



The science of well-being is examined in the summer issue of *Stanford Medicine* magazine.

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New view of evolution informs cancer research

By Jennie Dusheck

In his office, at the Canary Center at Stanford for Cancer Early Detection, Parag Mallick, PhD, played a video on a computer: It showed a flock of birds wheeling in a blue sky. An assistant professor of radiology, Mallick said the way birds in flight moved like a single, giant, living thing is key to an emerging view of the way cancer cells behave.

Such group behavior, whether in birds, fish or cells, arises from simple rules governing the behavior of each individual.

In a flock of birds, the rules might include how each bird always flies in the same direction as nearby birds and always stays close, though not too close, to them. But the coordinated, dancelike behavior of flocks can't be predicted by studying one bird at a time. Complex behaviors that only emerge in groups are called "emergent properties." For example, no single molecule has a temperature, but groups of them do.

What triggers metastasis?

At the Canary Center, Mallick and other researchers are building on such insights to develop a computer model of cancer. Working with a team that includes the center's director, Sam Gambhir, MD, PhD, professor of radiology; computer scientist Christopher Ré, PhD; and interns from local high schools, Mallick is looking at how cancer cells behave in order to discover what triggers their sudden transformations, or state changes, from quiet and comparatively harmless tumor cells into peripatetic, metastatic cells that migrate all over the body, invading and altering other tissues.

Just as hundreds of birds can suddenly take flight together and head off in one direction, swooping and turning in unison, tumor cells can perform similar feats.

When cancer cells transition to metastatic behavior, it can happen quite suddenly, said Mallick. Non-metastatic tumor cells might sit quietly inside a tumor with a clear boundary. But when metastasis starts, the same cells become lethal; they aggressively break through the



NORBERT VON DER GROEBEN

Parag Mallick is working with colleagues to develop a model of how cancer cells behave in order to discover what triggers their sudden transformations from quiet and comparatively harmless tumor cells into peripatetic, metastatic cells that invade other tissues.

wall of the tumor and launch themselves out into the rest of the body. "Cancer cells will spontaneously start to move in one direction," he said. But what makes cancer cells suddenly get the travel itch? And more generally, added Mallick, "What are the origins of such state changes? How do you describe them? How do we model them? What's governing their behavior?"

Of course, the behavior of cancer cells, like that of healthy cells, is hugely complex. For example, cells might behave in a cancerous way for reasons that are deep in their genes, or the change could be driven by signals from the environment. And metastatic cells might circulate in the blood for long periods before beginning to colonize other

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Technique could permit chemo-free bone-marrow transplant, study finds

By Christopher Vaughan

Blood stem cell transplantation, widely known as bone marrow transplantation, is a powerful technique that potentially can provide a lifelong cure for a variety of diseases. But the procedure is so toxic that it is currently used to treat only the most critical cases.

Now, researchers at the School of Medicine have come up with a way of conducting the therapy that, in mice, dramatically lowers its toxicity. If the method eventually proves safe and effective for humans, it potentially could be used to cure autoimmune diseases like lupus, juvenile diabetes and multiple

sclerosis; fix congenital metabolic disorders like "bubble boy" disease; and treat many more kinds of cancer, as well as make organ transplants safer and more successful.

"There is almost no category of disease or organ transplant that is not impacted by this research," said Irving Weissman, MD, a co-author of the research and professor of pathology and of developmental biology at Stanford. A paper describing the technique was published Aug. 10 in *Science Translational Medicine*.

The paper's senior author is Judith Shizuru, MD, PhD, professor of medicine. The lead authors are research associate Akanksha Chhabra, PhD; former graduate student Aaron Ring, MD, PhD, who is now on the faculty at Yale; and Kipp Weiskopf, MD, PhD, a former graduate student who is now a resident at Brigham and Women's Hospital.

Noxious treatment

To successfully transplant blood stem cells, a patient's

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SCIENCE PHOTO / SHUTTERSTOCK.COM

In hunt for safe but powerful painkiller, a promising new compound emerges

By Bruce Goldman

Investigators at the School of Medicine and their collaborators at three other institutions have identified a novel compound that appears to exhibit painkilling power comparable to morphine but lacks that drug's most lethal property: respiratory suppression, which results in some 30,000 drug overdose deaths annually in the United States.

"This promising drug candidate was identified through an intensively cross-disciplinary, cross-continental combination of computer-based drug screening, medicinal chemistry, intuition and extensive preclinical testing," said Brian Kobilka, MD, professor of molecular and cellular physiology.

Scientists at the University of California-San Francisco, the University of North Carolina and the Friedrich Alexander University in Erlangen, Germany, were pivotal to the work, described in a study published Aug. 17 in *Nature*.

Kobilka, a senior author of the study, credited Aashish Manglik, MD, PhD, a recent graduate of Stanford's Medical Scientist Training Program, as driv-

ing the study from the Stanford side. Manglik is one of the study's three lead authors.

The new compound's potential is enhanced by promising early signs, in mouse studies, that it may be less addictive than morphine and related drugs. While this reduced addiction potential

remains to be demonstrated definitively in other animal studies, it's strongly suggested by, among other things, the experimental mice's indifferent attitude toward solutions containing the compound compared with otherwise identical solutions lacking it.

A drug with these characteristics would come as good news to physicians, patients and public-health authorities deeply concerned about a growing epidemic of addictive-painkiller abuse.

"Opium and its derivatives are perhaps the oldest drugs in the pharmaceutical formulary," said Manglik, who is now the School of Medicine's first-ever Stanford Distinguished Fellow, which enables him to have his own laboratory and independent funding. "There's some evidence that their use predates written history."

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Brian Kobilka

Stanford, Google team up to harness data science for health care

By Jennie Dusheck

Stanford Medicine and Google are working together to transform patient care and medical research through data science.

The new collaboration combines Stanford Medicine's excellence in health-care research and clinical work with Google's expertise in cloud technology and data science. Stanford's forthcoming Clinical Genomics Service, which puts genomic sequencing into the hands of clinicians to help diagnose disease, will be built using Google Genomics, a service that applies the same technologies that power Google Search and Maps to securely store, process, explore and share genomic data sets.

Stanford Medicine includes the Stanford School of Medicine, Stanford Health Care and Stanford Children's Health. Together, Stanford Medicine and Google will build cloud-based applications for exploring massive health-care data sets, a move that could transform patient care and medical research.

"Stanford Medicine and Google are committing to major investments in preventing and curing diseases that afflict ordinary people worldwide. We're proud to be setting this milestone for the future of patient care and research," said Lloyd Minor, MD, dean of the School of Medicine.

The agreement — considered key to Stanford Health Care's development of the Clinical Genomics Service — makes Google Inc. a formal business associate of Stanford Medicine. As such, Google and Stanford will both comply with the Health Insurance Portability and Accountability Act, a federal law that regulates the privacy and security of medical information. HIPAA requires that Stanford Medicine patient data stored on Google Cloud Platform servers stay private. Patient information will be encrypted, both in transit and on servers, and kept on servers in the United States.

Analyzing genetic data

With Google Genomics, Stanford Medicine will build its new Clinical Genomics Service on the Google Cloud Platform, expanding genomics research and establishing new methods of real-time data analysis for efficient patient care. "We are excited to support the creation of the Clinical Genomics Service by connecting our clinical care technologies with Google's extraordinary capabilities for cloud data storage, analysis and interpretation, enabling Stanford to lead in the field of precision health," said Pravene Nath, chief information officer for Stanford Health Care.

The Clinical Genomics Service will enable physi-

cians at Stanford Health Care and Stanford Children's Health to order genome sequencing for patients who have distinctive or unusual symptoms that might be caused by a wayward gene. The genomic data would then go to the Google Cloud Platform to join masses of aggregated and anonymous data from other Stanford patients. "As the new service launches," said Euan Ashley, MRCP, DPhil, a Stanford associate professor of medicine and of genetics, "we'll be doing hundreds and then thousands of genome sequences."



Lloyd Minor



Euan Ashley

The Clinical Genomics Service aims to make genetic testing a normal part of health care for patients. "Genetic testing is built into the whole system," said Ashley. A physician who thinks a genome-sequencing test could help a patient can simply request sequencing along with other blood tests, he said. "The DNA gets sequenced and a large amount of data comes back," he said. At that point, Stanford can use Google Cloud to analyze the data to decide which gene variants might be responsible for the patient's health condition. Then a data curation team will work with the physician to narrow the possibilities, he said.

"This collaboration will enable Stanford to discover new ways to advance medicine to the benefit of Stanford patients and families," said Ed Kopetsky, chief information officer at Lucile Packard Children's Hospital Stanford and Stanford Children's Health. "Together, Stanford Medicine and Google are making a major contribution and commitment in curing diseases that afflict chil-

dren not just in our community, but throughout the world. It's an extraordinary investment, and we're proud to play such a large role in transforming patient care and research."

