application of basic science. The Purzners didn't start off looking for a protein involved in the hedgehog pathway. But once they did, Teresa Purzner embarked on shepherding this finding all the way from a basic science investigation to preclinical tests that have now launched a clinical trial."

Getting to the clinical trial wasn't easy, however.

What to do next

"I had this beautiful, targeted small molecule inhibitor of CK2 that works in animals," Purzner said. "But I had absolutely no idea how to go from there to get it to patients. This was far outside my realm of experience."

Enter Stanford SPARK. The program matches academic researchers with volunteers from the pharmaceutical, biotech and financial industries to streamline drug development and make it faster and cheaper. SPARK was founded in 2006 by Daria Mochly-Rosen, PhD, professor of chemical and systems biology at Stanford, who co-directs the program with Kevin Grimes, PhD, professor of chemical and systems biology.

"They started setting me up with world leaders — experts in every part of the drug development process to help me understand step-by-step what would be required to go from my discovery in the lab to a patient in the clinic," Purzner said. "It went from a seemingly impossible task to something difficult but achievable. And then we started just tackling each milestone one after the other."

Important steps included convincing a Taiwanese company called Senhwa Biosciences Inc., which was producing the only human-tested CK2 inhibitor, CX-4945, for use in a trial of basal-cell carcinoma, to agree to provide their drug for a pediatric clinical trial. Purzner was also able to secure the involvement of the Pediatric Brain Tumor Consortium, formed by the National Cancer Institute to improve the care of children with brain tumors across the country.

"Teresa wrote an investigative new drug study for the Food and Drug Administration, and, importantly, convinced the Pediatric Brain Tumor Consortium to do a clinical trial with this drug given to her by Senhwa, which is unheard-of," Mochly-Rosen said. "Companies that are running a clinical trial with a drug never give that drug to another clinical study because if something happens it can affect their clinical study as well."

The FDA approved the phase 1-2 clinical trial of CX-4945 in children with hedgehog-pathway dependent medulloblastoma on Jan. 4, and the consortium's Central Institutional Review Board signed off on Feb. 28. The study opened on March 1.

'An absolute triumph'

"It's so exciting," Purzner said. "This took hundreds of hours and dozens of people to accomplish because in many ways it was not a typical trial to put together. There were at least two or three times I thought, 'This could be the end. All of our work could be for nothing, and these kids are never going to get to see this drug.'"

Scott said, "This was an absolute triumph of the translation of a series of basic scientific discoveries into a clinical trial. In 1980, we identified the first mutations in hedgehog and patched in fruit flies.

"Sixteen years later we reported a connection with cancer; 16 years later we had our first FDA-approved drug targeting the hedgehog pathway in basal-cell carcinoma. So it took 32 years from pure, curiosity-driven 'Huh, that's interesting' — when we found some genes that control patterning in fly larva — to a point where patients were being treated. Now, 32 years is either way too long, or not too bad in the big picture of drug development. But Teresa did it in five," he said.

"We benefited from an amazing cast of collaborators."

The Purzners have returned to Canada to complete their neurosurgical residencies.

It remains to be seen whether the CX-4945 will be safe and effective in children with hedgehog-dependent medulloblastoma. A success in mice doesn't always translate to humans. But Teresa Purzner's intensive approach to solving the problem has led to a promising new target in the field.

"Having my own children gave me a very sobering perspective about what these families are going through," she said. "I didn't fully grasp just how heart-wrenching it would be to have a child with a serious medical issue until I had my own children. Getting to this clinical trial has been very emotional. And I'm not an emotional person. It is just such a huge relief to get to this point and know that I did what I came to do." ISM

Osteoarthritis

continued from page 1

in human cells and tissues.

Osteoarthritis, by far the most frequently occurring variety of arthritis, is characterized by cartilage breakdown and inflammation in joints, which can be further aggravated by excess bone growths called osteophytes.

