

A close-up photograph of two medical syringes. The syringe in the foreground has a blue plunger and a yellow band around the barrel. The syringe in the background has a red plunger. The background is blurred, showing more of the syringes and some green and blue elements.

LIFE-CHANGING
SCIENCE

2020 RESEARCH HIGHLIGHTS

LUDWIG
CANCER
RESEARCH



Edward A. McDermott Jr.
President and
Chief Executive
Officer

WELCOME

Each year we select a few Ludwig-affiliated researchers whose labs have reported important discoveries in the previous year and profile them, describing the arc of their scientific journey, the logic and role of serendipity in its progression and its importance to cancer research and care. This year, we realize, our Annual Progress report arrives at a strange and profoundly difficult time.



Chi Van Dang
Scientific
Director

Since it emerged in late 2019, the new coronavirus has spread across the globe, leaving heartbreak and economic devastation in its wake. Meanwhile, in the U.S., and to some measure beyond, the death of George Floyd in May has provoked a broad protest movement and a reckoning with racial injustice in all its pernicious forms. All this unfolded as COVID-19 deaths mounted horribly and the lockdowns required to stem the tide of the pandemic disrupted nearly every aspect of our private lives, research activities, clinical cancer care and public endeavors. We are proud to say that through all this the Ludwig Cancer Research community has carried on with good humor, grit, collegiality and a renewed commitment to opposing racism.

In one of Ludwig's recent Scientific Insights webinars, four Ludwig researchers discussed how they and their labs have adapted to the new normal. Each noted a silver lining to the coronavirus crisis. Reengineering operations to adapt to the demands of lockdowns and 'social distancing,' they observed, has not only exposed inefficiencies and superfluous practices in prevailing models of biomedical research, but also improved their own approach to scientific research and collaboration in ways likely to long outlast the pandemic.

While COVID-19 and the rippling effects of George Floyd's death legitimately consume our attention these days, cancer is and will remain for the foreseeable future a leading cause of human suffering and death. The life-changing science supported by Ludwig Cancer Research will be of value long after this pandemic has passed. To be sure, that science is ultimately in service to cancer prevention, diagnosis and care. But it is also a very human enterprise, one animated by a spirit of wonder, fascination, adventure and compassion. The profiles in this report bear that out in full. We hope you enjoy reading them.

Sincerely,

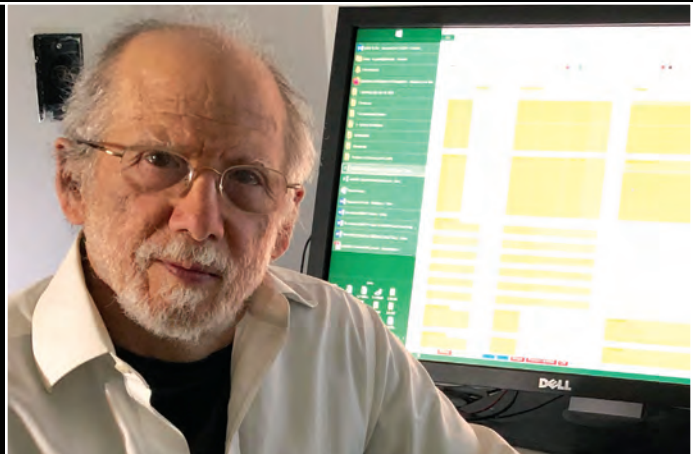
Edward A. McDermott Jr.

Chi Van Dang

BERT VOGELSTEIN

**THE
CANCER
DETECTOR**

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OF MALIGNANT
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Bert Vogelstein

LUDWIG WORKING FROM HOME

“We thought, ‘This is a way our lab can contribute **right now.**’ That’s what we should be focusing on.”

The cancer detector

IN EARLY 2020, BERT VOGELSTEIN watched with growing dread as the novel coronavirus infection (COVID-19) that emerged from China swept across the globe and infiltrated all 50 states of the U.S. By early April, COVID-19 had surpassed heart disease and cancer as the country's leading cause of death per day and brought the nation's economy to a standstill. But Vogelstein's distress turned to resolve when he realized that he and his team were in a unique position to help. A drug they had previously found might quell a dangerous over-reaction of the immune response known as a "cytokine storm" could potentially prevent the same condition in people with severe COVID-19.

"We thought," says Vogelstein, "This is a way our lab can contribute right now. That's what we should be focusing on."

Though infectious disease is not quite Vogelstein's bailiwick, the proposed intervention bore some similarity to his team's primary focus these days: secondary cancer prevention—the application of cancer genomics to catching cancers early, before they spread and turn deadly. To that end, the team he leads as Co-director of Ludwig Johns Hopkins has in recent years designed and evaluated minimally invasive tests (or "liquid biopsies") to screen patients for multiple undiagnosed malignancies. In 2019, they reported the advantages of using one such test to screen colorectal cancer patients for disease recurrence; In April this year, they published the results of the first clinical evaluation in the general population of a blood-based screening test for multiple cancers. Now Vogelstein hopes to similarly nip in the bud a potentially lethal complication of COVID-19. "What we're trying

“What we’re trying to do is prevent the severe consequences of COVID-19, not treat them—which is very similar to a major focus in our cancer research.”

to do,” says Vogelstein, “is prevent the severe consequences of COVID-19, not treat them—which is very similar to a major focus in our cancer research.”

Preempting a storm

Cytokines are small signaling proteins produced by immune and other cells to put the body on high defensive alert, usually in response to infections. In some instances, the immune system fails to switch off this protective response even after the infection has been brought under control, and instead pumps out more and more cytokines. Their unbridled release causes intense, systemic inflammation and other corrosive physiological responses that can devastate internal organs and cause often lethal pneumonia. The complication is associated with a variety of conditions, including transplant rejection, certain cancer immunotherapies, bacterial infections and viral diseases such as influenza, SARS—and now COVID-19.

“It’s not the virus destroying the lungs, but the body’s reaction through these cytokines that is too much,” explains Vogelstein. “It’s too vigorous a response, and that ends up causing more problems than the infection itself.”

A study Vogelstein led with Ludwig Johns Hopkins researchers Vernea Staedtke and Shibin Zhou and published in *Nature* in 2018 described a series of cascading events mediated by immune cells that precipitated such storms. That study showed that drugs known as alpha-1AR antagonists, including the cheap and widely available drug prazosin, could squelch cytokine storms in mice. To assess the applicability of those findings to humans, Vogelstein’s team and their colleagues did a retrospective analysis of patients hospitalized for acute respiratory distress (ARD), which is often caused by cytokine storms in COVID-19 and other diseases. It revealed that men diagnosed with ARD who had been taking prazosin had a 36% lower risk of requiring a ventilator or dying than those who had not. In May, Vogelstein and his colleagues began a clinical trial to test whether the drug might also be effective in preventing cytokine storms when given preemptively to COVID-19 patients.

Know thy enemy

None of this is to say Vogelstein has lost sight of his main quarry, which for the past four decades has been cancer. When he was a young medical student in the late 1970s, the causes of malignancies were largely a mystery. “Cancer was basically a black box,” he says. “It was like an alien that came from outer space and invaded people’s bodies.”

During a pediatrics internship in 1974, Vogelstein encountered a family whose four-year-old daughter had leukemia. Vogelstein had no answer when the father asked him, “Why did this happen to my beautiful little girl?” The question haunted Vogelstein and factored into his decision to switch from



Vogelstein with Ludwig Johns Hopkins Co-director Ken Kinzler.

Photo by Flynn Larsen

pediatrics to full-time research. Thanks in good measure to his own efforts, Vogelstein now has some answers.

In fact, we now know more about the root causes of cancer than we do about many other diseases. "That's been a giant first step," Vogelstein says. "Yet, we're still unable to prevent cancer or cure it to the degree that we'd like. Eventually, it'll come — the translation to patient benefit. But in the intervening years, it's frustrating that you understand so much about the disease but can't really do much about it for most patients."

Cancer is primarily caused by the sequential accumulation of mutations in cells. "Obviously, there are a bunch of other factors involved, but, in essence, cancer is a genetic disease,"

Vogelstein says. "If you don't have mutations, you're not going to have a cancer."

Much of our modern understanding of cancer can be traced back to discoveries made in Vogelstein's lab, beginning with his methodical description of how mutations accumulate to drive the progression of colorectal cancers (CRCs). In 1989, his group showed that a gene called p53 was mutated in CRCs, and many other tumors besides. This study and their other work on the biochemistry of p53 led to the surprising discovery that it is not a tumor promotor, or oncogene, but rather a tumor *suppressor* gene whose protective function is disabled by mutations.

Over the next several years, Vogelstein's lab implicated many other genes and mutations

in not only colon cancer, but other cancers as well, including those of the breast and pancreas. Step by step, he and Kenneth W. Kinzler, who co-directs the Ludwig Center at Johns Hopkins, were slowly prying open the black box and laying the foundations for a new and deeper understanding of the origins of cancer and its progression.

In 2006, Vogelstein and Kinzler published the first comprehensive profile of all the expressed genes, or exome, in breast and colorectal cancers. It was a bold undertaking that some had considered impossible. This study, published in *Science*, and others revealed several new cancer genes, including PIK3CA, which, like P53, is one of the most commonly mutated genes across cancers. His team would go on to sequence the genomes of scores of other cancers, a feat that was significantly bolstered by Ludwig support following the establishment of the Hopkins Center in 2006.

Vogelstein said his lab didn't start out with such an audacious plan in mind—it was just that as their experience grew, so too did their ambition and confidence. “We started with one gene at a time, sequencing it in a group of cancers,” Vogelstein says. “Then we continued that with sequencing small groups of genes. We followed that with sequencing classes of genes. Then we said, ‘Well, now that we know how to do this, let’s go the extra mile. Why not look at all 20,000 genes?’ Remarkably, several trainees in our lab concurred that this was not crazy. In retrospect, it was totally crazy.”

A yen for translation

When it comes to cancer, Vogelstein has never been satisfied with just knowing something about why it develops and how it spreads. He's had another goal in mind from the start. “One way to look at it is we were not driven by intellectual curiosity. A lot of scientists are. We were not,” he says. “We were interested in trying to do something to reduce morbidity and mortality from cancer.

The basic research that we did was generally geared towards understanding enough so that we could formulate reasonable hypotheses and reasonable strategies to attack the disease. This line of thought and this view was integral right from the beginning.”

He attributes his way of thinking in part to his time as a pediatrician, when he first became aware that the most dramatic improvements in child health came not from treatments but from vaccinations and other public-health measures. “So, even though treating cancers is extremely important and worthwhile, in the end analysis I thought the best way to reduce cancer deaths would be through primary or secondary prevention,” Vogelstein says.