Ashley noted that medicine mostly deals in small data, such as lab tests. But genomic studies, patient health records, medical images from MRI and CT scans, and wearable devices that monitor activity, gait or blood chemistry involve huge amounts of data that can allow doctors and researchers alike to analyze myriad aspects of patient health in ways that lead to improved medical decisions and products that are tailored to the patient — the essence of a precision health approach.

Focusing on precision health

"In the past few years, the amount of available data about health care has exploded," said Minor. "While researchers are learning to integrate this big data, putting it to work for individual patients, in real time, is a huge challenge. Our collaboration with Google will help us to meet this challenge."

Sam Schillace, vice president of engineering for industry solutions at Google Cloud Platform, said, "I'm

excited because this agreement brings together expertise in three areas: data science, life science research and clinical care. The next decade of improvements in understanding and advancing health care is going to come from leaders in those three areas working together to build the next generation of platforms, tools and data."

It's all consistent with Stanford Medicine's focus on precision health. "You could imagine that, going forward, potentially every patient could be sequenced," said Michael Halaas, chief information officer for the School of Medicine. "The technology challenge we need to solve is how to derive useful insights from data and apply it directly to the care of a patient in near real time and also make progress on research."

Halaas said the Stanford-Google agreement does more than provide Stanford with server space. "It's not just stacks of servers," he said. "It includes layers and layers of innovative technology. This agreement allows us to do the analytics in a way that is fast and secure."

Minor said, "We'll be working with Google to build innovative technology that will enable Stanford to lead in precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill."

Data as the engine that drives research

Large-scale patient data is already helping answer research questions at Stanford. For example, Ami Bhatt, MD, PhD, an assistant professor of medicine and of genetics, is exploring changes in patient microbiomes that can precede symptoms of a disease such as cancer.

Another study is looking at alarm data from patient hospital rooms. The de-identified, or anonymized, data has been accumulating at Stanford's adult and children's hospitals for about 15 years, said Ashley, but until now no one has studied it. Hospitalized patients are typically hooked up to monitors that display their heart rate, blood-oxygen levels and other basic data, with alarms that go off if the measurements suggest something is wrong. The problem is that the alarms go off when nothing is wrong — sometimes when the patient just moves. Health-care providers often turn off the alarms so patients can rest and nurses can concentrate on people who need care. An artificial-intelligence approach in the works could use the alarm data to distinguish false alarms from real ones.

The analytics applications and virtual supercomputers available through Google Genomics could pave the way for other kinds of projects, as well. Working with Google's engineers, Stanford researchers could make advances in visual learning that might, for example, enable computers to distinguish malignant tumors from benign ones in medical images.

The Stanford-Google collaboration is a critical step on the path to precision health, Minor said. "This is the foundational work for bringing patient health information and other big data to the bedside," he said. ISM

One approach can prevent both teen obesity, eating disorders

By Erin Digitale

A single approach can prevent both obesity and eating disorders in teenagers, according to new guidelines from the American Academy of Pediatrics.

Scientific evidence summarized in the new recommendations shows that physicians and parents can ward off problems at both ends of the weight spectrum by avoiding focusing teens' attention on

weight or dieting, and instead encouraging a healthy, balanced lifestyle.

The guidelines, which were published online Aug. 21 in *Pediatrics*, were developed in response to growing concern about teenagers' use of unhealthy methods to lose weight. Teens who use these methods may not fit doctors' or parents' image of eating-disorder patients, since most are not excessively thin. However, their quick, substantial weight loss can

trigger medical consequences seen in people with anorexia nervosa, such as an unstable heart rate.

"This is a dangerous category of patient because they're often missed by physicians," said Neville Golden, MD, professor of pediatrics at the School of Medicine and a lead author of the new guidelines. "At some point, these patients may have had a real need to lose weight, but things got out of control."

Up to 40 percent of patients now admitted to some eating disorder treatment programs fit this easy-to-miss category, said Golden, who is also chief of adolescent medicine at Lucile Packard Children's Hospital Stanford and a physician with the hospital's Comprehensive Eating Disorders Program.

Evidence-based strategies

The new recommendations include five evidence-based strategies that pediatricians and parents can use to help teenagers avoid both obesity and eating disorders, and that apply to all teens, not

just those with weight problems. Three recommendations focus on behaviors to avoid: Parents and doctors should not encourage dieting; should avoid "weight talk," such as commenting on their own

weight or their child's weight; and should never tease teens about their weight. Two recommendations focus on behaviors to promote: Families should eat regular meals together, and parents should help their children develop a healthy body image by encouraging them to eat a balanced diet and to exercise for fitness, not weight loss.

"Scientific evidence increasingly shows that for teenagers, dieting is bad news," Golden said. Teens who diet in ninth grade are three times more likely than their peers to be overweight in 12th grade, for instance. And calorie-counting diets can deprive growing teenagers of the energy they need and lead to symptoms of anorexia nervosa, which may even become life-threatening. "It's not unusual for us to see young people who have rapidly lost a lot of weight but

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Neville Golden

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Computers trounce experts in diagnosing lung cancer type, severity

By Krista Conger

Computers can be trained to be more accurate than pathologists in assessing slides of lung cancer tissues, according to a new study by researchers at the School of Medicine.

The researchers found that a machine-learning approach to identifying critical disease-related features accurately differentiated between two types of lung cancers and predicted patient survival times better than the standard approach of pathologists classifying tumors by grade and stage.

“Pathology as it is practiced now is very subjective,” said Michael Snyder, PhD, professor and chair of genetics. “Two highly skilled pathologists assessing the same slide will agree only about 60 percent of the time. This approach replaces this subjectivity with sophisticated, quantitative measurements that we feel are likely to improve patient outcomes.”

The research was published Aug. 16 in *Nature Communications*. Snyder, who directs the Stanford Center for Genomics and Personalized Medicine, shares senior authorship of the study with Daniel Rubin, MD, assistant professor of radiology and of medicine. Graduate student Kun-Hsing Yu, MD, is the lead author of the study.

Although the current study focused on lung cancer, the researchers believe that a similar approach could be used for many other types of cancer.

“Ultimately this technique will give us insight into the molecular mechanisms of cancer by connecting important pathological features with outcome data,” said Snyder.

Assessing severity of cancer

For decades, pathologists have assessed the severity, or grade, of cancer by using a light microscope to examine thin cross-sections of tumor tissue mounted on glass slides. The more abnormal the

tumor tissue appeared — in terms of cell size and shape, among other indicators — the higher the grade. A stage is also assigned based on whether and where the cancer has spread throughout the body.

Often a cancer’s grade and stage can be used to predict how the patient will fare. They also can help clinicians decide how, and how aggressively, to treat the disease. This classification system doesn’t always work well for lung

cancer, however. In particular, the lung cancer subtypes of adenocarcinoma and squamous cell carcinoma can be difficult to tell apart when examining tissue culture slides. Furthermore, the stage and grade of a patient’s cancer doesn’t always correlate with their prognosis, which can vary widely. Fifty percent of stage-1 adenocarcinoma patients, for example, die within five years of their diagnosis, while about 15 percent survive more than 10 years.

The researchers used 2,186 images from a national database called The Cancer Genome Atlas obtained from patients with either adenocarcinoma or squamous cell carcinoma. The database also contained information about the grade and stage assigned to each cancer and how long each patient lived after diagnosis.

The researchers then used the images to “train” a computer software program to identify many more cancer-specific characteristics than can be detected by the human eye — nearly 10,000 individual traits, versus the several hundred usually assessed by pathologists. These characteristics included not just cell size and shape, but also the shape and texture of the cells’ nuclei and the spatial relations among neighboring tumor cells.

“We began the study without any preconceived ideas, and we let the software determine which characteristics are important,” said Snyder, who is the Stanford W. Ascherman, MD, FACS, Professor in Genetics. “In hindsight, everything makes sense. And the computers can assess even tiny differences across thousands of samples many times more accurately and rapidly than a human.”

Complementing ‘omics’ studies

The researchers homed in on a subset of cellular characteristics identified by the software that could best be used to differentiate tumor cells from the surrounding noncancerous tissue, identify

the cancer subtype, and predict how long each patient would survive after diagnosis. They then validated the ability of the software to accurately distinguish short-term survivors from those who lived significantly longer on another data set of 294 lung cancer patients from the Stanford Tissue Microarray Database.

Identifying previously unknown physical characteristics that can predict cancer severity and survival times is also likely

to lead to greater understanding of the molecular processes of cancer initiation and progression. In particular, Snyder anticipates that the machine-learning system described in this study will be able to complement the emerging fields of cancer genomics, transcriptomics and proteomics. Cancer researchers in these fields

study the DNA mutations and the gene and protein expression patterns that lead to disease.

“We launched this study because we wanted to begin marrying imaging to our ‘omics’ studies to better understand cancer processes at a molecular level,” Snyder said. “This brings cancer pathology into the 21st century and has the potential to be an awesome thing for patients and their clinicians.”

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.

Stanford co-authors of the study are former postdoctoral scholar Ce Zhang, PhD; professor of pathology Gerald Berry, MD; professor of bioengineering, of genetics and of medicine Russ Altman, MD, PhD; and assistant professor of computer science Christopher Re, PhD.