Some 30 million Americans have symptomatic osteoarthritis. By the time you're 60, your chances of exhibiting osteoarthritis symptoms exceed 30%. By age 80 or 90, your risk has risen to nearly 100%.

STEVE FISCH

William Robinson is the senior author of a study that found banishing immune cells called mast cells — or blocking signals from the most common stimulus activating them in real life, or disabling a cartilage-degrading enzyme they release when activated — protected mice from developing osteoarthritis.

"Almost all of us will ultimately suffer from osteoarthritis if we live to be old enough," said William Robinson, MD, PhD, professor of immunology and rheumatology, who is the study's senior author. Lead authorship is shared by research associate Qian Wang, MD, PhD; former MD-PhD student Christin Lepus, MD, PhD; and former postdoctoral scholar Harini Raghu, PhD.

Not just wear and tear

Osteoarthritis has traditionally been thought to be an inevitable result of wear and tear: the breakdown of cartilage over many years, ultimately resulting in grinding, bone-on-bone contact and degeneration in the affected joints. But the new study shows the essential involvement of the immune system in the genesis of osteoarthritis, while prying open a window through which researchers can see a way to designing drugs to prevent it. At present, there are no drugs that can prevent, slow or cure it.

"Even though the vast majority of us will develop osteoarthritis at some point in our lives, we don't have any disease-slowing therapies," Robinson said.

The chances of actually reversing damage to joints are slim, he said. Today's treatments for the pain and mobility consist of painkillers like ibuprofen, naproxen and acetaminophen; walkers; canes; and knee or hip replacements.

Mast cells are best known as the culprits that produce the histamines and other molecules responsible for allergic symptoms, ranging from the itch of eczema to the mucous explosions of hay fever to the throat constriction of asthma or food-triggered anaphylaxis. But mast cells also produce a degradative protein, tryptase, that can rip up collagens and other molecules that form the cartilage in joints.

Mast cells usually reside quietly within tissues throughout the body. But when they become activated, they secrete granules containing histamine, tryptase and other inflammatory substances. The classic trigger for that activation is the binding of a form of circulating antibody, or immunoglobin, called IgE to specialized receptors abounding on mast cells. The evolutionary purpose of IgE, mast cells and histamine is believed to be to fight off parasites, which have plagued humans and other animals throughout most of evolution but have become relatively rare in humans in recent decades, at least in industrialized countries.

While mast cells have been found lurking in joints of people with and without symptomatic arthritis, until now neither mast cells nor IgE have been definitively identified as risk factors for osteoarthritis.

Protection from the disease

In the study, Robinson's group used electron microscopy to show that mast cells in injured joints of humans who didn't yet have arthritic symptoms weren't releasing their histamine- and tryptase-laden granules, whereas mast cells residing in the joints of humans with arthritic symptoms were.

Several types of genetically altered lab mice whose mast cells were deficient or absent were highly resistant to the development of osteoarthritic features including joint inflammation, osteophyte development and joint breakdown after undergoing an experimental procedure to induce these symptoms, the researchers found.

The researchers also proved that impairing the action of tryptase, which is secreted almost solely by mast cells, had a similar protective effect. And they further demonstrated that depleting IgE or its binding to receptors on mast cells, or disabling those receptors' subsequent signaling to components within mast cells, all were protective.

In all, the scientists were able to get the same osteoarthritis-protective results using a number of genetic tricks, as well as three small-molecule compounds that each blocked a separate stage of the cascade via which IgE trips off mast-cell activation and secretion of granules containing collagen-chewing tryptase. One drug used to impede mast cells' survival, imatinib, is licensed by the Food and Drug Administration and marketed as Gleevec as a therapy for chronic myeloid lymphoma. But while imatinib is an acceptable drug for such life-threatening cancers, Robinson said, it's too toxic for sustained long-term use as a therapy in an indication such as osteoarthritis, which, although painful and mobility-reducing, is seldom directly life-threatening.

In any case, Robinson said, much of the joint damage caused by osteoarthritis is unlikely to be reversible.