In the early 1990s, he and Kinzler began focusing in earnest on transforming the genetic alterations they had discovered into tools for detecting cancers early. “Before that time, the cancer biomarkers that were used to follow patients with cancer were all relatively nonspecific. They were associated with cancer, but they weren't causative,” Vogelstein explains. “We thought that the mutations themselves—these mutations that are the proximate cause of cancer—could actually be used as biomarkers to detect cancer early because they're exquisitely specific.”

Their first major success in this area came in 1991 and 1992, when Vogelstein and his colleagues published papers showing that mutations in bladder cancers can be detected in the urine of patients with the disease and that colon cancer mutations are similarly detectable in the stool. This line of inquiry culminated in the first FDA-approved genetic test for the early detection of cancer, called Cologuard. “That was approved about five years ago and it's estimated that 40 million Americans will take that test over the next decade,” Vogelstein says.

As a first step toward improving secondary

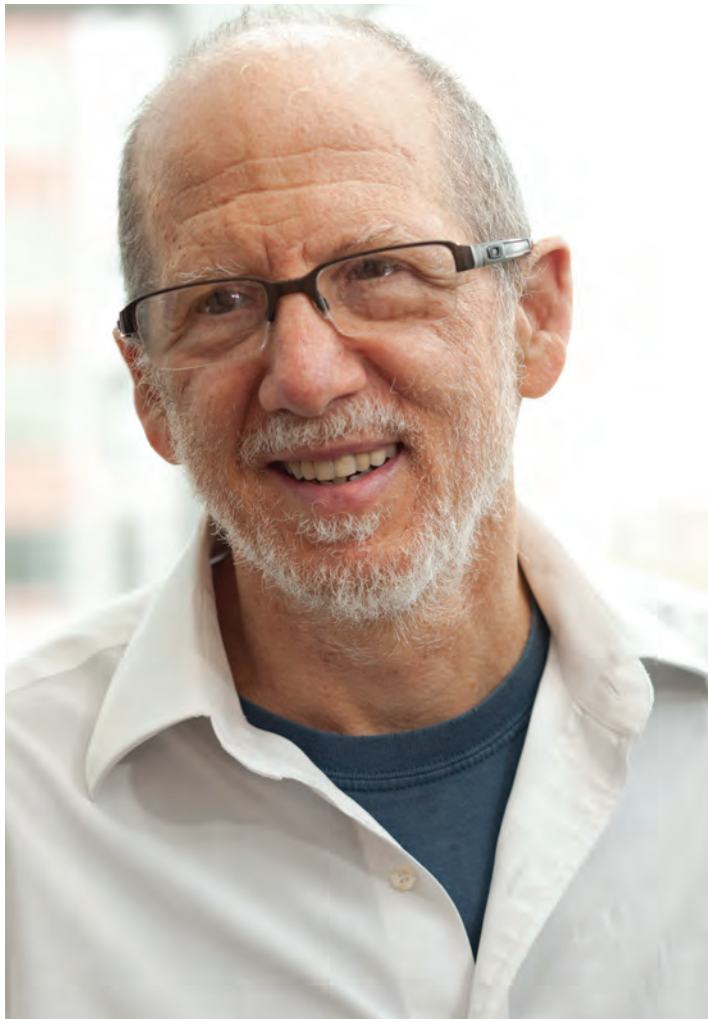


Photo by Flynn Larsen

“Many of these ideas are off the beaten path, some might even have seemed crazy at the time they were formulated. But occasionally, one pans out ...”

prevention, he and his colleagues focused on detecting cancer *recurrences* in patients. “Technically, that’s actually much less challenging than trying to detect cancers in completely asymptomatic people not known to have cancer,” Vogelstein says. Joining a five-year, \$10 million cancer prevention initiative launched by Ludwig and the Conrad N. Hilton Foundation, the Ludwig Johns Hopkins team examined the use of liquid biopsies to monitor CRC patients for such recurrences, working in partnership with Ludwig-supported researchers in Australia.

In two studies published in 2019 in *JAMA Oncology*, a collaboration between the labs of Vogelstein, Kinzler, and Peter Gibbs, a

Ludwig alumnus in Melbourne, showed that circulating tumor DNA (ctDNA) in the blood of cancer patients could be used to not only detect colorectal cancer recurrence earlier but also as a real-time monitor of the effectiveness of chemotherapy given after surgery for cancer. “Peter told us that in colon cancer, if you treat people with micro-metastatic disease too small to be seen on X-rays, you can actually cure nearly 50 percent of them even though their tumors have already metastasized,” Vogelstein says.

Taking the leap

In the late 1990s, Vogelstein and Kinzler developed a new cancer mutation screening technology called digital PCR for the

detection of DNA shed by colon tumors. "Since then, we've been looking to extend that technique so we could look at more molecules," says Vogelstein. "Back then, we could only look at a few hundred at a time, but by the early 2000s, our lab had developed a way to look at millions at a time with a technique called BEAMing."

Building on that technology, the team published two papers in *Science Translational Medicine* in 2013 and 2014 demonstrating that they could detect the presence of most uterine tumors and a third of ovarian tumors in Pap smears, as well as many other tumors in ctDNA. They subsequently launched PapGene, a Baltimore-based biotech company established to develop liquid biopsies. In 2019, PapGene was acquired by Third Rock Ventures and incorporated into a new company named Thrive Earlier Detection, which raised \$110 million in Series A financing. Thrive's first priority is to further develop the most ambitious iteration of the Ludwig Johns Hopkins team's liquid biopsy technologies, CancerSEEK. Initially reported in *Science* in 2018, CancerSEEK evaluated the levels of eight proteins and a variety of mutations in DNA shed into the blood by tumors to detect malignancies that account for more than 60% of cancer deaths in the U.S.

"Support from Ludwig has been instrumental to our lab's success for more than a decade," says Vogelstein. "It has permitted us the freedom to pursue our ideas in an unfettered way. Many of these ideas are off the beaten path, some might even have seemed crazy at the time they were formulated. But occasionally, one pans out and has the potential to mitigate suffering and deaths from cancer in a new way. The freedom to pursue those ideas through focused research is precious—perhaps the greatest gift a foundation can provide to its scientific staff."

In April 2020, the Ludwig Johns Hopkins

team, working with the Geisinger Health System and their colleagues at Thrive, published the results of the first major test of their cancer-screening technology, a clinical trial involving nearly 10,000 women between the ages of 65 and 75. "It was the first prospective interventional trial of a multi-cancer blood test in individuals who were not known to have cancer," says Vogelstein.

Published in *Science*, the study found that the liquid biopsy more than doubled the number of cancers detected when added to traditional screening, safely detecting 26 previously undetected malignancies. Most of the cancers were localized by diagnostic PET-CT, and 12 could be surgically removed with the intent to cure. Combining the blood test with standard of care screening such as mammography and colonoscopy improved the sensitivity of detecting breast, colon and lung tumors from 47% to 71%. The blood test was also able to detect seven cancers for which screening tests do not exist, such as thyroid, kidney, and ovarian cancers. More than half of the cancers that occurred during the study were detected by either blood testing or traditional screening.

Vogelstein says the launch of Thrive Earlier Detection is a "giant leap forward" toward his dream of making cancer screening a routine part of annual medical exams—but there's more work to be done. "The dream will only be realized when the tests can be made available to the public outside of a research study, which will need regulatory approval. It will require people actually getting the test and the demonstration that it actually helps them—something we and our colleagues at Thrive are diligently working on. Until then, it's research," Vogelstein says. "The vision that Ken and I had no longer seems like science fiction, but we haven't landed on the moon yet." ■

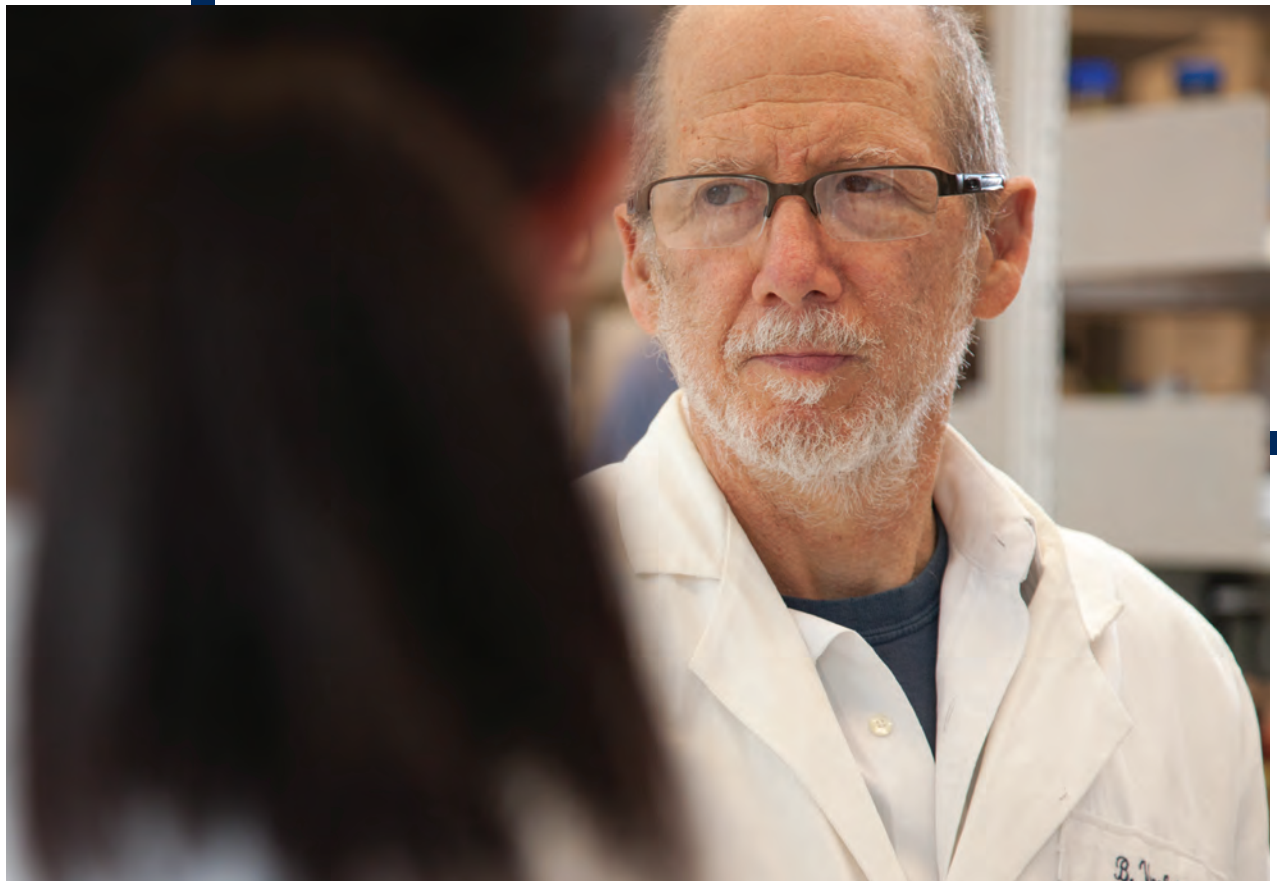


Photo by Flynn Larsen



Chi Van Dang

LUDWIG WORKING FROM HOME

“We really didn’t know which oncogenes would be most prominent in human cancer. It was **pure luck** that I wound up focusing on Myc.”