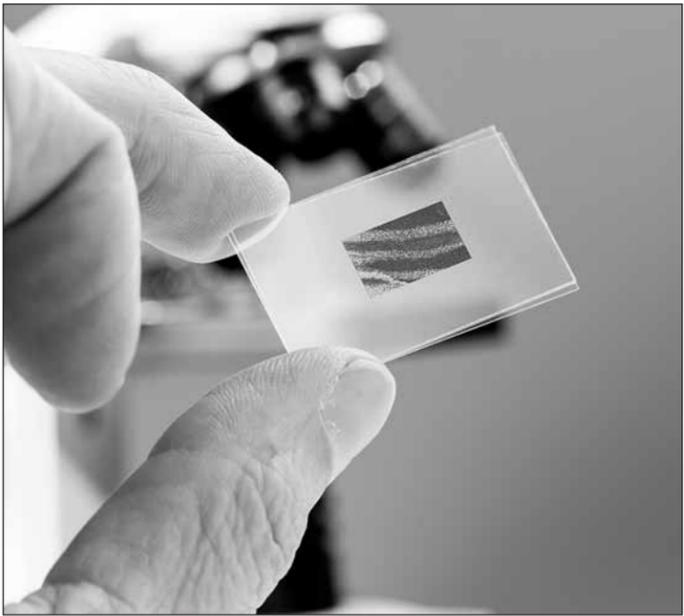
The study was supported by the National Cancer Institute and the National Institutes of Health.

Stanford’s Department of Genetics also supported the work. ISM



Michael Snyder

SCIENCE PHOTO / SHUTTERSTOCK.COM



For decades, pathologists have assessed the severity of cancer by using a light microscope to examine thin cross-sections of tumor tissue mounted on glass slides.

Anti-doping course offered for continuing medical education credit

The U.S. Anti-Doping Agency and the School of Medicine have developed the first anti-doping course that offers continuing medical education credit.

The online course, “HealthPro Advantage: Anti-Doping Education for the Health Professional,” is free for health-care professionals and can be taken at the Stanford Center for Continuing Medical Education website.

The course covers anti-doping roles and responsibilities, the World Anti-Doping Agency prohibited list, therapeutic-use exemptions, dietary supplements, the sample-collection process and specific anti-doping information for major games.

“USADA is thrilled to be working in partnership with Stanford, a prestigious academic institution and a leader in the field of online continuing medical education,” said Matthew Fedoruk, PhD, the agency’s science director.

USADA and the School of Medicine entered into a close collaboration, making it Stanford’s first partnership with an external organization for jointly provided continuing medical education.

“For our first experience jointly providing online CME, we couldn’t have picked a better partner for the journey,” said Linda Baer, MSPH, director of the Stanford Center for Continuing Medical Education. “USADA was positive and patient as we worked together to navigate through the technical and legal intricacies involved in producing a jointly-provided certified CME activity.”

Jason Dragoo, MD, associate professor of orthopaedic surgery at Stanford and co-director of the course, said, “It is timely, very well-done and a valuable course for any practitioner working with competitive athletes.” ISM

Guidelines

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are not healthy; they end up in the hospital attached to a heart monitor with unstable vital signs,” Golden said.

Negative comments about weight can also be detrimental to a teen’s health, Golden said. “Mothers who talk about their own bodies and weights can inadvertently encourage their kids to have body dissatisfaction, which we see in half of teen girls and a quarter of boys,” Golden said. Such dissatisfaction is associated with lower levels of physical activity and with use of vomiting, laxatives and diuretics to control weight.

Eating together

Family meals, on the other hand, protect against weight problems. The mechanism isn’t certain, but Golden thinks it may be partly due to the opportunity for teenagers to see their parents modeling healthy eating. “Pediatricians can encourage families to have family meals as often as possible,” he said. “It doesn’t have to be every night.”

The new advice is important in part because, although childhood obesity rates have begun to drop, obesity rates in adolescents have not declined. Helping teens maintain healthy weights without veering toward obesity or an eating disorder is more challenging than it is for young children. “Adolescents are also dealing with other issues, such as teasing from peers

and body-image concerns,” Golden said. “A 3-year-old may not be worried if she’s a bit overweight, whereas an adolescent may try unhealthy weight-loss methods like fasting or diet pills and end up in a vicious circle of more weight gain.”

Other lead authors of the new guidelines are Marcie Schneider, MD, who represented the AAP Committee on Adolescence, and Christine Wood, MD, who represented the AAP Section on Obesity. Additional experts from the American Academy of Pediatrics’ Committee on Nutrition, Committee on Adolescence and Section on Obesity also contributed to the guidelines. ISM

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Study: Automating genome comparison can identify diseases

NORBERT VON DER GROEBEN

By Erin Digitale

When Shayla Haddock was born in 1997, her parents immediately realized something was wrong. The sixth of seven children, Shayla had unusual facial features. She had club feet and shorter-than-normal limbs. She was smaller than most newborns. Hearing tests showed she was deaf.

As her parents, Cheryl and Levko Siloti, searched for answers about her condition, they worried: Had some preventable event during Cheryl's pregnancy caused Shayla's symptoms? Could identifying her diagnosis improve her treatment options? If Shayla's siblings wanted to become parents someday, would their children be at risk for the same illness?

"It was kind of an emotional roller coaster," Cheryl Siloti said. Over the years, doctors suggested many diagnoses for Shayla, but medical tests repeatedly disproved their theories. "We would get these possibilities and then hear 'Nope, that's not the answer.'"

The Stockton, California, family's quest for answers illustrates the challenges of diagnosing rare genetic diseases, and illustrates how and why scientists at the Stanford University School of Medicine are devising new approaches to help.

As much as Shayla's parents longed for a diagnosis, they almost didn't get one. On Aug. 10, 2012 — only two weeks after Shayla's doctors at Lucile Packard Children's Hospital Stanford concluded that they could not match her genetic patterns and symptoms to a disease — a scientific report about a newly discovered link between a genetic defect and a rare disease was published that would have allowed them to diagnose her. But at the time, genetic-testing results were not routinely re-analyzed to take into account new knowledge. The family and doctors remained unaware that the answer was out there.

Genetic re-analysis

Last year, as part of a scientific study, Shayla's parents agreed to have her genome re-analyzed. This time, Stanford computer scientists used new compu-

tational tools they had developed to compare Shayla's gene sequences to the scientific literature. They found the 2012 scientific report and predicted that Shayla had a rare genetic disease called Wiedemann-Steiner syndrome, which her doctors confirmed.

"With each passing month, more of the world's genetic diversity is represented in scientific databases, and each time more information is there, it's easier to interpret the next thing you see," said Jon Bernstein, MD, Shayla's clinical geneticist at Packard Children's and an author of the new report, which was published online July 21 in *Genetics in Medicine*. Ten percent of the patients in the study — four individuals, including Shayla, out of 40 who did not receive diagnoses after their first genetic analysis — were diagnosed with various rare diseases based on recent discoveries, even though the initial analyses had been conducted an average of only 20 months earlier.

These "near misses" highlight a big challenge in the realm of precision health: Although the speed, cost and effort involved in obtaining individuals' genetic sequences has dropped dramatically in recent years, it still requires about 20 to 40 hours of work by trained experts to match a patient's rare mutations to information in the scientific literature that might reveal a diagnosis. Among patients suspected of having a rare genetic disease,

75 percent aren't diagnosed the first time they have their DNA analyzed. And yet the knowledge base is growing fast. Each year, researchers discover the cause of about 250 genetic diseases and also find 9,200 links between specific gene variants and known diseases.

Too many to diagnose by hand

"Our study demonstrates that re-analysis of patients' gene-testing results is useful because there's a steady rate of discovery," said Bernstein, who is also an associate professor of pediatrics at the School of Medicine.

"But there is no way we'll have enough manpower to continue to do all the analysis manually, as clinicians and scientists have done in the past," said



Shayla Haddock was diagnosed with Wiedemann-Steiner syndrome, a rare genetic disease, after she had her genome reanalyzed in 2015.

Gill Bejerano, PhD, senior author of the study and associate professor of developmental biology, of computer science and of pediatrics.

Bejerano led the computer scientists who devised the automated approach used in the new research. Several million Americans may have some form of rare genetic disease, he noted — too many to diagnose by hand. "Rather than continuing to invest dozens of hours in each patient's analysis, our team thought it made more sense to spend that time building computer science tools that can do much of the work for us," he said.

Comparing genes

In the new study, the scientists tested whether automated comparisons between undiagnosed patients' genomes and existing gene databases could accelerate diagnosis. The approach worked.

"The genome is ultimately a programming language," Bejerano said. "We really would like to use machine learning and other approaches to build computer systems that leave as little as possible work for the human expert. A computer is going to be weaker than a human at doing this, but we think we can take the process 80 to 90 percent of the way by computer and provide a huge time savings for the human in the loop."