"A major goal in my career is to find a way to prevent people from getting osteoarthritis," he said, adding that he wants to identify drugs with excellent safety profiles and the ability to prevent, rather than treat, osteoarthritis. "These drugs will have to be safe enough for large numbers of people to take for decades without problems."

Robinson is an affiliate of the Stanford Institute of Immunity, Transplantation and Infection; a member of Stanford Bio-X, the Stanford Maternal & Child Health Research Institute and the Stanford Cancer Institute; and a staff physician and investigator in the Geriatric Research Education and Clinical Center at the Veterans Affairs Palo Alto Health Care System.

Other Stanford co-authors are former postdoctoral scholar Laurent Reber, PhD; senior research scientist Mindy Tsai, DMSc; research assistants Heidi Wong, Nick Hu and Eileen Elliott; MD-PhD student Ericka von Kaeppler; former research assistant Nithya Lingampalli; scientific writer and project manager Michelle Bloom, PhD; professors of orthopaedic surgery Nicholas Giori, MD, PhD, Stuart Goodman, MD, PhD, and Constance Chu, MD; former clinical assistant professor of medicine Jeremy Sokolove, MD; and Stephen Galli, MD, professor of pathology and of microbiology and immunology.

Researchers at the University of Padova, in Italy, and Virginia Commonwealth University School of Medicine contributed to the work.

The research was funded by the National Institutes of Health and the U.S. Department of Veterans Affairs.

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

Memory

continued from page 1

to get into the brain itself.

"We can now try to treat brain degeneration using drugs that typically aren't very good at getting through the blood-brain barrier — but, in this case, would no longer need to," Yousef said.

Different way of reaching the brain

The researchers focused on the mouse hippocampus, a well-studied brain structure that's essential to memory and learning and whose architecture and function are similar in mice and humans. The hippocampus is also one of the very few sites in the adult mammalian brain where neurogenesis, the creation of new nerve cells, occurs; those new cells are critical to the formation of new memories.

Since his lab first began reporting several years ago that unknown factors in old blood can accelerate cognitive decline and, conversely, that factors in young blood can rejuvenate old brains, Wyss-Coray, the D.H. Chen Professor II, has sought to identify those factors. But he and his colleagues took a different tack in the new study.

He said the roughly 400 miles of blood vessels that pass through the human brain differ from those elsewhere in the body in one important respect: They're much more selective about what gets in and what comes out.

"The blood-brain barrier excludes most bloodborne cells and substances," he said. "We wondered if, instead of entering the brain and monkeying with brain cells directly, something in circulating blood could be communicating directly with the brain's endothelial cells."

A few years ago, Wyss-Coray and his colleagues compared blood from young and old people to pinpoint substances whose abundance changes with age. In the new study, they narrowed their search to just those age-associated bloodborne substances that are in some way directly related to vascular function. Topping the list was a circulating form of a protein constantly produced within endothelial cells and displayed on their surfaces.

The protein, VCAM1, is well known to immunologists. It's a docking station for circulating cells of the immune system — a first stop in a passport-punching process that under certain relatively rare conditions grants those immune cells permission to migrate across the brain's otherwise tightly closed border.

This protein gets sawed off of endothelial cell surfaces and dumped into the bloodstream by lawnmowerlike enzymes at pretty much the same rate it gets produced, so its population size on blood vessels remains relatively constant. But VCAM1's abundance on blood vessel surfaces jumps markedly in the event of local injury or infection. That snags immune cells, which combat infectious pathogens and are essential to the healing process.

"At any given time, levels of circulating VCAM1 are a good proxy for the total amount of VCAM1 on the body's blood-vessel endothelial cell surfaces," Wyss-Coray said. Previous studies have linked high circulating VCAM1 levels to cancer, heart disease, stroke, Alzheimer's disease, epilepsy and other inflammatory disorders.