Master of malignant metabolism

PEOPLE TEND TO DISMISS THE ROLE OF serendipity in their successes. Not Chi Van Dang. “My research career has been paved with luck,” Ludwig’s scientific director says with a chuckle. It was certainly there when Dang arrived in 1985 at the University of California, San Francisco, fresh from his medical residency at Johns Hopkins, for a fellowship in hematology-oncology and in molecular oncology, which he would do under the physician-scientist William Lee and two pioneers of the field, Harold Varmus and J. Michael Bishop.

“Varmus asked me what I wanted to work on,” Dang recalls, “I said, ‘oncogenes.’ He looked at me and said, ‘Which one?’ Dang confessed he had no idea. Unfazed, Varmus told Dang to go interview junior faculty on the team and pick one. “I hit it off with Bill Lee, and he was studying Myc,” says Dang. “At that

point we really didn’t know which oncogenes would be most prominent in human cancer. It was pure luck that I wound up focusing on Myc—it turned out to be a really important oncogene.”

Luck, for its part, found in Dang an amply prepared mind. Over the next three decades, his elucidation of Myc biology would expose the protein’s starring role in the orchestration of cellular metabolism and reveal how its dysfunction drives the malignant transformation of cells. Dang’s discoveries spurred a revival of the long-dormant field of cancer metabolism and led him into studies of phenomena as seemingly disparate as cellular oxygen sensing and chronobiology. In 2019, Dang’s Ludwig laboratory at the Wistar Institute in Philadelphia, in collaboration with researchers at Stanford University, added a new chapter to the winding tale of Myc

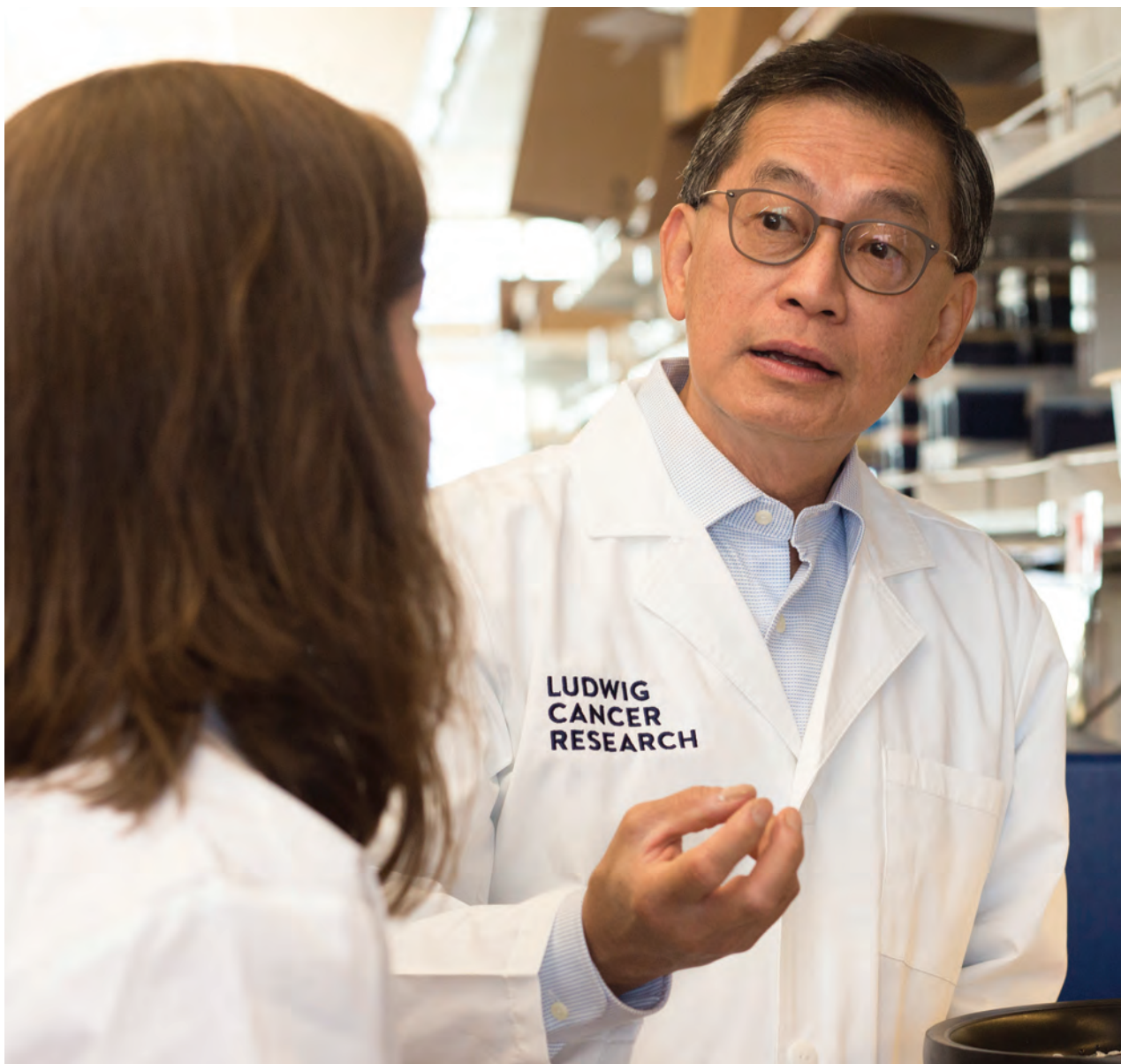


Photo by Flynn Larsen

and cancer metabolism. They reported in *Cell Metabolism* that cancer cells driven by Myc shut down their importation of fats and become highly dependent on their internal lipid-making machinery. That dependency, they showed, might be exploited for cancer therapy.

Lucky breaks

Dang was born in the former Saigon, now Ho Chi Minh City, in southern Vietnam. "My

mother was probably the best mother I know," says Dang, "because she managed to keep track of ten children." His father, Chieu Van Dang, was Vietnam's first neurosurgeon and Dean of the Saigon School of Medicine. "He was a curious, eclectic man, very up about education," Dang recalls. "He told us, 'I won't have a lot of money to leave you, but I will leave each of you an education.' That was always his mantra."

Dang's family frequently hosted foreign doctors and, as the Vietnam war heated up, an orthopedic surgeon from the U.S. who had stayed with them in 1960 and remained a friend offered to take a couple of the Dang children into his home in Flint, Michigan. Because they attended an English medium school, Dang and his brother Chuc were picked to go in 1967.

A bookish boy of 12, Dang found a welcoming community in Flint, learning about American culture from friendly neighbors and excelling in his studies. After a spell in refugee camps, the rest of his family migrated in 1975 to California, where Dang's father completed an internship and residency at the age of 56 to obtain a U.S. medical license. "That influenced me," says Dang, "the illustration that you can recover from adversity, recoup and get back on your feet."

Dang graduated with highest honors in 1975 from the University of Michigan, Ann Arbor, where he had majored in chemistry. But the fall of Saigon that year left him stateless and, despite his topping test scores and grades, medical schools put him on their waitlists as they puzzled over his immigration status. Ultimately, Georgetown University accepted Dang into its graduate program in chemistry with the understanding that he would join its medical school after earning his PhD.

But upon completing his graduate studies, Dang transferred in 1978 to Johns Hopkins University, where he earned his MD and continued honing his research skills, working on cell biology before moving into blood coagulation research. Both those experiences, along with exposure to cancer patients during his internship and residency at Hopkins, drew Dang to oncology. And so, in 1985, Dang and his new wife, Mary, packed up their belongings and drove across the U.S. to San Francisco, where Dang planned to start his career as a clinical and research oncologist—by learning, first, what makes a gene an oncogene.

"My mother was probably the best mother I know, because she managed to keep track of ten children."

Profiling Myc

At the time, Myc was known to be a viral oncogene, but very little was known about the version of the protein encoded by cells. Working with Lee, Dang tested a leading hypothesis—that Myc was somehow involved in replicating DNA—and proved, with some disappointment, that it is not.

The two continued their collaboration after Dang was recruited back to Hopkins in 1987. Soon after he started his lab, Dang received a call from the molecular biologist Steve McKnight, whose team had noticed that another DNA-binding protein named C/EBP had some similarities to Myc. Dang and McKnight put their heads together and noticed that these and other cancer-driving proteins that bind DNA share a structural feature. McKnight's team argued in a landmark analysis in *Science* in 1988 that this feature, which they dubbed the "leucine zipper," allowed DNA-binding proteins to zip up with a partner protein bearing the same structure and so bind DNA.

Dang, meanwhile, returned to his lab to prove that Myc's leucine zipper indeed performed such a function. That work, reported in *Nature* and co-authored with Lee, experimentally validated McKnight's hypothesis, cementing a basic principle of