Another key finding from the new research, according to Bernstein and

Bejerano, is that comparing patients' gene sequences to those of their parents greatly speeds the diagnostic process. Such comparisons help turn up new disease-causing mutations that occurred in the patients but are not present in their parents. "These things stand out more easily if you have the parents' data in front you," Bernstein said.

In Shayla's case, her diagnosis brought her family the answers they'd long been seeking. She doesn't share her disease-causing mutation with her parents; instead, it occurred spontaneously in her. It wasn't preventable, nor is there any expectation it would affect her siblings' children. "It really relieves a lot of worry to know that," Siloti said.

The diagnosis also has helped the Silotis find other families whose children have the same diagnosis. They share stories on a Facebook group and feel they've found a new sense of support and community. "We've always believed that knowledge is power," Siloti said. "It is wonderful to have some answers, especially after such a long search."

The co-lead authors of the new study are research scientists Aaron Wenger, PhD, and Harendra Guturu, PhD. The research was funded by Stanford's Department of Pediatrics, the Stanford Discovery Fund, the Defense Advanced Research Projects Agency and the National Institutes of Health. **ISM**



Jon Bernstein

Heart muscle made from stem cells could aid precision medicine

By Krista Conger

Heart muscle cells made from induced pluripotent stem cells faithfully mirror the expression patterns of key genes in the donor's native heart tissue, according to researchers at the School of Medicine. As a result, the cells can be used as a proxy to predict whether a patient is likely to experience drug-related heart damage.

The discovery validates the use of such cells to test the potential cardiotoxicity of certain drugs and to devise new therapies for conditions like cardiomyopathy. Pinpointing people who are likely to suffer heart damage before they undergo treatment could increase the safety profile of many medications, the researchers believe.

"Thirty percent of drugs in clinical trials are eventually withdrawn due to safety concerns, which often involve adverse cardiac effects," said Joseph Wu, MD, PhD, director of Stanford's Cardiovascular Institute and professor of cardiovascular medicine and of radiology. "This study shows that these cells serve as a functional readout to predict how a patient's heart might respond to particular drug treatments and identify those who should avoid certain treatments."

Wu is the senior author of the study, which was published online Aug. 18 in *Cell Stem Cell*. Cardiovascular medicine instructor Elena Matsa, PhD, is the lead au-

thor of the research.

The ability to create stem cells from easily obtained skin or blood samples has revolutionized the concept of personalized medicine and made it possible to create many types of human tissue for use in the clinic. Researchers have wondered, however, whether the process of creating stem cells, and subsequently coaxing those stem cells to become other tissues, might affect the patterns of gene expression and even the ways the specialized cells function. If so, these changes could limit their clinical usefulness.

Testing the tissue

Matsa, Wu and their colleagues created heart muscle cells, or cardiomyocytes, from iPS cells from seven people not known to be genetically predisposed to cardiac problems.

They sequenced the RNA molecules made by the heart muscle cells to learn which proteins the cells were making, and how much. They then compared the results within individuals — looking at the gene expression patterns of cardiomyocytes derived from several batches of iPS cells from each person — as well as among all seven study subjects.

They also investigated how the cardiomyocytes from each person responded to increasing amounts of two drugs, one called rosiglitazone that is sometimes used to treat Type 2 diabetes and another called tacrolimus that

serves as an immunosuppressant to inhibit the rejection of transplanted organs. Each of the two drugs has been associated with adverse cardiac effects in some people, but it has not been possible to predict which patients will experience heart damage.

"We found that the gene expression patterns of the iPS cell-derived cardiomyocytes from each individual patient correlated very well," said Matsa. "But there was marked variability among the seven people, particularly in genes involved in metabolism and stress responses. In fact, one of our subjects exhibited a very abnormal expression of genes in a key metabolic pathway."

Identifying an unusual response

Heart muscle cells from this person, the researchers found, responded differently than the others to exposure to rosiglitazone. Concerns about its effect on cardiac function have caused the drug to be withdrawn from the market in Europe and have strictly limited its use in the United States.

"This person's cells produced abnormal amounts of reactive oxygen species, were unable to regenerate their mitochondria and contracted much more weakly when exposed to rosiglitazone than cells derived from the other subjects," said Matsa.

Although the researchers were unable to identify a specific genetic mutation likely to cause such an outcome, they were able to pinpoint an important metabolic pathway involved

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5 QUESTIONS

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

Robert Malenka discusses Ecstasy research

Neuroscientist Robert Malenka, MD, PhD, the Nancy Friend Pritzker Professor in Psychiatry and Behavioral Sciences, has

conducted trailblazing inquiries into the nature of the brain's reward circuitry. This archipelago of interacting brain structures is responsible for generating sensations of pleasure in connection with survival-enhancing behaviors, such as mating and eating, but also with self-destructive behaviors, such as the use of addictive drugs.

In a recent commentary in *Cell*, Malenka and Boris Heifets, MD, PhD, an instructor of anesthesiology at Stanford, called for focused study of the empathogenic drug 3,4-methylenedioxymethamphetamine, or MDMA.

This call is controversial because MDMA is illegal. Science writer Bruce Goldman asked Malenka about the drug, its origins, its clinical value and why neuroscientists are interested in researching it.

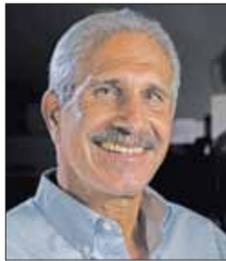
1 What is MDMA, and how and when did it get ensconced in the popular culture?

MALENKA: MDMA — also known by its street name, Ecstasy — was first synthesized by Merck in the early 1900s. Merck decided not to pursue the drug's possible therapeutic actions. But in the 1970s, a San Francisco Bay Area organic chemist, Alexander Shulgin, noted that it had a structure similar to both amphetamine and the hallucinogen mescaline. Shulgin was part of a community of like-minded individuals who were willing to ingest substances they made and see what they did. This small group quickly realized that MDMA's effects were qualitatively different from those of other drugs they had taken. It caused a powerful prosocial effect, greatly facilitating warm feelings of compassion and empathy toward all others with whom the users crossed paths. It helped stimulate a willingness to discuss personal issues and a desire to talk to others and get to know them. Fairly soon, a small group of therapists were trying out MDMA as an adjunct to therapy, with the idea that it might facilitate open communication and increased comfort in the therapeutic setting.

By the early 1980s, MDMA use had spread among the youth culture. People started taking MDMA in bars and nightclubs. Soon MDMA became a staple at large parties called raves, and its popularity spread throughout the country. However, reports started popping up of dangerous overheating and fatal electrolyte imbalances occurring among individuals on MDMA at densely packed parties. These types of events led the Food and Drug Administration to make MDMA a Schedule 1 drug. Recreational use continues to this day, but any effort to study it scientifically has become difficult.

2 What does "Schedule 1 drug" mean?

MALENKA: It's illegal. The Drug Enforcement Agency classifies drugs according to their medical utility and perceived dangerousness. Schedule 1 drugs are considered the most "dangerous" in that they're believed to have a high potential for abuse and no currently accepted medical use. It's illegal to manufacture, distribute or possess MDMA. This makes it very difficult to obtain small quantities even for strictly research purposes.



Robert Malenka

Like all psychoactive drugs, including legal substances like alcohol and nicotine and prescribed drugs such as antidepressants, MDMA can be dangerous if taken at high doses and frequently. Related drugs, such as amphetamine and cocaine, do have high abuse potential. And like any amphetamine derivative, MDMA may have longer-lasting deleterious effects.

However, even drugs like amphetamine have therapeutic uses — for example, treatment of attention deficit hyperactivity disorder — while cocaine is a very useful local anesthetic for certain types of surgery. It's not rational to demonize any drug. Each needs to be evaluated in a rigorous scientific manner to determine how dangerous it can be and whether it might be used for therapeutic purposes. Then rational decisions need to be made as to whether that drug should be allowed to be prescribed by physicians or should be made completely illegal.

3 What causes a chemical to be psychoactive?

MALENKA: Drugs are psychoactive because they affect specific proteins in the brain and, by modifying those proteins' functions, they alter activity in specific parts of the brain. This in turn can affect how the person feels or thinks or behaves. Just think about the effects of having a beer or two or three or having several strong cups of espresso. Those psychoactive effects are because the alcohol in the beer or the caffeine in the espresso is modifying the activity in specific parts of the brain.

4 What's MDMA's clinical value?

MALENKA: MDMA is beginning to be tested as an adjunct to therapy in post-traumatic stress disorder, as well as in people with certain forms of autism in which social interactions are not normal. In very early studies, MDMA appears to be helping individuals with PTSD learn from their therapy and get relief from their often devastating symptoms. It's too early to know whether MDMA will prove useful for people with autism spectrum disorders or for other types of disorders which involve dysfunctional social behavior. But I believe it is certainly worthwhile to perform rigorous, careful and ethical clinical trials to find out whether MDMA might have therapeutic benefit.