Identifying the source of dysfunction

In the study, the researchers showed that VCAM1's abundance on the endothelial cells comprising blood vessel walls in the mouse brains rises in old age, as well as in the brains of younger mice that are given infusions of older mice's plasma, the cell-free, liquid portion of blood. Likewise, the researchers observed increased signs of inflammation in the older mice's cells.

Wyss-Coray suspects that the tethering of immune cells to blood-vessel surfaces — particularly if immune cells are in an activated state due to an existing condition, such as injury or infection, or to old age — enhances the release of inflammatory proteins that penetrate blood vessel walls via specialized receptors on endothelial-cell surfaces.

Circulating VCAM1, though, wasn't the source of brain dysfunction. When the investigators depleted old mice's plasma of the protein before giving the plasma to young mice, they observed the same damaging effects in the hippocampus — reduced neurogenesis, increased microglial inflammation — they'd previously seen when young mice received old plasma.

Deleting the gene encoding VCAM1 in mice brains prevented the protein's production in the brain's endothelial cells. If this deletion was performed in young adulthood, the mice no longer suffered reduced neurogenesis or increased microglial inflammation when they grew older.

The researchers achieved the same results with monoclonal antibodies, specialized proteins that bind avidly and exclusively to their target. Three weeks of treatment with a monoclonal antibody directly targeting and blocking VCAM1 was enough to increase neurogenesis and diminish microglial reactivity in older mice's hippocampi.

These mice aced a battery of mental-acuity tests. One test, the Barnes maze, involves a table from which mice want to escape. The table has lots of holes through which the mouse can fall a short distance onto the floor (although not far enough to cause an injury). But one hole connects to a tube mounted horizontally under it, providing a comforting escape to the mice. The mouse must learn and remember how to get to the "safety" hole.

Once they were fully trained, older mice treated with this antibody reached the escape hole in the Barnes maze as quickly as young mice.

"Blocking VCAM1 in the brain wound up making these mice smarter," Wyss-Coray said. "In all the time I've been working on this, I've never seen such performance before." ISM

New tool enables powerful molecular analysis of tissue samples

By Christopher Vaughan

Single-cell RNA sequencing is emerging as a powerful technology in modern medical research, allowing scientists to examine individual cells and their behaviors in diseases like cancer. But the technique, which can't be applied to the vast majority of preserved tissue samples, is expensive and can't be done at the scale required to be part of routine clinical treatment.

In an effort to address these shortcomings, researchers at the School of Medicine invented a computational technique called CIBERSORTx that can analyze the RNA of individual cells taken from whole-tissue samples or data sets. "We believe this technique has major implications for biomedical discovery and precision medicine," said Aaron Newman, PhD, assistant professor of biomedical data science.

A paper describing their method was published online May 6 in *Nature Biotechnology*. Newman is the lead author of the paper; Ash Alizadeh, MD, PhD, associate professor of medicine, is the senior author.

Pinpointing cells and their states

CIBERSORTx is an evolutionary leap from the technique the group developed previously, called CIBERSORT. "With the original version of CIBERSORT, we could take a mixture of cells and, by analyzing the frequency with which certain molecules were made, could tell how much of each kind of cell was in the original mix without having to physically sort them," Alizadeh said.

"We made the analogy that it was like analyzing a fruit smoothie," Newman said. "You don't have to see what fruits are going into the smoothie because you can sip it and taste a lot of apple, a little banana and see the red color of some strawberries."

CIBERSORTx takes that principle much further. The researchers start by doing a single-cell RNA analysis of a small sample of tissue. They might take a cancerous tumor, for instance, separate the cells in the tumor and look closely at the RNA (and therefore the proteins) that each cell makes. From this they produce a "bar code," a pattern of RNA expression, that identifies not only the kind of cell they are looking at, but also the subtype or mode it's operating in. For instance, Alizadeh said, the immune cells infiltrating a tumor act differently and produce different RNA and proteins — and therefore a different RNA bar code — than the same kind of immune cells circulating in the blood.