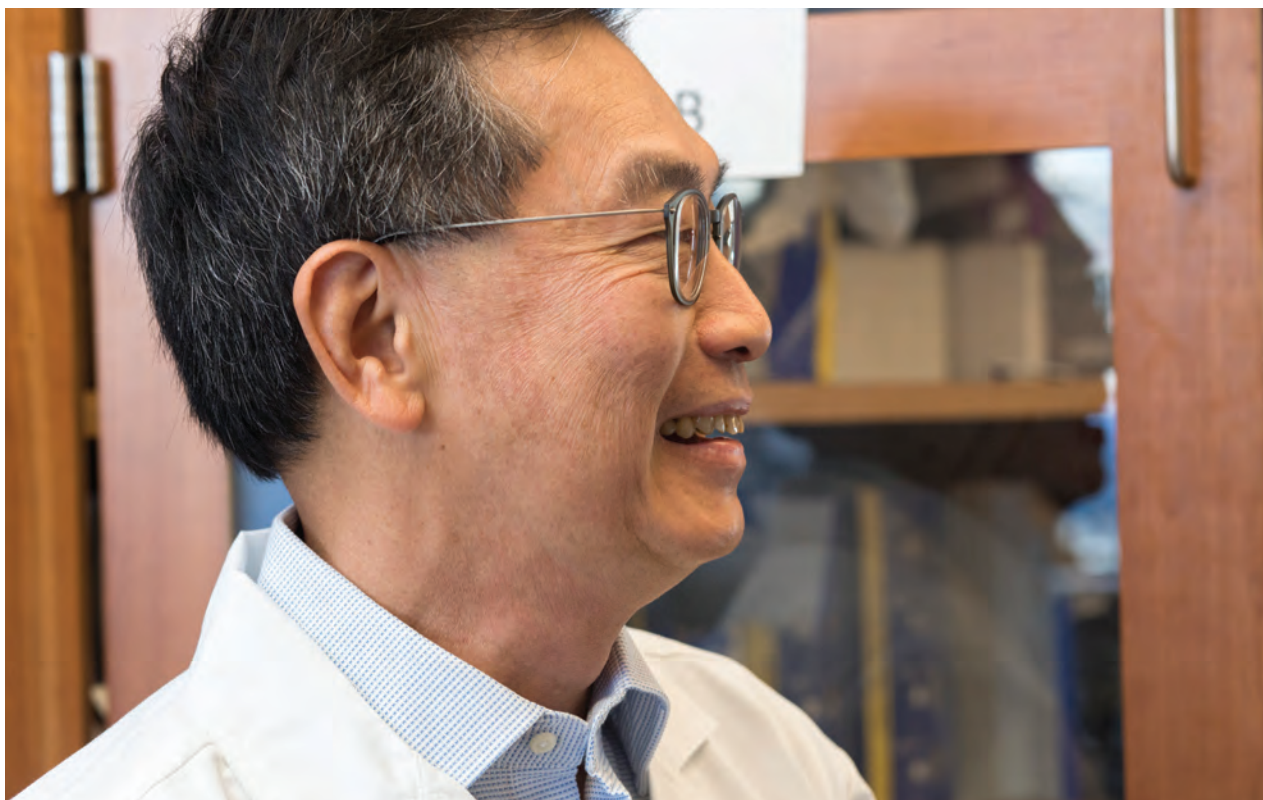


Photo by Flynn Larsen

molecular biology. “It was totally by luck that we made this discovery,” says Dang. “If McKnight hadn’t contacted me, we’d have been off working on something else.” Dang and Lee also discerned the molecular bar code on Myc that directs it into the nucleus and, most notably, proved that Myc is a transcription factor—a protein that controls the expression of genes.

Into malignant metabolism

Now the race was on to discover the genes turned on by Myc. Dang and others found that Myc activates many genes essential to cell division. But an entirely different kind of Myc-activated gene piqued Dang’s curiosity. “That gene was for lactate dehydrogenase A (LDHA), a metabolic enzyme that is seemingly very boring, just a housekeeping gene,” says Dang. Yet the discovery suggested to Dang an exciting possibility.

Cancer cells must rewire their metabolism to generate the extra energy and raw materials required to duplicate themselves. One way they do that is by switching on a metabolic pathway for burning sugar, known as glycolysis, in which LDHA is involved. Glycolysis is ordinarily employed only by oxygen-starved cells, but cancer cells keep it going regardless—a hallmark of cancer first identified the 1920s by the biologist Otto Warburg. Cancer cells like glycolysis because, though it generates relatively little energy, it produces large amounts of a key cellular building block, lactate, the acidic byproduct that makes overexerted muscles burn.

In 1997, Dang and his colleagues reported that Myc boosts the expression of LDHA in cancer cells, providing the first mechanistic link between an oncogene and the classical Warburg effect. Over the next several years, Dang’s lab would describe the myriad ways in which Myc controls metabolism by

modulating the production of key enzymes and the generation of cellular components like mitochondria—the powerhouses of cells—and ribosomes, which are required for the synthesis of proteins.

“The function of Myc is to turbocharge the production machinery of the cell so it can assemble all the building blocks required to double in size, copy DNA and then divide,” explains Dang. “If you get rid of Myc, cells can’t do this, so Myc is often permanently switched on in cancer cells. That’s a summary of about three decades of research on how Myc actually functions.”

Further afield

While Dang was exploring Myc’s rewiring of cellular metabolism, his Hopkins colleague Gregg Semenza had been investigating how cells adapt to oxygen starvation, or hypoxia. Semenza, who with Ludwig Oxford’s Peter Ratcliffe and Harvard’s William Kaelin won the 2019 Nobel Prize for that body of work, noticed that HIF, a transcription factor central to the cell’s hypoxic response, also activates glycolysis. In 1999, he and Dang began a fruitful collaboration exploring HIF’s influence on hypoxic metabolism and its interaction with Myc in cancer.

Hypoxia is a common feature of tumors and, in 2008, Dang and Semenza demonstrated that the pharmacological inhibition of LDHA could slow the growth of certain cancers, and worked for a few years to develop a drug for that purpose. Dang also explored the inhibition of another metabolic pathway activated by Myc—one involving the amino acid glutamine, to which many cancers cells are addicted—as a cancer therapy. Both efforts, which continued after Dang was appointed director of the Abramson Cancer Center at the University of Pennsylvania in 2012, were encouraging. Yet they have so far yielded mixed results in early trials and animal studies conducted by other researchers and companies.

“We think that if we make a drug that targets the cancer cell’s metabolic pathways, we can kill the cancer,” says Dang. “The mistake we made conceptually is that many cells, not just cancer cells, use those metabolic pathways. Most important, immune cells also depend on them.” Dang has thus adjusted his approach to look for metabolic interventions less likely to disrupt the immune response to tumors. (As it turns out, the targeting of glutamine metabolism seems to pass that test.)

Dang’s move to UPenn in 2012, meanwhile, exposed him to a cluster of chronobiologists, who study how circadian rhythms affect physiology. Those rhythms are coordinated by a central clock in the brain, subsidiary clocks in other organs and a network of clock-associated genes in cells.

Normally, cellular metabolism is in sync with the circadian clock, active during the day and slow at night. But ceaselessly proliferating cancer cells presumably do not rest. Dang and his colleagues wondered whether Myc—which binds to the same DNA sequences as a pair of proteins that control clock gene expression—has a hand in that circadian dysfunction. In 2015, they reported in *Cell Metabolism* that it does. Myc, it turns out, indirectly suppresses one of those genes to disrupt the cellular clock and reprogram metabolism to support cancer cell proliferation.

A basic discovery

Dang and his team were now curious about whether HIF too disrupts clock genes in the oxygen-starved cells of tumors, since HIF and Myc bind to similar DNA sequences. Their studies indicated, unexpectedly, that it does not. But the graduate student working on the project, Zandra Walton, found that acidity—caused by the lactate generated by glycolysis—suffices to disrupt the circadian clock and that the effect could be reversed by neutralizing the medium around hypoxic cells.

Figuring out how that happened continued

as Dang joined the Ludwig Institute for Cancer Research as scientific director and became a member of The Wistar Institute in Philadelphia in 2017. The following year, he and his team detailed in *Cell* a surprising molecular mechanism by which the acidity—caused by glycolysis in hypoxic tumors—pushes cancer cells, and all other cells, into a dormant state. The discovery has implications for cancer therapy because dormant cells in tumors cannot typically be killed by chemotherapy and are a major source of drug resistance and disease recurrence.

They also found that the effect, caused by the disabling of a protein complex named mTORC1, could be easily reversed. “In tumors grafted into mice, we saw mTOR activity in spotty places where there’s oxygen,” says Dang. “But when we added baking soda [which neutralizes acid] to the drinking water given to those mice, the tumor would light up with mTOR activity. The prediction would be that by reawakening these cells, you could make the tumor more sensitive to therapy.” Dang and his team also found that the activation of the immune system’s T cells, essential to most immunotherapies, is similarly compromised by acidity. “We’re looking now at whether that can modulate immunotherapy,” says Dang.

Back to basics

Dang and his team were at the time also examining how Myc influences the production of lipids—fat molecules that build cell membranes and play many other important roles in proliferating cells. A protein named SREBP1 normally monitors lipid levels and, when more are needed, activates the expression of genes involved in their synthesis. A graduate student in Dang’s lab, Arvin Gouw, discovered that Myc ramps up the production of SREBP1, putting it into overdrive. “We also found that MYC then binds to the same genes as SREBP1, and the two collaborate to push lipid synthesis to even higher levels,” says Dang. Those findings were

reported in a *Cell Metabolism* paper published in 2019 and led by Dang and Stanford University researchers Richard Zare and Dean Felsher—whose lab Gouw subsequently joined as a postdoc.

Myc, the researchers showed, controls the gene expression required for almost every stage of lipid synthesis in proliferating cells. Further, studies on mice engineered to develop Myc-driven cancers of the blood, lungs, kidneys and liver revealed that cells of such tumors are highly dependent on synthesizing their own fats rather than importing them. Inhibiting an early step of lipid synthesis led to the regression of the induced tumors and of Myc-driven human tumors implanted in mice. Even tumors primarily driven by other oncogenes are susceptible to the inhibition of fatty acid production if they indirectly activate Myc. The findings suggest strategies for developing drugs that could treat multiple tumor types, as Myc is overexpressed or activated in more than half of all cancers.

Dang remains eager to translate these and other such discoveries into cancer therapies, but now in a more nuanced way. “We’re still interested in metabolic inhibitors but are particularly careful about examining how they affect other cells in the tumor microenvironment,” says Dang. He and his colleagues are also engineering immune cells to withstand the enervating acidity of the tumor microenvironment, with the aim of improving cellular immunotherapy, and exploring how the circadian clock affects the anti-tumor immune response.

“These are the kind of projects where you take a shot at something that’s a little crazy, and then leverage your early results to compete for more traditional external funding—something I am encouraging all our Ludwig researchers to do,” he says. “That’s what’s important about Ludwig support—it allows you to innovate and not be fearful of trying something that’s way out there.” ■