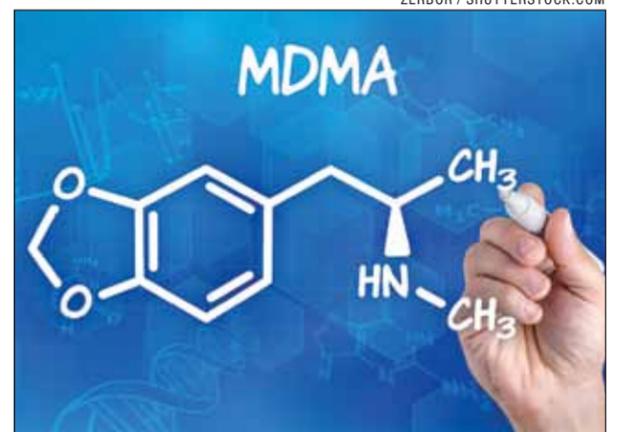
5 You've called for intensive research on how MDMA works in the brain. What makes it of special interest to neuroscientists?

MALENKA: The rigorous scientific study of drugs can teach us about how the brain functions. I hope that by figuring out how MDMA works in the brain, we will learn how to make new, better drugs that will have some of the same potential therapeutic benefits of MDMA while minimizing its abuse potential and any toxic effects it might have.

I don't think it's useful to study psychoactive drugs indiscriminately. Each drug needs to be considered individually, and the value of its study needs to be weighed against its potential danger and the potential usefulness of the information that studying it will generate.

MDMA is special. Unlike amphetamine, cocaine, LSD, psilocybin, alcohol, heroin or any other

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known drug, it affects how one human being deals with another human being. After ingesting MDMA, it's extremely difficult if not impossible to feel anger or hostility toward another person. Studying how MDMA works in the brain might provide important insights into how the brain generates prosocial feelings and behavior. We neuroscientists hope to identify the specific molecules in the brain with which MDMA interacts and how those interactions modify activity in specific brain circuits to generate these powerful prosocial feelings and even empathy. At a time when hostility and irrational anger toward fellow human beings appears to be increasing, I cannot imagine a more important topic for neuroscientists to investigate. **ISM**

New issue of *Stanford Medicine* magazine examines well-being

By Kathy Zonana

Humans long for a sense of well-being. For thousands of years, everyone — from philosophers such as Aristotle, Epictetus and Buddha to the smooth-talking snake-oil salesman — has tugged at the problem of happiness, well-being and what makes for a good life.

Despite those endeavors, there is precious little data on what well-being means and how to attain it. "The vast majority of biomedical research has focused on treating diseases, while a much smaller part has focused on maintaining health and maybe some prevention efforts," said John Ioannidis, MD, DSc, professor of medicine and director of the Stanford Prevention Research Center. "There's very, very little research that has tried to look at the big picture — what makes people happy, resilient, creative, fully exploring their potential and living not only healthy, but more-than-healthy lives."

But that research is expanding — and it's the subject of a special report in the summer issue of *Stanford Medicine* magazine, "Strive, thrive and take five: The

science of well-being."

The report, produced with the support of the Stanford Prevention Research Center, includes a Q&A with author Laura Hillenbrand, who wrote the best-sellers *Seabiscuit* and *Unbroken* while grappling with severe chronic fatigue syndrome. The online version of the magazine includes audio of the conversation with Hillenbrand on what it means to be well when you have been unwell for decades.

Also in the special report:

- A feature on Stanford researchers' efforts to define, measure and improve wellness in 30,000 people in the Bay Area, China and Taiwan.

- A piece on "stealth health," and why tapping into people's broad environmental, ethical or cultural motivations may be more successful than just telling them to eat better and exercise more.

- A story on how the local American Indian community asked Stanford researchers to help develop a culturally informed approach to diabetes prevention.

- An article on why research studies must be robust and reproducible if med-

ical science is to keep us well.

- A look at how empowerment programs for girls and boys in Kenya could reduce the incidence of sexual assault.

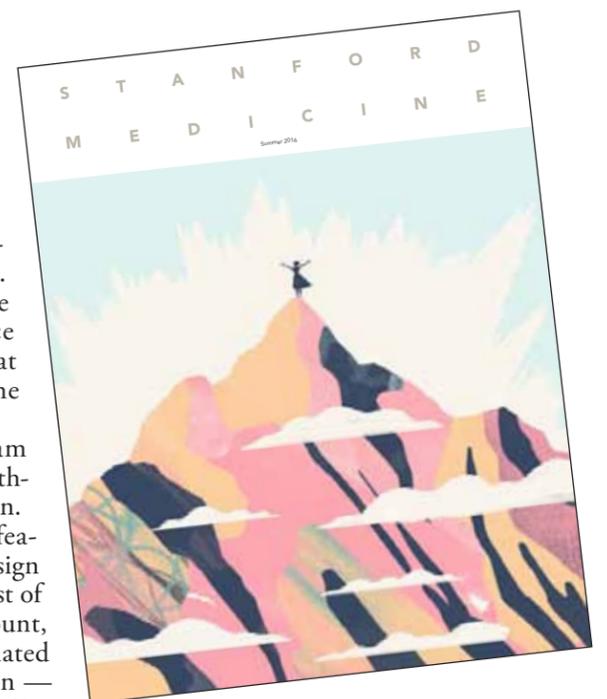
- A feature about innovative ways to convince people to quit smoking, using new technologies and policy approaches.

- An article about how the frequency of cash assistance payments can influence what low-income people buy at the market.

- A quick look at a program that aims to convince college athletes they should wear sunscreen.

The issue also includes a feature about the Stanford Biodesign program's efforts to take the cost of medical innovations into account, and an article on how age-related chronic systemic inflammation — aka "inflammaging" — may affect your heart.

The magazine is available online at <http://stanmed.stanford.edu/2016summer.html>. Print copies are



being sent to subscribers. Others can request a copy at 723-6911 or by sending an email to medmag@stanford.edu. **ISM**

Cancer

continued from page 1

parts of the body.

Yet we do know that no single governor gives a top-down order to all the cells; instead, just like a flock of birds taking wing, the cells all begin moving at once, responding to one another.

Building a model

In an attempt to detect, predict and prevent such transitions, Mallick and his colleagues are building a massive computer model of cancer that includes every level of organization, starting from molecular processes and the behavior of individual cells to the growth of whole tumors and their metastasis, as well as immune responses throughout the body. "We're working on coalescing all of that information into what, in our mind, is the first-ever truly multiscale data set," he said.

Mallick's forte is finding ways to connect all these different levels of organization. One connection is the sudden transition from the independent behavior of cancer cells to group behavior. Another might be a nutrient gradient across a tumor that connects the effects of nutrients on individual cells with those on the whole tumor.

"If you are modeling water," he said, "there's a particular sort of math that you use to describe the behavior of single atoms, and a very different sort of math for describing the flow of rivers." For a multiscale model of water, you would need a way for those two models to connect.

Putting the pieces together means accepting that the very theory of how cancer works is evolving.

An evolving theory of cancer

Decades of work had researchers convinced that cancer resulted from genetic mutations in individual cells. The theory was that a carcinogen, such as asbestos or cigarette smoke, induced mutations in a cell's DNA that eventually caused it to become cancerous. That bad cell multiplied and spread.

But it has turned out that most of the things that cause cancer, including tobacco smoke and asbestos, don't cause mutations. Rather than modifying the genes themselves, smoke and asbestos alter the activity of genes through a collection of processes called epigenetics.

Epigenetics consists of tiny modifications — either to the DNA itself or to proteins called histones that wrap around the DNA and change the activity of the genes. For example, if you spend every weekend gardening, changes in the activity of genes in the skin cells of your hands will produce callouses.

Our callouses might seem very ordinary to us; they come and go depending on what we've been up to recently. But what if the genes whose activity changes to produce them could mutate so that our callouses became permanent? What if some babies were born with calloused hands?

Amazingly, modern evolutionary biologists are moving to the view that that's exactly how wild plants and animals often evolve.

It all starts with the phenotype, which is every single trait of an organism or cell other than the genome itself. The phenotype includes the actual enzymes encoded by genes, myriad metabolic pathways, the shape of a nose or the hands, a vast repertoire of behavior and even memories of an equation or a loved one.

We already know that the same genes can produce alternate phenotypes, depending on just how the genes are expressed. That phenotypic plasticity delivers different castes of ants, all from the same genotype; hands that look different from our feet, even though they have the same genotype; and identical twins of different heights and personalities. All these changes arise from the way the immediate environments of cells, or of organs, or of whole individuals interact with genes. The differences in gene activity are mediated by an array of hormones, transcription factors and other mechanisms.

'Genes are followers, not leaders'

Evolutionary biologist Mary Jane West-Eberhard, PhD, one of the leaders of the movement to reframe evolution, has laid out the experimental evidence showing that the plasticity of an organism's characteristics, or phenotype, foreshadows its evolution. In essence, you can start with an epigenetic variant — think calloused hands — and later that particular trait can become permanently fixed in the genes.

Famously, West-Eberhard said, "Genes are followers, not leaders, in evolution." Now that same idea is invading the theory of cancer. It seems that cancer cells, too, can first begin to change through temporary epigenetic

changes, instead of by means of mutations in the DNA.