"What CIBERSORTx does is let us not just tell how much apple there is in the smoothie, but how many are Granny Smiths, how many are Red Delicious, how many are still green and how many are bruised," Alizadeh said. "Similarly, starting with a mix of RNA barcodes from a tumor can give us insights into the mix of cell types and their perturbed cell states in these tumors, and how we might be able to address these defects for cancer therapy."

CHRISTOPHER VAUGHAN

Aaron Newman (left) and Ash Alizadeh worked on developing a computational technique called CIBERSORTx that can analyze the RNA of individual cells taken from whole-tissue samples or data sets.

Being able to identify not only the types of cells, but also their state or behaviors in particular environments, could lead to dramatic new biological discoveries and provide information that could improve therapies, the scientists said.

The group analyzed over 1,000 whole tumors with the technique and found that not only were cancer cells different from normal cells, as expected, but immune cells infiltrating a tumor acted differently than circulating immune cells — and even normal structural cells surrounding the cancer cells acted differently than the same type of cells in other parts of an organ. "Your cancer cells are changing all the other cells in the tumor," Newman said. The researchers even showed that the immune cells infiltrating one type of lung cancer were different from the same type of immune cells infiltrating another type of lung cancer.

A major strength of CIBERSORTx is that it can be used on tissue samples that have been "pickled" in formalin and stored in paraffin, which is true of the vast majority of diagnostic tumor samples. Most of these samples cannot be analyzed through single-cell RNA sequencing because the cell walls are often damaged or the cells can't be separated from each other. This makes single-cell RNA analysis impractical or impossible for most large studies and clinical trials, where information about how cells are behaving is crucial.

The researchers also tested the tool's diagnostic power by analyzing melanoma tumors. One of the most effective therapies for metastatic melanoma and some other cancers are drugs that block the production of proteins called PD-1 and CTLA4 in the T cells that infiltrate and attack the tumors. But these "checkpoint inhibitor" drugs work well in a minority of patients, and there has been no easy way to tell which patients will respond.

Confirming a hypothesis

One prior hypothesis has been that if a patient has high levels of PD-1 and CTLA4 in the T cells infiltrating their tumor, these drugs are more likely to work, but researchers have had difficulty ascertaining whether this was true. CIBERSORTx allowed the team to explore this question. After training their algorithms on single-cell RNA data from a few melanoma tumors, they analyzed publicly available data sets from previous studies on bulk melanoma tumors and tested fixed samples. They confirmed the hypothesis, finding that high levels of expression of PD-1 and CTLA4 in certain T cells was correlated with lower mortality rates among patients being treated with PD-1-blocking drugs.

CIBERSORTx may also allow the discovery of new cell markers that will provide other pathways for attacking cancer, the researchers said. Using the tool to analyze stored tissues and correlating cell types with clinical outcomes may point to genes and proteins that are important for cancer growth, they said. "It took 30 years to identify PD-1 and CTLA4 as important proteins, but these markers just jump out of the data when we use CIBERSORTx to correlate gene expression of cells in tumors with treatment outcomes," Alizadeh said.

"We see so many new molecules that could prove interesting," Newman said. "It's a treasure trove."

As with the original tool, the scientists plan to let researchers from around the world use CIBERSORTx algorithms on computers at Stanford through an internet link. Newman and Alizadeh think they will see a lot of online traffic. "We expect to see smoke coming out of the computer room," Alizadeh said. **ISM**

Humanwide

continued from page 1

able to focus on the whole human: who they are when they're working, who they are when they're playing, who they are when they're at home," she said. "This program demonstrates how we can zero in on what matters to a patient, to craft the entire care plan around their goals."

Redesigning primary care

Humanwide was built on the foundation of Primary Care 2.0, the health care practice redesign effort at Stanford Medicine, where patients communicate regularly, in-person and virtually with members of a care team consisting of a primary care physician, nutritionist, behavioral health specialist and clinical pharmacist, depending on their health needs.

Patients in the Humanwide pilot represented a diverse mix of ages, races/ethnicities, genders and medical complexities.