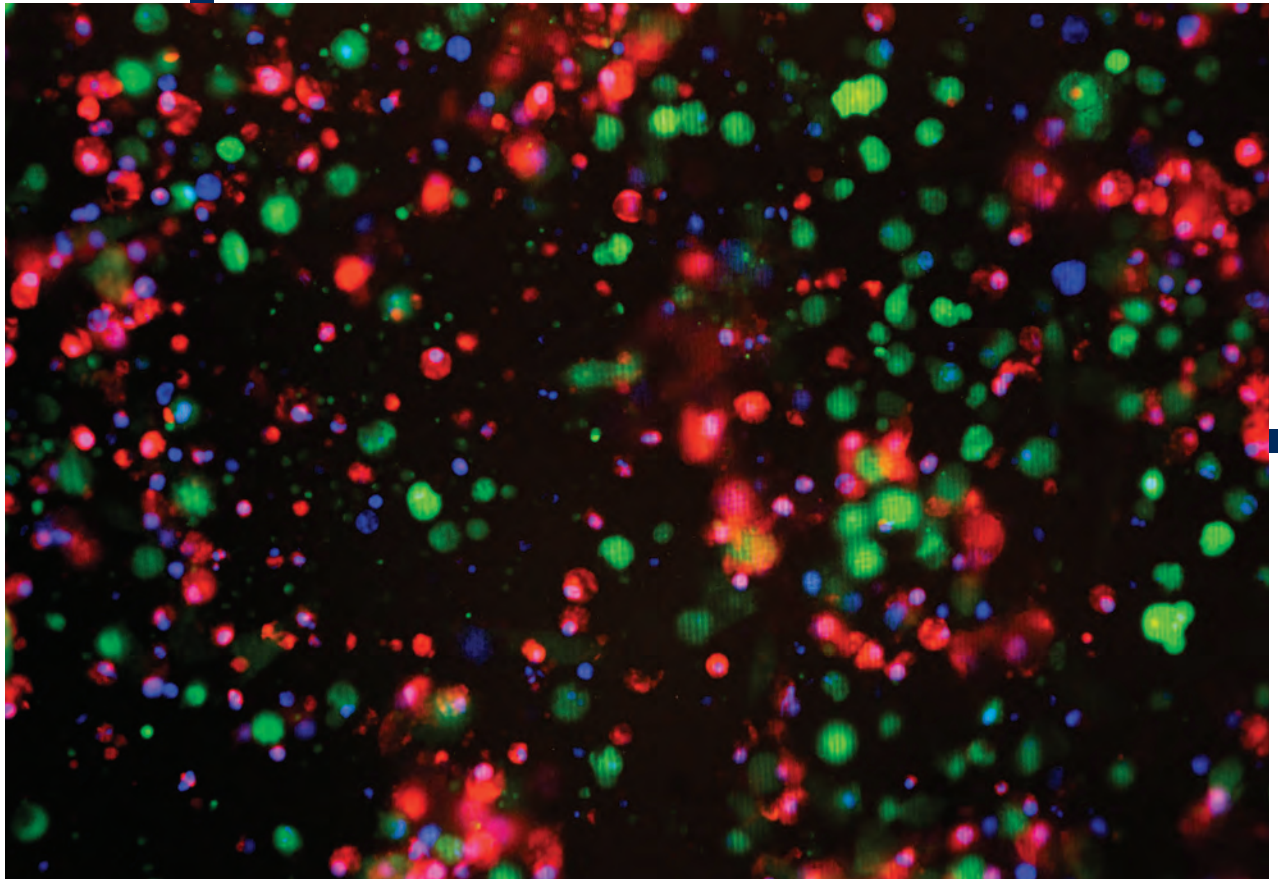


Photo by Flynn Larsen



Irv Weissman

LUDWIG WORKING FROM HOME

"All four of the **'don't eat me'** signals that we know of were discovered from my lab, and they were all funded by Ludwig."

The stem cell pioneer

FOLLOWING THE END OF HIS MEDICAL school training in 1965, Irv Weissman faced what should have been a life-changing decision: continue with an internship and medical residency or become a full-time researcher.

As it happened, the choice was a no-brainer. Weissman had, in fact, already made his decision by age 10, when he got hooked on science after reading *Microbe Hunters* by Paul de Kruif and deciding he'd like nothing more than to follow in the footsteps of the book's protagonists. "The people in the book not only made discoveries about microbes, they immediately applied those discoveries to medicine," says Weissman, who is today director of the Ludwig Center at Stanford University. "I knew then that's what I wanted to do."

It didn't take him long to get started. While still in high school, Weissman talked his way into a laboratory run by a physician in his hometown of Great Falls, Montana, and was soon contributing to experiments that would ultimately help pave the way for the first successful skin and organ transplants. Then, as a researcher at Stanford, he led the first isolation of a tissue stem cell—the hematopoietic stem cell—and went on to describe the steps by which it generates all blood cells. The discoveries he made along the way, and continues to make today, promise to transform transplantation medicine and the treatment of ailments ranging from autoimmune diseases to cancer. They include his characterization of "don't eat me" signals exploited by cancer cells to evade immune attack, a body of work that is already being applied by a company he co-founded to translate that work into a

While in medical school, Weissman recruited other students to work with him, researching how the immune system develops to distinguish “self” from “non-self.”

cancer therapy. In 2019, he and his colleagues reported in *Nature* a fourth such signal and showed how its blockade could prompt an immune attack on malignancies, including ovarian and triple-negative breast cancers.

The novice

Weissman was 15 when he first heard about Ernst Eichwald, a doctor and researcher who had recently moved from the University of Utah to work as a pathologist at Montana Deaconess Hospital in Great Falls. In what Weissman often describes as the most important event in his life, he approached Eichwald to ask for a job. “He was partially deaf and spoke with a thick German accent, and our conversation went nowhere until I said I’d work for free,” Weissman says. “We settled on \$25 a month.”

As Eichwald’s research assistant, Weissman

learned not just the fundamentals of science, but also how to puzzle things out for himself. Eichwald once told him, for example, that about a quarter of the skin grafts he had conducted on his mice had been rejected, and asked Weissman to speculate on the cause. “At first, I thought it had something to do with the age of the mice, but that wasn’t it,” Weissman says. “Eventually, I asked, ‘Were the skin grafts from males rejected by females?’ and he said ‘Yes.’ That experience helped me realize that I can think and do science.”

After high school, Weissman continued working in Eichwald’s lab as a college student attending what is now Montana State University (MSU). By that point, he was conducting his own experiments to understand why adult mice rejected tissue from nonmatching donors, while fetal mice exposed to blood-forming cells from adult mice of a different strain accepted transplanted tissue from that strain for the rest of their lives.

After graduating from college, Weissman joined the medical school at Stanford, drawn there by its unique five-year medical program. “Stanford divided the two years of basic science that every medical student takes into three years,” Weissman says. “That meant that, every day, we had half a day free.” At the end of his first year at Stanford, Weissman joined the lab of Henry Kaplan, a professor of radiology. In an unusual move, Kaplan gave the young Weissman a shared lab of his own and the support of a research assistant.

By his junior year, Weissman had recruited other medical students to work with him, researching how the immune system develops to distinguish “self” from “non-self.” In 1964, he spent nine months in the UK working in the lab of immunologist Jim Gowans at Oxford University. While there, Weissman performed a landmark experiment in which he showed that the thymus, rather



Photo by Flynn Larsen

than merely producing hormones to aid immune cell development at a distance, actually matured T lymphocytes before sending them out to lymphoid organs.

The experience at Oxford affirmed for Weissman that he wanted to pursue a career in scientific research. "That important discovery that the thymus was the place that made T cells made me decide that, as much as I loved medicine, I wasn't going to do an internship and residency," Weissman said.

A hard lesson

Over the next two decades, Weissman and his lab at Stanford identified where many of the different cell types of the immune system are made and how they work. In 1988, he isolated purified hematopoietic stem cells for the first time from mice. Shortly after, his lab replicated the achievement with human

tissue, and went on to trace the steps leading from the stem cell to each of the many types of mature cells found in blood, and identify how they run awry in many blood diseases and cancers.

These discoveries opened up the possibility of using a patient's own stem cells to regenerate tissues, organs and cells damaged by disease. But in 2001, the Bush administration placed strict limits on the use of federal funds for human embryonic stem cell research. In response, Weissman worked with real estate developer Robert Klein to write a proposition to provide \$3 billion for stem cell research in California. In 2004, 59% of California voters approved Proposition 71: the California Stem Cell Research and Cures Initiative, leading to the establishment of the California Institute for Regenerative Medicine (CIRM).



Photo by Flynn Larsen

CIRM's funding mechanism was set up to avoid a painful business lesson Weissman learned in the 1990s, after forming a company called SyStemix Inc. to test the use of purified blood-forming stem cells to reconstitute the immune system of cancer patients. The company's clinical trial was abruptly ended in 2000 after the pharmaceutical company Novartis bought Systemix and shut down its stem cell programs. "To this day, the stem cell transplants we did as part of Systemix's clinical trial is the only instance of people being cured of metastatic breast cancer, but it's not a standard practice of medicine," Weissman says.

With that experience in mind, Weissman

stipulated in Prop 71 that the state agency should fund not only stem cell science, but its development for medical applications as well—through early clinical trials. In the 16 years since Prop 71 passed, CIRM has funded or supported research that has led to more than 60 clinical trials to study the use of stem cells to combat a host of diseases, including diabetes, spinal cord injury, various cancers and—most recently—COVID-19.

"I hope this will be an enduring legacy by the California voters—a new way to advance discoveries through clinical trials without having the risks that both venture capital and big pharma now avoid," Weissman says.

Weissman, for his part, has made enormous headway with his Stanford colleagues in harnessing stem cells to transform bone marrow transplantation, which currently requires the use of harsh chemo- and radiotherapy to destroy the recipient's existing, and diseased, blood stem cells. In 2019, for example, he and his colleagues described in *Cell Stem Cell* a gentle method—involving treatment with six antibodies—by which mice could be transplanted with blood stem cells from an immunologically mismatched donor. Further, they showed that recipient animals could then accept an organ or tissue transplant matching that of the (mismatched) donor stem cells without requiring ongoing immune suppression. If the findings are replicated in humans, the work could transform the treatment of immune and blood disorders, and vastly expand the pool of available organs for transplantation.

The 'don't eat me' era

During their investigations of hematopoietic stem cells and human leukemia stem cells in acute myelogenous leukemia, Weissman and his team had noticed that a protein, CD47, was expressed at higher levels in the leukemia stem cells than in normal bone marrow hematopoietic stem cells, or in their multipotent daughter cells—the stage at which most leukemia stem cells are found. They began investigating it in earnest after a Swedish group showed that red blood cells in mice that didn't exhibit this surface protein were removed by macrophages, immune cells that gobble up potential threats. CD47 was, in effect, a “don't eat me” signal.

In 2009, Weissman and his team reported in *Cell* that CD47 overexpression is linked to worse outcomes in acute myeloid leukemia and suggested it is a potential therapeutic target for the cancer. Their subsequent studies showed that the protein is abundantly expressed in nearly every human cancer. With support from Ludwig Cancer Research, Weissman's group also developed an antibody that blocked CD47 and showed that it

Weissman and colleagues have made enormous headway in harnessing stem cells to transform bone marrow transplantation.

restored the ability of macrophages to engulf cancer cells and, in immune deficient mice transplanted with human primary leukemias, lymphomas and other cancers, inhibit or eliminate a variety of tumors.

In a 2013 study published in the *Proceedings of the National Academy of Sciences*, his group showed that after engulfing the cancer cells, the macrophages presented molecular markers, or antigens, of the cancer cells to killer T cells, recruiting them into the attack on cancer cells as well. An antibody targeting CD47 is now in clinical trials—alone and in combination with other immunotherapies—in people with several types of blood and solid cancers. In March 2020, a biotech company, Forty Seven Inc., that Weissman co-founded to develop CD47-based immunotherapies was purchased by the pharmaceutical company Gilead for \$4.9 billion.

A fourth signal

Meanwhile, the finding that not all patients respond to anti-CD47 antibodies motivated Weissman to look for alternative don't-eat-me signals that might also stump macrophage attack. The hunch paid off. Over the next several years, the team uncovered two other such signals exploited by cancer cells. One of them is PD-L1, a protein that also scuttles T cell attack and is targeted by

“We don’t know yet, but you could imagine that some macrophages will have all four ‘don’t eat me’ receptors, some will have three, some two, and some only one.”

checkpoint blockade immunotherapies; the other is a protein associated with the major histocompatibility class 1 complex that cells use to present antigens to T cells. In 2019, Weissman’s group reported in *Nature* that a fourth protein, CD24—which ordinarily plays a part in controlling the severity of certain immune responses—also transmits a don’t eat me signal to macrophages.