In cancer biology, the role of epigenetics is gaining acceptance, but it's still meeting resistance from researchers who may have spent a lifetime with the idea that cancer cells are primarily the result of individual mutations, said Alexander Anderson, PhD, chair of integrated mathematical oncology at the Moffitt Cancer Center, in Tampa, Florida. "There's still definitely an old-school crowd who think if we just sequence deep enough, we'll solve all the problems."

A recent article in *The New Yorker* about epigenetics by Siddhartha Mukherjee, MD, DPhil, triggered a storm of complaints from molecular biologists who felt that standard genetics had been ignored. But while it's possible to quibble about whether Mukherjee, an assistant professor of medicine at Columbia University, should have put his discussion of epigenetics in context, there's no question that epigenetics is deeply altering our understanding of both evolution and cancer. "There's a feeling in the field that we have to start thinking more holistically," said Anderson. And the key to that, he said, is math.

A systems approach

Mallick, said Anderson, is one of a few researchers with a strong understanding of both cancer biology and the mathematics needed to build a model of cancer based on a systems approach.

Said Mallick, "We just had a paper accepted where we found that when you treated cells with a chemotherapeutic drug over long periods of time, you could make cells that were 40 times more drug-resistant. Yet the cells had no genetic alterations." Instead, all the changes were epigenetic. "If you treated the cells with the drug, they were like, 'Oh, OK, let me change my histones,'" he said. "It's a crazy thought."

While the mechanisms for changes may be modifications to the histone proteins or the DNA, the driver of change is the environment. It is now well-established that epigenetic changes play a role in both cancer initiation and progression. The same processes may also determine if cells are cancerous or healthy, metastatic or not.

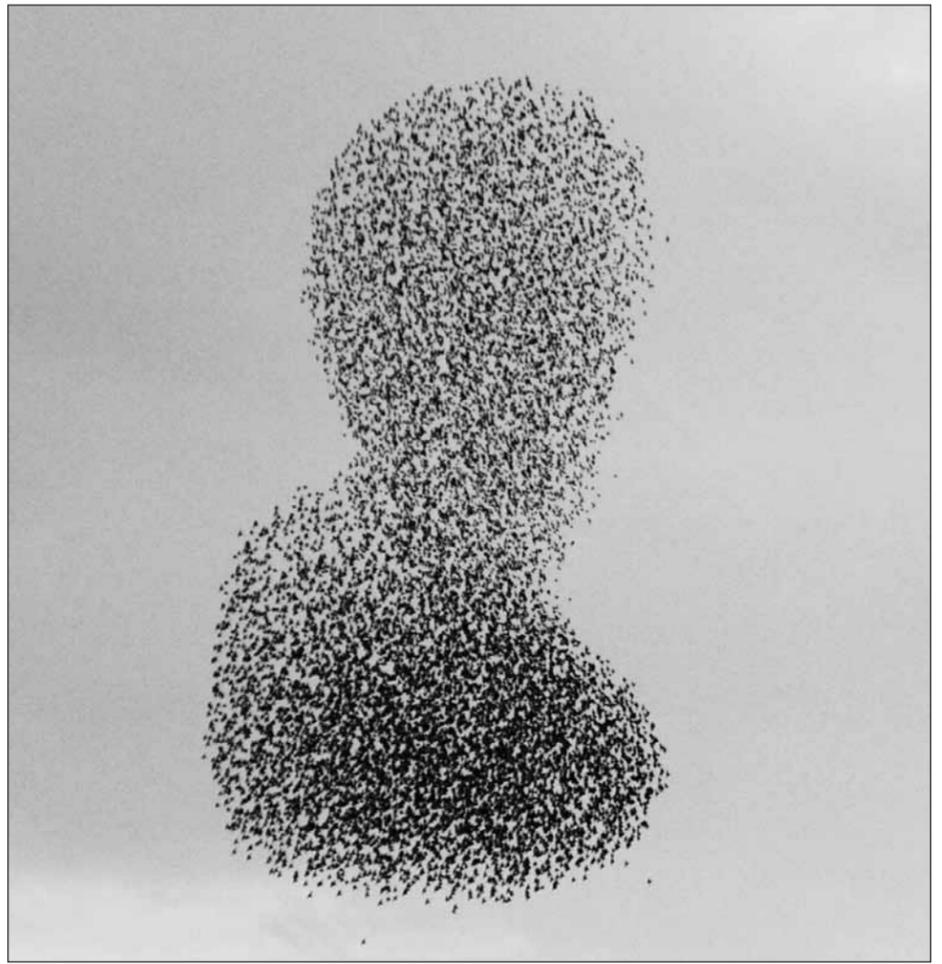
Cancer, explained renowned developmental biologist Scott Gilbert, PhD, of Swarthmore College, can result not only from bad cells but from a bad cellular environment.

For cancer cells, said Mallick, that means where the cells live in a tumor, how close they are to nutrient-rich blood vessels, the behavior of nearby cells and where the cells are in the body. Each of these situations can induce a range of epigenetic reactions that can impact, for example, how resistant or sensitive the cells are to chemotherapy drugs or how likely the cells are to begin to metastasize.

A tumor comprises an array of ecological niches, each of which can induce a different kind of behavior or phenotype in the cancer cells that live there, said Anderson. But just as a tropical rainforest functions similarly whether it's on one continent or another, different kinds of tumors share common rules that govern their overall behavior and the phenotypes of individual cells in different parts of the tumor.

Animals and other organisms can pass epigenetically mediated traits to multiple generations without any change in the genes themselves. And at least some of these phenotypic traits can become permanently fixed in the genome, as demonstrated in lab studies. It makes sense then that cancer cells could do the same.

Mallick said the epigenetic changes that incite tumor cells to resist deadly drugs are passed on to daughter cells. Although no one has witnessed it happen, it's pretty clear that the right mutation could turn the trait



A flock of starlings flies over Great Yarmouth in England. Mallick says that emergent properties, such as this group behavior, may help explain how populations of tumor cells become malignant.

for drug resistance from plastic to permanent, making the trait part of the cancer cells' permanent genetic repertoire.

As Gilbert said, "You start off with an epigenetically induced phenotype. And then if any mutations occur that allow this to be fixed into the genome, it goes for it."

Markerville

This new way of understanding evolution is the theoretical engine that drives Mallick's research. Viewing cancer as a dynamically evolving adaptive system, his team's big focus is the giant model of cancer behavior that integrates all the different levels. "Our entire purpose in life is to build a virtual model of cancer," said Mallick.

The ultimate goal of the model is to explain cancer, but the model also has immediate medical uses. For instance, Mallick is using the model as a tool to help identify markers of important transitions in the life of populations of cells — to cancer, to drug resistance or to metastasis. Such markers are essential to developing tests for diagnosing cancer and for investigating how patients respond to treatment over the course of their disease.

Mallick and his colleagues are on the verge of launching a publicly accessible, interactive database and model of cancer called Markerville. "It includes both a model of cancer and a collection of data we've pulled from the literature about each protein," he said. Markerville will tell users everything known about a particular protein and also how it might be expected to serve as a marker in a given cancer. "Our goal is to build a computer program that you could come to with any protein and say, 'OK, I'm interested in this protein and I'm looking at this cancer. Do you think it has potential to be a good biomarker?'"

Our understanding of cancer biology has taken off in recent years, but it's not yet clear where it's leading researchers. Just as it's difficult to see which way the individual birds in a flock will turn from moment to moment, it's difficult to predict which discoveries will transform our understanding of cancer. But changes in the understanding of both basic evolutionary biology and systems biology are helping researchers see things in new ways. **ISM**

"There's a feeling in the field that we have to start thinking more holistically."

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Transplant

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own population of blood stem cells must be killed. Currently, this is done using chemotherapy or radiotherapy, treatments that are toxic enough to damage a variety of organs and even result in death. “The chemotherapy and radiation used for transplant damage DNA and can cause both immediate problems and long-term damage to many tissues in the body,” Shizuru said. “Among the many known toxic side effects, these treatments can cause damage to the liver, reproductive organs and brain, potentially causing seizures and impairing neurological development and growth in children.” For these reasons, blood stem cell transplantation is used only when the risks of serious disease outweigh the complications from the transplant.

To avoid these terrible side effects, the Stanford researchers composed a symphony of biological instruments that clear the way for blood stem cell transplantation without the use of chemotherapy or radiotherapy.

Using antibodies

The scientists started with an antibody against a cell surface protein called c-kit, which is a primary marker of blood stem cells. Attaching the antibody to c-kit resulted in depletion of blood stem cells in immune-deficient mice. “However, this antibody alone would not be effective in immune-competent recipients, who represent a majority of potential bone marrow transplant recipients,” Chhabra said. The researchers sought to enhance the effectiveness

by combining it with antibodies or with biologic agents that block another cell surface protein called CD47. Blocking CD47 liberated macrophages to “eat” target cells covered with c-kit antibody, Chhabra said.