As part of the pilot, Humanwide patients:

Underwent genetic assessments and a pharmacogenomic screening, which evaluated their individual physiologic response to medications based on their genetic profile.

Used mobile monitoring devices, including a glucometer, pedometer, scale and blood pressure cuff, to regularly measure key health metrics. The data automatically uploaded to their electronic health records for remote monitoring by their health care team.

Worked with a certified health coach to identify wellness goals and create a plan for achieving them.

Through these initial assessments, regular interactions and continuous monitoring, health care teams gathered data on each patient on a variety of factors known to influence health: activity, behaviors, biometric measurement, genetic factors, biological markers, care-utilization data and environmental exposures.

Team members met regularly to review the multiple streams of data for each patient, and this continuous access to information and engagement with patients enabled them to pinpoint health risks and take preventive action.

"We saw this as an opportunity to bring in more data that was previously not available, so that we now have an unprecedented understanding of our patients' risks," Mahoney said. "Now we have the ability to proactively take care of them in a way we've never had before."

Improvements in patient health

Through Humanwide's comprehensive approach, Mahoney and her team detected and treated a range of health concerns previously overlooked. For example, among 33 women who were screened for breast cancer risk, five were identified as having a very high risk and in need of ongoing, enhanced surveillance.

Pharmacogenomics screening resulted in more than a dozen changes in medication prescriptions or dosages, including an adjustment that relieved pain for a patient experiencing persistent leg cramps from statins. Additionally, continuous readings from home-based devices helped providers identify early diabetes or hypertension in several patients and work with them to manage their risk. More extensive testing revealed masked hypertension in one patient who was at high risk of cardiovascular disease, and his team helped control his condition through medication and lifestyle changes.

Patients said they liked the strong connection with their care team and the opportunity to apply their personal data to their health.

"I loved the fact that you could get all of this precision health information to help your doctor and your caregiving team better pinpoint how to manage your health specific to you," said participant Debbie Spaizman. "I got everything out of it that I had hoped for, and more."

Another important finding involved provider satisfaction: Clinicians reported that they felt more engaged in their work when sharing the goal of caring for a patient with a like-minded team.

"Nearly half of all practicing physicians report at least one symptom of burnout, and that's a huge concern for me," Minor said. "That's why it's so important for programs like Humanwide to consider the experiences of both patients and physicians."

David Entwistle, president and CEO of Stanford Health Care, said the pilot also paves the way for a new mindset about patient wellness.

"Looking at genomic data and other factors that actually predict patient health allows us to be proactive instead of waiting for something to happen and having to react to that," he said. "Humanwide is an opportunity to build a deep understanding of each patient in a unique way."

In their paper, Mahoney and Asch noted that the Humanwide pilot demonstrates the feasibility of creating a comprehensive, patient-centered, data-driven environment, and that both patients and health care providers are receptive to using new tools and data streams to transform primary care. Mahoney added that the project offers insights for the future use of detailed population health data to benefit individual patients.

Asch, who led evaluation of the pilot, said, "Humanwide is the future of primary care. It's a future that looks at the patient as a whole person. It's a future that collects data very broadly. And most importantly, it's a future that helps patients achieve their goals, rather than treating them like a collection of diseases."

For videos, podcasts and other information about the pilot, visit humanwide.stanford.edu. **ISM**

Tobacco, e-cig promotions spark teens' use of nicotine products, study finds

By Erin Digitale

Owning items that promote e-cigarettes and other alternative tobacco products doubles the likelihood that a young person will try these products, a new study led by the School of Medicine has found. The finding illustrates the influence of such marketing on teenagers.

The study, which was published online May 17 in *JAMA Network Open,* followed 757 California teens for a year. At the beginning of the year, participants had never used alternative tobacco products, including e-cigarettes, chewing tobacco, cigars, cigarillos, pipes and hookahs.

Bonnie Halpern-Felsher

But some participants owned marketing materials for these products, such as coupons, samples and branded hats or T-shirts.