“All four of the ‘don’t eat me’ signals that we know of were discovered from my lab, and they were all funded by Ludwig,” Weissman says. So far, it seems that only CD47 is found on the surface of all cancer cells—something that is not true for the other three signals. “We were lucky we discovered CD47 first, and I emphasize the word ‘lucky,’” Weissman says.

In their 2019 paper, Weissman and his team showed that blocking the CD24 signal in mice implanted with human breast cancer cells allows immune cells to attack the cancers. The team also found that ovarian and triple-negative breast cancer, both of

which are very difficult to treat, are especially vulnerable to macrophage attack when their CD24 signals are blocked.

Interestingly, CD24 and CD47 operate in seemingly complementary ways. Some cancers, like those of the blood, appear to be highly susceptible to CD47 blockade, whereas others, such as ovarian cancer, are more vulnerable to CD24 blockade. Weissman suspects the same is likely true for the other “don’t eat me” signals. “We don’t know yet, but you could imagine that some macrophages will have all four ‘don’t eat me’ receptors, some will have three, some two, and some only one,” he says.

Thus, it might be that most cancers will be susceptible to attack by blocking one of these signals, and that cancers may be even more vulnerable when more than one signal is blocked. “Let’s imagine that you have an ovarian cancer and you have 50,000 CD24 molecules per cell and 80,000 CD47 molecules per cell,” Weissman says. “Even if we block all of the CD47 molecules, that cell still has a lot of don’t-eat-me signals left, so the chances that it will be eaten are not great.”

Weissman envisions a future where doctors will be able to fine tune a cancer patient’s immune response with a precisely tailored cocktail of antibodies that can revive the macrophage attack. “We know from previous experiments that we have to block at least 80 percent of all of the signals for the cancer cells to be eaten,” Weissman says. Doctors might even check whether the composition of the “don’t eat me” signals changes over the course of therapy, and then tweak the cocktail as needed.

Such precision therapy would likely require the screening of tumor-associated macrophages for the corresponding receptor of each signal. “It is my fond desire that we will get to that point,” Weissman says. If he’s on the case, we probably will. ■



Photo by Monty Rakusen



Bradley Bernstein

LUDWIG WORKING FROM HOME

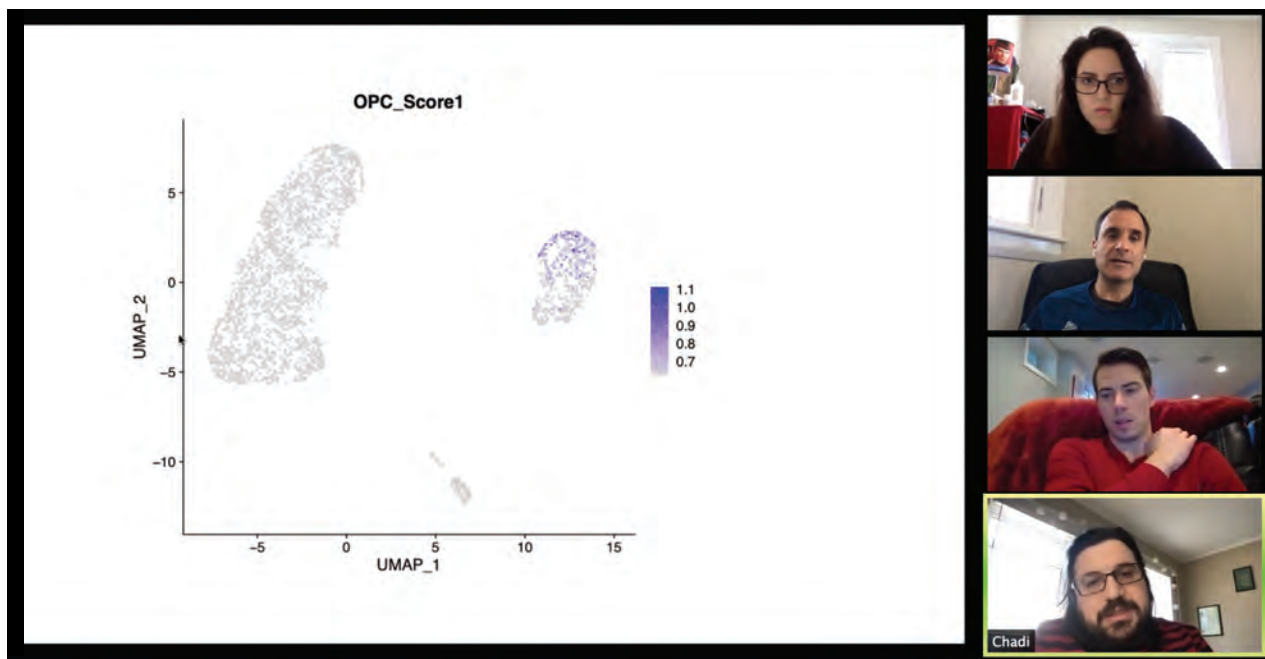
“The human genome is about six feet in length, and it has to fit into this tiny nucleus inside a cell. But it also has to fit in a way that makes all the **right genes** accessible.”

Surveyor of genome structure

GROWING UP IN SEATTLE, WASHINGTON, Ludwig Harvard's Bradley Bernstein always knew what he wanted to do when he grew up. "For as long as I can remember, at least from the time I was five years old, I wanted to be a physician," he recalls. There was a phase in college, at Yale University, when physics caught his fancy. But it didn't last. "Around quantum mechanics, I realized that I might not be cut out to be a theoretical physicist," he says dryly. One thing he retained from this early training, however, was a love of quantitative analysis, which he channeled into structural biology as a novice researcher in the Yale laboratory of Thomas Steitz, who won the Nobel Prize in chemistry in 2000. "It really grabbed me," he says, "this application of math and physics to biology."

The fascination endured, ultimately propelling Bernstein into a career detailing and mapping

the chemical and structural changes to DNA and its protein packaging—or chromatin—that govern gene activity. His pioneering work in this field has helped illuminate how such processes orchestrate human development and how their dysfunctions fuel cancer. In one study published in *Cell* in 2019, Bernstein and his colleagues generated an atlas of cell states in acute myeloid leukemia (AML) that could inform new treatments for the aggressive cancer. In another, done in collaboration with Center Co-director George Demetri and reported in *Nature*, Bernstein described how a structural change to chromatin—as opposed to a classical mutation to a growth-promoting gene—drives a subtype of abdominal tumors known as GISTs. The study revealed a possible new strategy for treating these sarcomas and furnished further proof for a surprising mechanism of carcinogenesis.



Bernstein leads his team through a virtual research meeting.

Functions of structure

After graduating from Yale, Bernstein enrolled in an MD/PhD program at the University of Washington, Seattle, where his doctoral research under the guidance of structural biologist Wim Hol focused on the structure of an enzyme expressed by the trypanosome parasite. Upon the suggestion of pathologist Stephen Schwartz—who in March 2020 died of complications from COVID-19—Bernstein picked pathology as his medical specialty, moving to Brigham and Women’s Hospital in Boston, Massachusetts, for his residency. “Pathology seemed very connected to disease mechanism and close to the type of science that fascinated me,” he says.

Bernstein then joined the Harvard University laboratory of Stuart Schreiber, where as a postdoctoral fellow he developed new technologies to elucidate the structure of chromatin in yeast. When the complete sequence of the human genome was reported in 2003, Bernstein saw a golden opportunity to map human chromatin on

a large scale—and to get a step closer to linking his scientific interests to his medical ones.

“The human genome is about six feet in length, and it has to fit into this tiny nucleus inside a cell,” says Bernstein. “But it also has to fit in a way that makes all the right genes accessible.” Cells do this by winding DNA around protein spools and packing away unneeded stretches, while unraveling and opening for business genes that are essential to their identity and function. Targeted chemical—or epigenetic—tags placed on chromatin determine which stretches of the genome are open and which are closed. Distinct epigenomic landscapes are an essential part of what make, say, a pulsing heart cell so different from a firing neuron or a crawling immune cell. Epigenetic aberrations, on the other hand, can cause disease, not least cancer.

In 2005, Bernstein and Schreiber, in partnership with MIT’s Eric Lander and other researchers, reported in *Cell* the

first large-scale map of human chromatin structure, charting the distribution of a pair of epigenetic tags on two chromosomes and providing an early glimpse of how epigenetics regulates gene expression. Later that year, Bernstein joined the faculty of Harvard Medical School, set up his own lab at the Massachusetts General Hospital and became a member of the Broad Institute of MIT and Harvard.

In 2006, Bernstein, Schreiber, Lander and their colleagues published another landmark study in *Cell* on how genes that orchestrate embryonic development are epigenetically tagged to perform their functions. “At the time, people thought a gene sits in either an open or a closed state,” says Bernstein. “What we showed was that embryonic stem cells keep their options open by ensuring that master developmental genes exist in this dynamic, bivalent state, poised to either switch on or stably turn off, depending on which lineage their progeny choose.”

Into the cancer genome

Around then, researchers were noticing that tumor progression too seemed to depend on stem-like cells. Eager to parlay his experience in stem cell biology into more applied medical research, Bernstein began working with MGH colleagues to chart the regulatory circuits that push the stem-like cells of the brain cancer glioblastoma (GBM) into a proliferative state. They reported in *Cell* in 2014 four transcription factors—master regulators of gene expression—responsible for that capability. Their over-expression, the team showed, could turn an ordinary GBM cell into a cancer stem cell. Another study Bernstein and his team published in *Science* that year profiled global gene expression of individual GBM cells. The tumors, they found, are often driven by several distinct stem-like cancer cells, explaining in part the brain tumor’s notorious resistance to a variety of individual therapies.

“At the time, people thought a gene sits in either an open or a closed state. What we showed was that embryonic stem cells keep their options open ...”

In exploring the mechanisms underlying GBM, Bernstein also applied his team’s expertise in mapping regulatory elements of DNA, which encode no proteins but instead switch genes on and off or modulate the intensity of their expression. There are about a million such switches, known as enhancers and repressors, scattered across the genome.

In 2016, Bernstein and his colleagues discovered a novel way in which one such switch, through the agency of disrupted chromatin structure, drives a subtype of brain tumor. The tumors in question puzzled researchers because they lack mutations in any of the usual growth-promoting genes that cause cancer. They are instead characterized by mutations to a metabolic enzyme named IDH.

“Cancer has traditionally been thought of as a genetic disease ... But here we were showing that you can have a nongenetic mechanism ... that switches on an oncogene.”