With the CD47 marker blocked and the antibody attached to c-kit proteins, the immune system effectively depleted the animals’ blood-forming stem cells, clearing the way for transplanted blood stem cells from a donor to take up residence in the bone marrow and generate a whole new blood and immune system.

Comparing blood stem cell transplants to planting a new field of crops, Shizuru noted that the researchers not only found a safer way to clear the field for planting, but “we also used safer techniques to seed the new blood-generating cells.” Currently, bone marrow transplants involve a mix of cells that includes blood stem cells as well as various immune cells from the donor, which can attack the tissue of the transplant recipient. This immune attack results in what is called graft-versus-host disease, which can damage tissues and even kill patients.

Building on knowledge gained from previous research, the team purified the donor tissue so that it contained only blood stem cells and not the other immune cells that cause graft-versus-host disease.

The success of these techniques in mice raises hopes that similar techniques will succeed in human patients. “If it works in humans like it did in mice, we would expect that the risk of death from blood stem cell transplant would drop

from 20 percent to effectively zero,” Shizuru says.

‘New era in disease treatment’

“If and when this is accomplished, it will be a whole new era in disease treatment and regenerative medicine,” said Weissman, who is director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, as well as the director of the Ludwig Center for Cancer Stem Cell Research and Medicine.

Once a patient’s blood and immune system can safely be replaced, any disease caused by the patient’s own blood and immune cells could potentially be cured by a one-time application of blood stem cell transplantation, they said. Safely replacing a patient’s blood and immune cells will get rid of the cells that attack their own tissues and produce disease like rheumatoid arthritis and Type 1 diabetes.

A method of safely doing blood stem cell transplants would also potentially make organ transplantation safer and easier, the researchers said. Currently, people who get an organ transplant must for the rest of their lives stay on drugs that keep their immune systems from attacking the transplanted organ. “Even if you are on immunosuppressants, most organ transplants diminish in function or fail over time, and the immunosuppressive drugs themselves make the patient more susceptible to life-threatening infections or newly forming cancers,” Weissman said.

But if blood and immune stem cells from the organ donor can be transplanted at the same time as the organ, the new immune system will recognize the donated

organ and not attack it, the researchers said. “The transplanted cells, the donated organ and the patient’s own tissues all learn to coexist,” Shizuru said. “The donor blood stem cells re-educate the immune system of the patient, and the transplanted organ doesn’t get kicked out.”

Blood and immune stem cell transplants may also be critical to making the new era of regenerative medicine a success. If stem cells for organs or tissues like heart or liver are grown for general transplantation — that is, not designed specifically for one patient — the patient will require immune conditioning through blood stem cell transplantation so that the stem cells are not rejected as foreign bodies, the researchers said.

Other Stanford-affiliated co-authors of the work are graduate student Sydney Gordon; research assistant Alan Le; research associate Hye-Sook Kwon, PhD; former medical fellow Nan Guo Ring, MD; Jens-Peter Volkmer, MD, an instructor at the Institute for Stem Cell Biology and Regenerative Medicine; former research assistants Serena Tseng and Peter John Schnorr; and former research assistant Po Yi Ho.

Support for this research came from the Virginia and D.K. Ludwig Fund for Cancer Research, the California Institute for Regenerative Medicine, the National Institutes of Health, the Stanford Medical Science Training Program, the Tom and Stacy Siebel Foundation, the Stinehart-Reed Foundation, the Gunn/Oliver Research Fund, and the HL Snyder Medical Foundation.

Stanford’s departments of Medicine, of Pathology and of Developmental Biology also supported the work. **ISM**

Painkiller

continued from page 1

A natural extract of the opium poppy, morphine was, in the 19th century, the first natural substance purified to homogeneity for medical use, Manglik said.

But respiratory suppression remains a general drawback of opioids, which in addition to morphine include the prescription painkillers codeine, oxycodone, oxycontin, hydrocodone and fentanyl, as well as illicit drugs such as heroin. Designing a safer molecule required close collaboration between Stanford and scientists at three other institutions.

The hunt for a safer painkiller

The new compound’s identification made use of the three-dimensional structure of the mu opioid receptor determined by Manglik and colleagues in the Kobilka lab in 2012. The receptor, via which morphinelike drugs exert the bulk of their potent painkilling effect, is a member of a family of structurally similar cell-surface proteins found throughout the brain and spinal cord. When bound by morphine or one of its many natural or synthetic analogs, these receptors initiate signaling processes that alter the activities of other proteins inside the cells on which they sit.

Earlier work by other researchers established that morphine-resembling drugs’ analgesic effect is brought about by a particular cascade of downstream chemical reactions set in motion when these drugs bind to the mu opioid receptor, while their respiration-suppressing effect is induced by a separate molecular pathway tripped off by the same binding event.

Safely reproducing morphine’s benefits meant finding a way to separate those two effects. The trick was to activate the mu opioid receptor but not any of the other opioid receptors — and, having done so, to stimulate only the molecular pathway responsible for inducing analgesia and not the pathway responsible for respiratory suppression.

“The field had wondered whether a small molecule with just the right chemical features to trip off one pathway, but

not the other, could be designed,” said Manglik. Determining the mu opioid receptor’s structure enabled detailed analysis of the receptor’s binding pocket, into which opioids fit like a hand in a glove. This, in turn, propelled an interdisciplinary collaboration with scientists at UCSF, UNC and FAU.

Using a ‘virtual medicine cabinet’

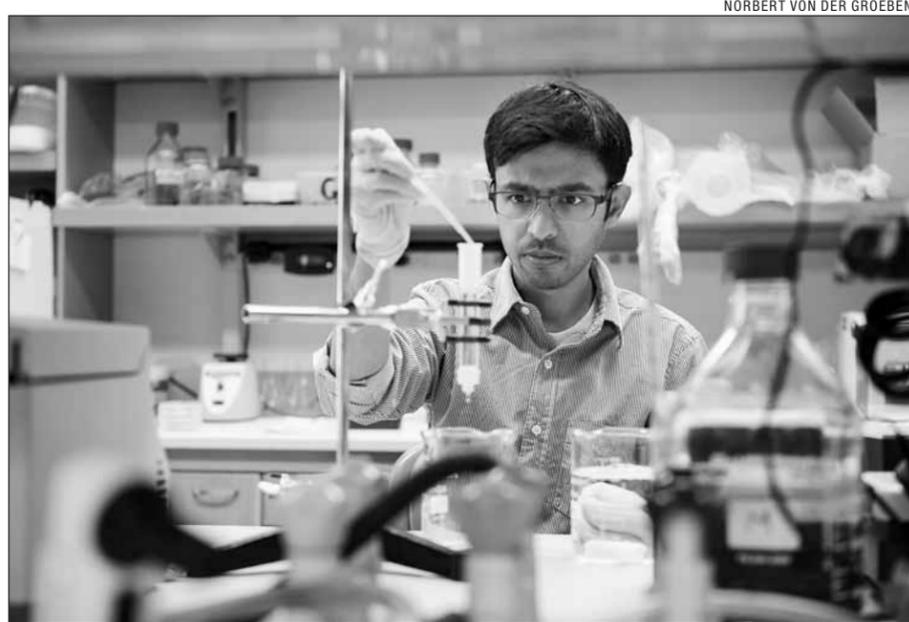
Manglik and Kobilka enlisted Henry Lin, PhD, then a graduate student in the lab of UCSF pharmaceutical chemistry professor Brian Shoichet, PhD. (Lin is a lead author and Shoichet is a senior author of the study.) After computationally screening about 3 million commercially available or easily synthesized compounds in a “virtual medicinal-compound cabinet” created by Shoichet’s group, Manglik and Lin focused on 2,500 compounds that, computer simulations suggested, may bind to the mu opioid receptor. From those, they culled a few dozen that looked like especially good candidates for further inspection.

After testing 23 of these compounds and narrowing the field to seven, Lin and Manglik returned to the Shoichet group’s online database, searched for similar compounds worth testing and found another dozen or so.

A dose of intuition

These compounds were sent to the laboratory of Bryan Roth, MD, PhD, a professor of pharmacology and of medicinal chemistry at UNC, who analyzed them further and found that one strongly activated the “good” downstream molecular pathway without significantly recruiting the “bad” pathway. Though promising, the compound was not sufficiently potent to work as a therapeutic. To optimize its properties, the group enlisted the help of Peter Gmeiner, PhD, chair and professor of medicinal chemistry at FAU. Gmeiner’s group created numerous versions of the compound, and identified one that bound the mu opioid better than its predecessor.

An intuitive insight on Manglik’s part led to a final tweak: the addition, in Gmeiner’s lab, of a chemical feature



NORBERT VON DER GROEBEN

Aashish Manglik helped lead a collaborative effort to identify a compound that appears to be similar to morphine in its painkilling power but less addictive.

called a hydroxyl group that would stabilize the molecule’s “fit” inside the receptor’s binding pocket. The resulting molecule, which the investigators named PZM21, had a mu opioid-binding strength about 1,000 times that of the compound in the original database from which it was derived.