"We wanted to see how owning promotional materials would affect young people's use later on," said the study's lead author, Hoda Magid, PhD, a postdoctoral scholar in health research and policy. Among teens, cigarette smoking rates have dropped in recent decades, but their use of e-cigarettes and other tobacco products has risen sharply. "The increase in use of alternative tobacco products poses a threat to the decades of hard work that public health experts have done to reduce tobacco use," Magid said.

"We need to know trajectories of use of alternative tobacco products," said the study's senior author, Bonnie Halpern-Felsher, PhD, professor of pediatrics. Understanding when and why youth start using such products is important for stemming the tide of addiction to them, she said.

Restrictions on conventional smokes

While marketing to minors and providing samples is illegal for all tobacco products, providing coupons and branded promotional items such as T-shirts and hats is not illegal for most alternative tobacco products, except smokeless tobacco. Enforcement of all policies is lacking, Halpern-Felsher said.

"The problem is that the FDA has been very slow to enact new or enforce existing laws and regulations for e-cigarettes," Halpern-Felsher said. "Right now, the FDA is not going after manufacturers who have not put in an application to market e-cigarettes." The Family Smoking Prevention and Tobacco Control Act requires all tobacco companies to submit premarket applications to FDA and receive agency authorization before putting a product on the market, but the compliance dates have been extended to 2021 or 2022, depending on the product. This means thousands of e-cigarettes and other alternative tobacco products are being marketed without any FDA review.

When the study began, participants were 13 to 19 years old and attended California high schools. They completed questionnaires asking whether they had ever used traditional cigarettes or any alternative tobacco products, and whether they or their friends owned promotional items — such as coupons, free samples, T-shirts, posters and hats — for any type of nicotine product. The researchers asked similar questions about the use of cigarettes and alternative tobacco products a year later and analyzed changes in the teens' use of e-cigarettes and other tobacco products as a function of owning or receiving promotional materials.

At the start of the study, 81 of the 757 participants owned items that promoted tobacco products, including 52 who owned promotional items for e-cigarettes. Over the course of the study, 129 participants, or 17 percent, began using alternative tobacco products but not traditional cigarettes. Twelve participants began using traditional cigarettes alone or in combination with alternative tobacco products. Before adjusting for confounding factors, teens who owned promotional items were found to be 2.23 times as likely to try alternative tobacco products as those who did not own such items. After adjusting for age, gender, race/ethnicity, maternal education level and baseline alcohol and cigarette use, the teens who owned promotional materials were 2.13 times as likely as their peers to begin using alternative tobacco products. When teens who had tried both alternative tobacco products and cigarettes during the year were included in the analysis, the influence of owning promotional materials did not reach statistical significance.

> "We need to know trajectories of use of alternative tobacco products."

Study finds strong association

The findings provide evidence that ownership of marketing materials is strongly associated with more young people using e-cigarettes and other alternative tobacco products, the researchers said. The findings clearly show that no tobacco company, including any e-cigarette company, should be allowed to provide coupons, free samples or other marketing materials to teens, and suggest that the FDA should further restrict and enforce such marketing techniques, Halpern-Felsher said.

"Manufacturers say they're not marketing to teens, but teens are reporting owning these promotional items, and they're reporting use of alternative tobacco products," Magid said. Current restrictions and laws that make marketing cigarettes and other tobacco products to minors should be enforced for all nicotine-containing products, she added.

Halpern-Felsher is a member of Stanford's Maternal & Child Health Research Institute and the Stanford Cancer Institute. Researchers from the University of California-Berkeley and the University of California-San Francisco also contributed to the study. Magid was a graduate student at UC-Berkeley at the time the research was conducted.

The research was supported by grants from the National Cancer Institute and the Food and Drug Administration.