How this might fuel cancer was unclear, but one clue was that the DNA in such tumors bristled with an abnormal number of epigenetic tags known as methyl groups. This increased methylation of the DNA, Bernstein and his colleagues reported in *Nature*, disrupts a recurrent element of genomic structure, known as an insulator, that partitions entire neighborhoods of the genome from each other. “When the insulator is knocked out, the genome refolds in such a way that a giant ‘on’ switch comes in contact with an oncogene called PDGFRA, turning it on and driving tumor growth,” says Bernstein. The researchers also showed that a chemotherapy that reverses methylation could suppress the growth of the tumors in culture.

“Cancer has traditionally been thought of as a genetic disease, in which a mutation to DNA creates an oncogene that drives the formation of a tumor,” says Bernstein. “But here we were showing that you can have a nongenetic mechanism—this is, an epigenetic one—that switches on an

oncogene.” Most exciting for Bernstein is that the findings have led to the launch of a clinical trial to evaluate the use of DNA demethylating drugs for the treatment of brain tumors.

Structure and dysfunction

Since aberrant methylation of DNA has long been associated with cancer genomes, the chances were high that similar epigenetic mechanisms might drive other cancers as well. One likely candidate emerged in Bernstein’s conversations with Ludwig Harvard Co-director George Demetri, an authority on sarcomas. A type of sarcoma known as a gastrointestinal stromal tumor (GIST) is often driven by mutations that activate the oncogenes KIT and PDGFRA. These can be treated by therapies Demetri helped develop. But one GIST subtype lacked any discernable oncogenic mutation. Its genome, however, was known to be aberrantly methylated.

Bernstein, Demetri and their colleagues reported in *Nature* in 2019 that the DNA



Photo by Flynn Larsen

methylation dissolved an insulator in the genomes of these GIST cells and allowed a potent enhancer to move in three-dimensional space such that it could access the gene for FGF4, a known activator of oncogenic signaling. A second disruption of an insulator in these tumors had a similar effect on KIT. Mouse models of such GISTs showed that a new class of drugs that inhibit FGF signaling caused significant tumor regression, an effect amplified when existing KIT inhibitors were added to the mix. The researchers are now planning clinical trials to evaluate FGF inhibitors as a therapy for this subtype of GIST.

"When I first started in this field, we knew so little. The joke was that if you didn't

"A lot of my path over the years has been figuring out how to bring my interests in basic science and medicine together."

“When I first started in this field, we knew so little. The joke was that if you didn’t understand some biological phenomenon, you said, ‘it must be epigenetic’.”

understand some biological phenomenon, you said, ‘it must be epigenetic’,” says Bernstein. “What’s so exciting now is that I can show you with great precision how epigenetic mechanisms are driving certain cancers, and we can generate ideas about how to treat it.”

Malignant hierarchies


Another study led by Bernstein and reported in *Cell* in 2019, done in collaboration with Ludwig Harvard’s John Aster and Andrew Lane and colleagues at the Broad Institute, MGH and the Dana Farber Institute, echoed Bernstein’s 2014 profiling of GBM tumors. In this case, the researchers profiled the cellular constituents of acute myeloid leukemia (AML), a blood cancer that originates in the bone marrow.

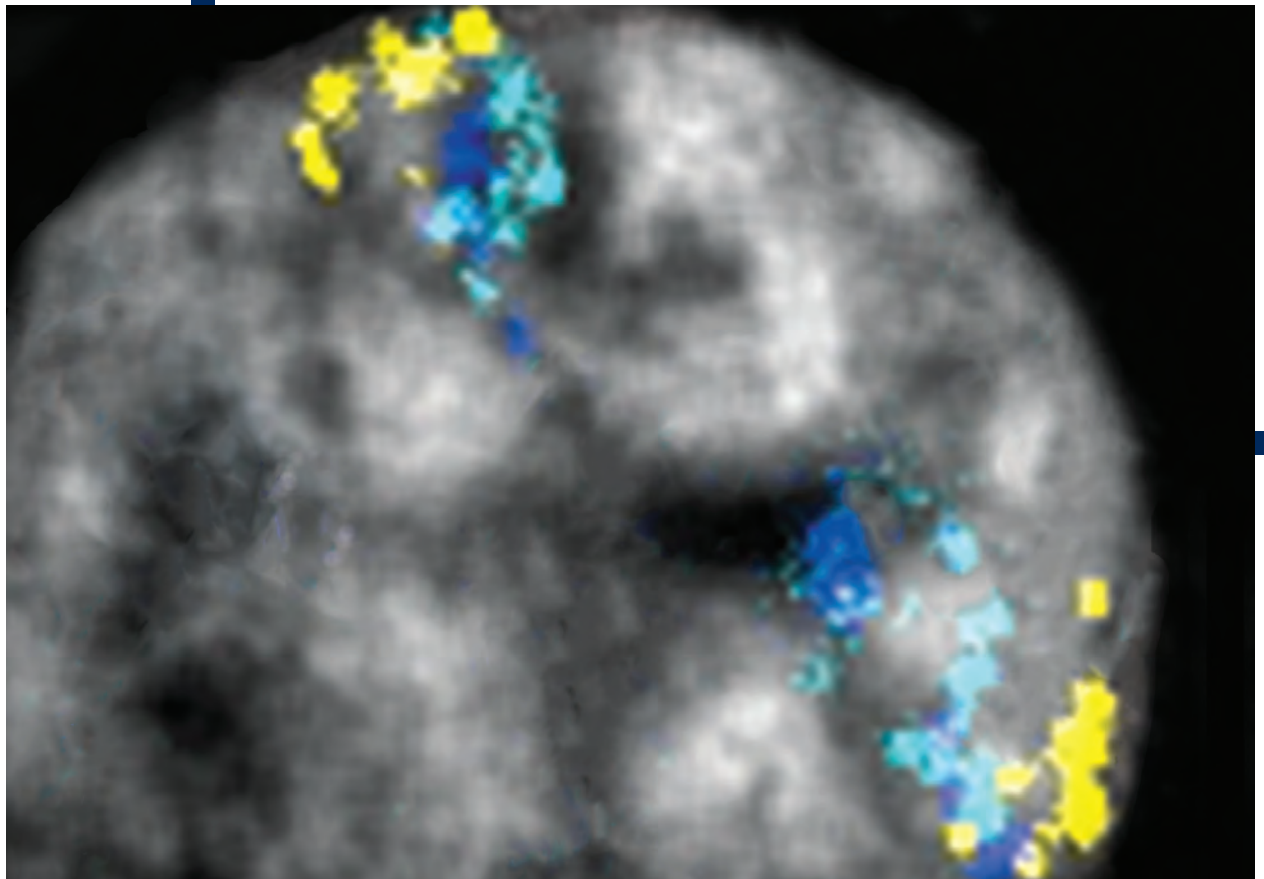
AML tumors are highly complex. They harbor a variety of normal and malignant cell types, including primitive cancer cells that closely resemble healthy blood stem cells and others that parallel various stages of normal blood cell formation. They also mutate frequently, forming lineages derived from “subclones.”

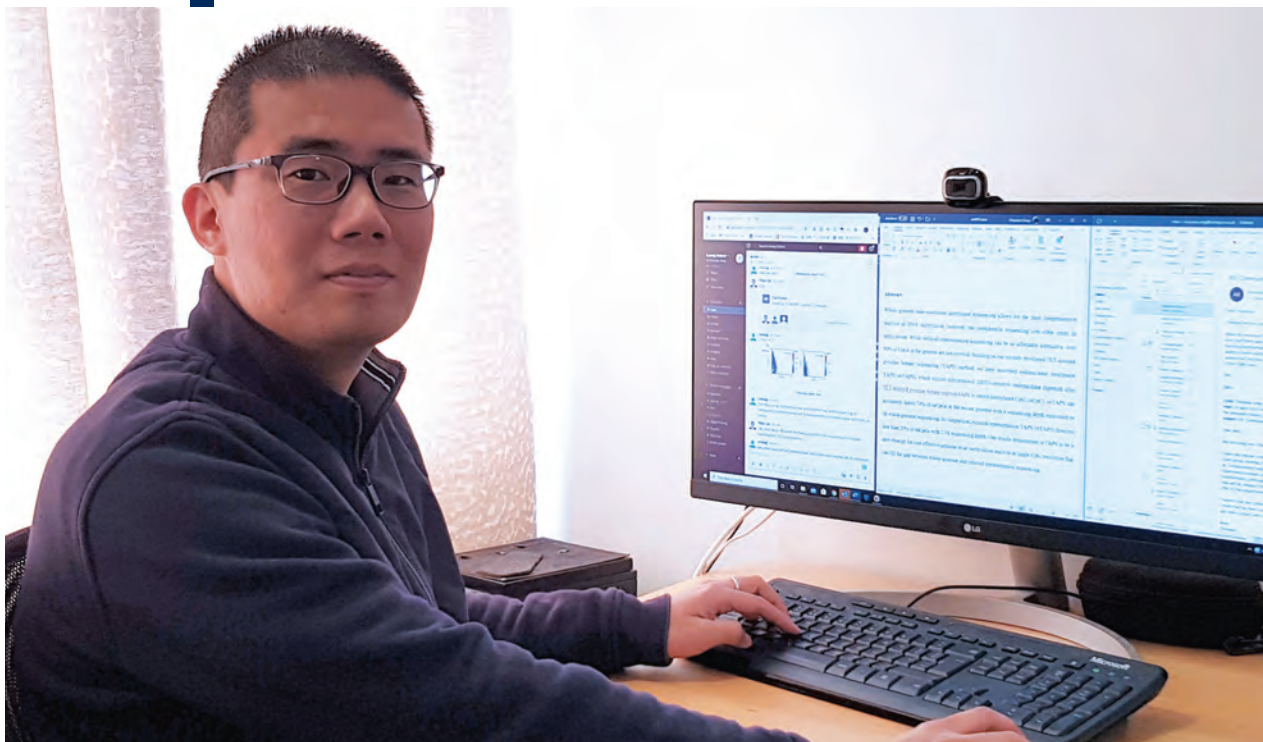
Bernstein and his colleagues harnessed a bank of AML tissue established by Ludwig Harvard, applying technologies used in the GBM study as well as a new method of DNA sequencing and machine learning software to profile nearly 40,000 individual bone

marrow cells from 16 AML patients and five healthy donors. The result was a revealing hierarchical atlas of AML cells, their gene expression programs and the relationship of those programs to patient prognoses.

The analysis also revealed one likely reason AML has so far thwarted immunotherapies. Many tumors draw in normal immune cells called ‘monocytes’ and coerce them to suppress immune responses against the tumor. AML takes a different tack to the same end. “While some AML cells rapidly divide and fuel the tumor,” says Bernstein, “others differentiate into monocyte-like cells that prevent an immune response.” The finding, says Bernstein, offers a clue to devising immunotherapies for AML and should be valuable to an interdisciplinary group starting up at Ludwig Harvard focused on immunosuppressive monocytes in a variety of cancers.