Still more tests in the Roth lab showed that PZM21 not only didn’t cause any significant activity in other opioid receptors but actually prevented activity in one of them, the kappa receptor, whose activation is associated with uneasiness and, sometimes, hallucinations.

Experiments in mice by co-lead author Dipendra Aryal, PhD, a research associate in the Roth lab, bore out predictions of PZM21’s analgesic efficacy — it was as powerful as morphine — and its benign character with respect to the suppression of breathing, compared with morphine. Given a choice between two chambers, one paired to an injection of a solution containing PZM21 and the other to an otherwise identical solution that lacked PZM21, the mice showed no preference for either chamber. By comparison, if one of the chambers is

paired with morphine, mice are known to spend substantially more time in the morphine-paired chamber.

Other experiments performed in the Stanford laboratory of Gregory Scherrer, PhD, assistant professor of anesthesiology, perioperative and pain medicine and of neurosurgery, showed that PZM21 had no effect on mice bioengineered to lack the mu opioid receptor, confirming the compound’s mechanism of action.

Gmeiner and Roth also share senior authorship of the study. Another Stanford co-author is postdoctoral scholar Gregory Corder, PhD.

A biotechnology company, Epiodyne, that Kobilka, Shoichet, Gmeiner, Roth and Manglik have formed is negotiating PZM21’s licensing from the four academic institutions for further development.

The study was funded by the National Institutes of Health, the Stanford University Medical Scientist Training Program, the American Heart Association and the German Research Foundation

Stanford’s Department of Molecular and Cellular Physiology also supported the work. **ISM**

Karl Deisseroth wins 2016 Massry Prize for optogenetics work

By Bruce Goldman

Karl Deisseroth, MD, PhD, professor of bioengineering and of psychiatry and behavioral sciences at Stanford, has been named a recipient of this year's Massry Prize for his pioneering efforts in the development of a breakthrough technology called optogenetics.

Deisseroth, who is the D.H. Chen Professor, shares the prize with two other researchers: Peter Hegemann, PhD, professor and chief of biophysics at the Humboldt University of Berlin, Germany; and Gero Miesenboeck, MD, professor of physiology at the University of Oxford in the United Kingdom. The three scientists will split the \$200,000 honorarium accompanying the award.

"It's a wonderful honor to share this prize with my colleagues Peter and Gero, and to recognize here also the students and postdocs in my lab over the past 12 years who have so creatively discovered, de-

veloped and applied the fundamental elements of optogenetics," Deisseroth said.

Optogenetics entails the installation of light-sensitive proteins, derived from microbes and delivered via gene vectors, on the surface of selected cells in a living, freely moving mammal. As a result, these cells can be either excited or inhibited by specific frequencies of laser light, which is delivered via a surgically implanted optical fiber.

The ability to turn on or turn off electrical activity in a precisely defined set of cells in selected parts of the brain allows researchers to gain insights into not only the causal mechanisms behind the organ's normal workings but also the defects in function that accompany brain disorders such as Parkinson's disease, depression and schizophrenia. Optogenetics has also been used to turn on and off electrical activ-



Karl Deisseroth

ity in heart and kidney cells, as well as in other tissues.

Deisseroth has received many previous awards for his work in optogenetics, most recently a \$3 million Breakthrough Prize, initiated by a consortium of Silicon Valley entrepreneurs, in 2015.

The Massry Prize, sponsored by the Meira and Shaul G. Massry Foundation and administered by the University of Southern California, has been awarded annually since 1996. The list of previous winners includes three Stanford School of Medicine faculty members: James Spudich, PhD, professor of biochemistry; Andrew Fire, PhD, professor of pathology and of genetics; and Roger Kornberg, PhD, professor of structural biology. Twelve Massry Prize recipients, including Fire and Kornberg, have gone on to receive a Nobel Prize. **ISM**

OF NOTE

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

EDDY ALBARRAN, a graduate student in neurosciences, has received a Gilliam Fellowship for Advanced Study from the Howard Hughes Medical Institute. The fellowships were created to increase diversity in the scientific workforce. Albarran will receive \$46,000 a year for up to three years.

JONATHAN BEREK, MD, the Laurie Kraus Jacob Professor and professor and chair of obstetrics and gynecology, was appointed as one of two U.S. representatives to the International Federation of Obstetrics and Gynecology Gynecologic Oncology Committee, which is responsible for the staging of gynecologic cancers. He is the director of the Stanford Women's Cancer Center, of communication and special programs at the Stanford Cancer Institute and of the Stanford Health Care Communication Program.

BERNARD DANNENBERG, MD, the Davies Family Director of Pediatric Emergency Medicine and clinical professor of emergency medicine, has received the 2016 Martha Bushore-Fallis Advanced Pediatric Life Support Award from the American Academy of Pediatrics. Dannenberg was honored for championing pediatric emergency medicine among pediatric and emergency physicians in Northern California.

ELIZABETH EGAN, MD, PhD, and **JOSHUA KNOWLES**, MD, PhD, have received 2016 Clinical Scientist Development Awards from the Doris Duke Charitable Foundation. Awardees receive \$495,000 over three years to launch their research programs and to help balance their



Eddy Albarran



Jonathan Berek



Bernard Dannenberg



Elizabeth Egan



Joshua Knowles

clinical and research roles. Egan, an assistant professor of pediatrics, is studying host genetic susceptibility to malaria. Knowles, an assistant professor of medicine, is examining the risk factors and mechanisms of statin-associated diabetes.

MIRIAM GOODMAN, PhD, was promoted to professor of molecular and cellular physiology, effective June 1. Her research focuses on the molecular and genetic bases of the sensation of touch and on the ability of peripheral neurons to withstand mechanical stress using *C. elegans* nematodes as a model system.

JILL HELMS, DDS, PhD, professor of surgery, received the 2016 Distinguished Scientist Isaac Schour Memorial Award from the International Association for Dental Research. The honor recognizes her outstanding contributions in tissue regeneration and stem cell biology.

ANN HSING, PhD, was appointed professor (research) of medicine, effective Dec. 1, 2015. She is the co-leader of the Stanford Cancer Institute Population Science



Miriam Goodman



Jill Helms



Eric Knudsen

Program. Her research focuses on clarifying the etiology of prostate and hepatobiliary cancers and on international studies on the etiology of cancer. She is developing a cancer prevention and control model for the San Francisco Bay Area that reflects its diverse population.

ERIC KNUDSEN, PhD, the Edward C. and Amy H. Sewall Professor and professor emeritus of neurobiology, has been elected to the American Philosophical Society. Founded in 1743, the society promotes useful knowledge in the sciences and humanities. Knudsen's research examines the mechanisms of attention, information processing and learning in the brain. **ISM**

Victor Carrion and Kari Nadeau named to endowed professorships

Two Stanford Medicine faculty members have been appointed to endowed professorships.

VICTOR CARRION, MD, professor of psychiatry and behavioral sciences, was appointed the John A. Turner, MD, Endowed Professor for Child and Adolescent Psychiatry, effective Feb. 23. Carrion's research and clinical work focus on the treatment of stress-related conditions in children and adolescents.

KARI NADEAU, MD, PhD, professor of medicine and of pediatrics, was appointed the



Victor Carrion



Kari Nadeau

Naddisy Foundation Professor of Pediatric Food Allergy, Immunology and Asthma, effective June 9. Nadeau directs the Sean N. Parker Center for Allergy and Asthma Research. Her research focuses on understanding the immune system dysfunction that produces asthma and allergies. **ISM**

Stem cell

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in the response to the drug by comparing the subject's gene expression profile with that of the others whose cells were unaffected. They were also able to correct the defect by using a genome editing technique to boost the expression of a gene in the pathway and restore normal function.

Finally, although the researchers showed that meaningful variability exists in the gene expression patterns of the seven individuals, they couldn't yet be certain that the iPS-derived cardiomyocytes faithfully replicated each person's native heart tissue. To investigate, they created iPS cells from another three people who had undergone either heart biopsies or transplants. They then compared the iPS-derived cardiomyocytes with the matching native heart tissue and confirmed that the gene expression patterns correlated in many significant ways — particularly for genes involved in metabolic pathways critical to cardiac function.

"Many people talk about precision medicine or precision health, but there are only few examples of how to carry it out in a clinically meaningful way," said Wu. "I think the patient-derived iPS cell platform gives us a surrogate window into the body and allows

us to not only predict the body's function but also to learn more about key disease-associated pathways."

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 instructor of cardiovascular medicine Paul Burridge, PhD; graduate student Kun-Hsing Yu, MD; research assistant John Ahrens; postdoctoral scholars Vittavat Termglinchan, MD, Haodi Wu, PhD, Jared Churko, PhD, Chun Liu, PhD, Praveen Shukla, PhD, and Ningyi Shao, MD, PhD; instructor Nazish Sayed, MD, PhD; students Nicole Woo and Alexander Chao; former assistant director of translational research at the Stanford Cardiovascular Institute Joseph Gold, PhD; assistant professor of cardiothoracic surgery Ioannis Karakikes, PhD; and professor of genetics Michael Snyder, PhD.

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