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

OF NOTE

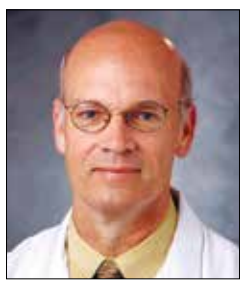
reports on significant honors and awards for faculty, staff and students

Rania Awaad

Heike Daldrup-Link

Maximilian Diehn

Carlos Esquivel



Donald Frush

Electron Kebebew

Abby King

Miquell Miller

Anca Pasca

Carla Pugh

RANIA AWAAD, MD, clinical assistant professor of psychiatry and behavioral sciences and director of the Stanford Muslim Mental Health Lab, received the community achievement award from Access California Services and Los Angeles County for exceptional commitment to promoting mental health well-being. The award was presented at Access California's 2019 Peace of Mind: A Family Wellness Event.

HEIKE DALDRUP-LINK, MD, professor of radiology, will receive the Harry Fischer Award for Excellence in Contrast Media Research at the 2019 Contrast Media Research Symposium in November. The award, which includes a $5,000 honorarium, recognizes a career of excellence in innovation, quality and leadership. It will be presented following her delivery of the Harry Fischer Lecture at the symposium.

MAXIMILIAN DIEHN, MD, PhD, associate professor of radiation oncology, is co-recipient of a Phillip A. Sharp Award for Innovation in Collaboration from Stand Up To Cancer. The two-year grant, totaling $225,000, will fund his work with Aaron Hata, MD, PhD, of the Massachusetts General Hospital Cancer Center, on analyzing cell-free RNA in non-small cell lung cancer in order to detect changes in cancer phenotypes during treatment.

CARLOS ESQUIVEL, MD, PhD, the Arnold and Barbara Silverman Professor in Pediatric Transplantation, professor of surgery and of pediatrics, and chief of the division of abdominal transplantation, was elected president of the International Pediatric Transplant Association. He will be president-elect for two years before taking office at the association's 2021 congress in Prague.

DONALD FRUSH, MD, professor of radiology, received the 2019 gold medal from the Society for Pediatric Radiology. The medal is awarded to pediatric radiologists and others who have contributed greatly to the society and the subspecialty of pediatric radiology as scientists, teachers, mentors and leaders.

ELECTRON KEBEBEW, MD, the Harry A. Oberhelman Jr. and Mark L. Welton Professor and chief of general surgery, has accepted the role of editor in chief of *Thyroid*, the official journal of the American Thyroid Association, starting with the January 2020 issue. He has served as an associate editor of the journal since 2012.

ABBY KING, PhD, professor of health research and policy and of medicine, will receive the inaugural lifetime achievement award for outstanding contributions to the field of behavioral nutrition and physical activity from the International Society of Behavioral Nutrition and Physical Activity. She will receive the award in June at the society's annual scientific conference in Prague.

MIQUELL MILLER, MD, resident in general surgery, was awarded the Claude H. Organ Jr., MD, FACS, Resident Research Award at the 2019 national conference for the Society of Black Academic Surgeons. Her abstract, "Racial disparities in surgical care and mortality across common tumor types," was recognized as one of the top three abstracts presented.

ANCA PASCA, MD, assistant professor of pediatrics, was a joint winner of the inaugural Bhatt-Ramanathan scholarship award from the California Association of Neonatologists. The award recognizes her accomplishments in advancing knowledge of brain injury from prematurity using human organoid models.

CARLA PUGH, MD, PhD, professor of surgery, was named the best plenary presenter at the Association for Surgical Education's 2019 meeting. Her presentation was titled "Use of error management theory to quantify and characterize residents' error recovery strategies."

Saul Rosenberg

SAUL ROSENBERG, MD, professor emeritus of medicine and the Maureen Lyles D'Ambrogio Professor in the School of Medicine, Emeritus, was one of 15 inductees into the 2019 Giants of Cancer Care awards program organized by OncLive, a group of specialized publications. He was recognized for his contributions in the clinical study of lymphoma. The Giants of Cancer Care program celebrates innovators who have made remarkable achievements in oncology research and clinical practice. ISM