“A lot of my path over the years has been figuring out how to bring my interests in basic science and medicine together, and I think the Ludwig Center at Harvard is helping me to do that,” says Bernstein. “It is building bridges, connecting labs with diverse expertise and resources and bringing people together to do science. It has drawn together so many pieces of the Harvard scientific community and hospitals. The benefits were abundantly clear in both the GIST and the AML study.” 





Chunxiao Song

LUDWIG WORKING FROM HOME

“Basically, everything around us can be represented in this very **simple and elegant** way. It seemed like the whole world could be written in simple formulas.”

The chemical biologist

IN 2008, CHUNXIAO SONG WAS JUST beginning his graduate studies in chemistry at the University of Chicago. It was not going well. A foreign student from China, Song had never traveled outside his country, and the pressure and loneliness were starting to get to him. “Graduate studies, especially for foreigners, can be tough,” Song says.

He was professionally adrift as well. During college at Peking University, Song had majored in organic chemistry. Now, eager to harness chemistry to probe the natural world, he had switched his focus to chemical biology. He was adept at designing reactions to create synthetic molecules without concern for their immediate utility. But in the biological world, chemistry is only useful to the extent that it explains or enables discovery.

A dozen years on, that lost feeling is a pleasantly dim memory for Song, who is now an assistant member of the Oxford Branch of the Ludwig Institute for Cancer Research. In 2019, Song, in collaboration with his Ludwig Oxford colleague Benjamin Schuster-Boeckler, published a study in the journal *Nature Biotechnology* that detailed a greatly improved method for mapping a key chemical—or “epigenetic”—modification made to DNA known as methylation. Epigenetic modifications play a critical role in controlling gene expression, and aberrant methylation across the genome has long been known to be a hallmark of cancer. In 2020, Song and his colleagues launched a company named Base Genomics to commercialize their new technology and apply it to minimally invasive cancer detection.



Song, left, with Ludwig Oxford colleagues Skirmantas Kriaucionis and Benjamin Schuster-Boeckler.

Photo by Monty Rakusen

“Ludwig’s generous funding support, the existing strength of the Oxford Branch in cancer epigenetics and the scientific vision of the Ludwig Institute were all important factors for me to pursue the development of this technology and are essential drivers of high-risk, high-reward projects in my lab to advance cancer diagnostics,” says Song.

Inspiration

Song’s journey to this happy outcome began in 2009, when scientists—including Skirmantas Kriaucionis, who later joined Ludwig Oxford—announced the discovery of a new DNA base, 5-hydroxymethylcytosine, or 5hmC, in human and mouse brains. Up to that point, scientists knew of five main bases, or “letters,” that make up DNA in the genomes of higher organisms. There

are the four canonical ones—adenine (A), thymine (T), guanine (G), and cytosine (C)—plus the product of epigenetic methylation, 5-methylcytosine. “People were calling 5hmC a sixth base,” Song says. “It was very exciting, and people everywhere were racing to understand the biological function of this new base.”

Determined to be one of those people, Song dove headfirst into epigenetics. For Song, the chemical groups involved in epigenetic modifications—found not only on DNA but on its protein packaging as well—were “a wonderland for a chemist to play with in an otherwise bland genome.”

Epigenetic analysis is also crucial to a deeper understanding of cancer. While

genetic information can be used to detect the mutations that drive cancer, it offers few clues about a tumor's tissue of origin or the regulation of its genome. In contrast, epigenetic information is very tissue- and disease-specific, since genes are switched on and off in early fetal development to form individual organs, and epigenetic aberrations are common in cancer.

"If not for that discovery in 2009, I probably would have worked on something else," Song says. "But as soon as I entered the field, I was blown away."

The wonders of chemistry

When Song was 10 years old, he happened upon a school textbook belonging to an older cousin that was filled with seemingly arcane symbols. His cousin explained that the symbols were a kind of shorthand for describing the world. NaCl, for example, was sodium chloride — common table salt. "Basically, everything around us can be represented in this very simple and elegant way," Song recalls. "It seemed like the whole world could be written in simple formulas. That was the first time I saw the wonder of chemistry."

By high school, Song was dragging his mother to the capital city of his province to purchase college chemistry textbooks so he could delve deeper into the subject. He won first-in-class in his province in a national chemistry competition that drew from college-level chemistry.

That gave Song an edge in the national college-entry exam in China. "That first-in-class award gave me an extra 20 points on the national exam. That's a huge, huge boost," Song says—one, in fact, that secured Song a spot in the best chemistry program in China, at Peking University.

In college, Song focused on organic chemistry. "The reactions I worked on were very interesting from a chemistry point of

For Song, the chemical groups involved in epigenetic modifications were "a wonderland for a chemist to play with in an otherwise bland genome."

view, but many wouldn't be useful for a very long time," Song says. "I wanted to change to another area of chemistry where my knowledge could have more immediate use." And so Song, whose second-favorite science was biology, applied to a chemical biology graduate program at the University of Chicago.

A detection tool

Following the discovery of 5hmC in 2009, Song's PhD advisor, Chuan He, tasked Song with devising a way to easily detect the new DNA base. The chemical structure of 5hmC and 5mC are so similar—the two molecules differ by just a single atom—that existing sequencing technologies could not distinguish between the two in the human genome.



Working from home during the pandemic can bring some added benefits.

"There was a chemical modification on the human genome that had never been seen before, but biologists couldn't sequence it," Song says. "Chuan He wanted to know, 'Can we use chemistry tools to detect it?'"

The standard method for detecting 5mC was to design custom antibodies to bind to and flag it on DNA. But that approach fell short with 5hmC modifications, since the modification is far rarer in the genome.

In 2011, Song and his colleagues published a paper in *Nature Biotechnology* detailing a detection method for 5hmC. It involved using enzymatic and chemical reactions to selectively attach a molecular tag to 5hmC modifications, making them easier to spot and target. Their method was eventually

made into commercial kits and is still widely used today.

Toward liquid biopsies

After earning his PhD in 2013, Song moved to northern California as a postdoctoral researcher in the bioengineering lab of Stephen Quake at Stanford University. There, amidst palm trees and perennially mild weather, he continued his efforts to harness epigenetic information for clinical applications, refining "liquid biopsy" tools Quake's lab was developing that looked for biomarkers in free-floating DNA in the blood.

Liquid biopsies are minimally invasive and altogether less risky for patients. A race is on today to use them as diagnostics that detect very rare bits of DNA shed by

tumors, which encode specific genetic alterations associated with various cancers. “At Stanford, I saw an opportunity to combine what I was doing before, which was epigenetic sequencing, with this cell-free DNA-based liquid biopsy,” Song says. “Before, scientists focused only on changes in the DNA sequence itself—mutations, for example—and ignored all the DNA modifications.”

It wasn’t that researchers weren’t interested in the epigenetic modifications of cell-free DNA—it was just very difficult to detect them. “Cell-free DNA is present in very minute amounts, and it’s highly degraded, so you need a very sensitive method to detect the modifications,” Song explains.

TAPS

On the strength of his postdoctoral work at Stanford, Song was recruited by Ludwig’s Oxford Branch in 2016 as an assistant member. At Ludwig, Song’s group has been developing technologies to study how epigenetic modifications to DNA, like 5mC and 5hmC, contribute to cancer. Those technologies could also be applied to develop liquid biopsies for early cancer detection, explore the heterogeneity of tumor cells and elucidate drug resistance mechanisms—all of which are primary goals of Ludwig Oxford.

In their 2019 *Nature Biotechnology* paper, Song, Schuster-Boeckler and their colleagues detailed a novel method for mapping DNA methylation. Called TET-assisted pyridine borane sequencing—TAPS for short—it’s less damaging and more efficient than the previous gold standard for mapping 5mC and 5hmC modifications in the genome. Biologists had relied on that method, bisulfite sequencing, for decades. But the approach is extremely destructive, degrading as much as 99% of the DNA in samples. This makes it unsuitable for analyzing cell-free DNA, which is only present in minute amounts in blood.

“Cell-free DNA is present in very minute amounts, and it’s highly degraded, so you need a very sensitive method to detect the modifications.”

Bisulfite sequencing can only detect 5mC and 5hmC indirectly, by selectively converting unmodified cytosine to another base, uracil (which is not found in DNA but only used by cells to transcribe genetic information into RNA). This approach is not only inefficient, it also complicates the computational analysis of the data.

TAPS is a two-step process that uses an enzyme called TET to gently convert 5mC and 5hmC to a third modification, 5-carboxylcytosine (5caC), which is then converted to thymine—a DNA base that can be read by ordinary sequencing machines. The Ludwig researchers demonstrated that TAPS can generate more accurate epigenetic sequencing data at a lower cost. They also developed two variations of the technique—TAPS-Beta and CAPS—which can be used to detect 5mC or 5hmC, respectively.

“Humans are diploid, meaning we inherit a genome from dad and a genome from mom. With conventional sequencing technologies, it’s very difficult to distinguish between the two copies because they are so similar.”

One run, two types of data

Song believes TAPS can replace bisulfate sequencing as the new standard in DNA epigenetic sequencing, and his group is now adapting the technique for various clinical and basic research applications. For example, they are exploring how TAPS might be used to perform single-cell epigenetic sequencing to study biologically significant differences between cell-types within tumors.

In March, Song’s group published a paper in *Genome Biology* describing how TAPS could be combined with other technologies to perform long-read epigenetic sequencing. Until recently, TAPS had only been used to read DNA sequences just a few hundred base pairs long. “In some parts of the genome, where you have repetitive regions and genome

rearrangements, this kind of sequencing does not work very well,” Song says.

But so-called third-generation sequencing technologies are able to read DNA sequences numbering tens of thousands of base pairs in length. “We’ve shown it’s possible to combine TAPS with third-generation technologies so we can do long-read epigenetic sequencing as well,” Song says.

This combination opens up new research possibilities. Not only will it allow researchers to map previously unmappable stretches of the epigenome, it will also enable them to study allele-specific methylations more easily. “Humans are diploid, meaning we inherit a genome from dad and a genome from mom,” Song explains. “With conventional sequencing technologies, it’s very difficult to distinguish between the two copies because they are so similar. But with long-read sequencing, we can actually distinguish between paternal and maternal genomes.”

Song believes scientists have only scratched the surface of what TAPS can do. He and Schuster-Boeckler are now exploring a way to collect both genetic and epigenetic information using TAPS. “If you remove the changes to the genome made by the TAPS chemistry and then use the dataset like you would normal whole-genome sequencing, you could use it for genotyping to identify mutations in cancer,” Schuster-Boeckler says.

The goal is to obtain, simultaneously, from one TAPS run, information about not only where the mutations are, but also about the epigenetic state of a sample.

“People and companies are now realizing that having just the genetic information is no longer enough,” Song says. “You need the epigenetic data as well.” ■



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