## LUDWIG CANCER RESEARCH



## 2021 RESEARCH HIGHLIGHTS



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### WELCOME

Golden jubilees may refer to the past, but they can also be occasions to celebrate the future. So, as 2021 was Ludwig's 50th anniversary, we thought it would be fitting to use our Annual Research Highlights Report to look ahead and profile three new Members at the Lausanne and Oxford Branches, and the three founding Members of our new Branch at Princeton University. A major commemorative event, the Ludwig Princeton Branch was established in the spring of this anniversary year and is dedicated to the study of cancer metabolism and its disruption for therapy.

In the pages that follow, you will read about Mikaël Pittet, who began his scientific journey at the Ludwig Branch in Lausanne as a graduate student and has now returned there, 23 years later, as a full Member. You will also learn about his colleague at Ludwig Lausanne, Distinguished Scholar Douglas Hanahan. A giant of cancer research, Hanahan single handedly developed one of the first mouse models of cancer and later co-authored, with Ludwig MIT Director Robert Weinberg, two of the most influential reviews of cancer biology. At Ludwig Oxford, we introduce you to Yang Shi, whose discoveries upended models of the epigenetic regulation of the genome and have since led to the development of new cancer therapies. Shi helped organize a lively 50th anniversary virtual Ludwig-Oxford Symposium on Cancer Early Detection and Epigenetics that underscored the growing significance of this field to cancer research.

And, of course, there are the three founding Members of Ludwig Princeton, beginning with Branch Director Joshua Rabinowitz, a pioneer of the burgeoning field of metabolomics. His investigations have shed new light on the metabolic chemistry of both healthy and cancerous cells, demolishing some long-held assumptions about metabolism along the way. We also introduce you to Ludwig Princeton's Associate Director Eileen White, who, after making landmark contributions to our understanding of how cancer cells evade programmed death, discovered that they also depend on a process of self-cannibalization known as autophagy to survive. Her work—often done in collaboration with Rabinowitz—continues to illuminate the physiological and immunologic effects of the phenomenon. Finally, you'll read in this report a profile of the third founding Member of the Ludwig Princeton Branch, Yibin Kang. An adept modeler of cancer metastasis, Kang has explored in mice virtually every aspect of the intracellular changes and cellular interactions that enable the spread of breast cancer. His discoveries have contributed to the development of five experimental cancer therapies—and counting.

We hope you enjoy learning more about our new colleagues, their lives and their careers in this report. We look forward to a hopeful future for cancer patients and are confident that the discoveries and innovations of clinical relevance made by scientists across the Ludwig Cancer Research community will contribute meaningfully to that goal.

Sincerely,

Edward a. Mengemon

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Chi Van Dang

### CONTENTS



Mikaël Pittet The immune cell profiler



Joshua Rabinowitz The metabolic portraitist



**Eileen White** 

The cancer cell cuisinologist



Yibin Kang Disruptor of metastasis



<mark>Yang Shi</mark> The epigenetic explorer



Douglas Hanahan Modeler of malignancy



# MIKAËL PITTET

# The immune cell profiler

When Mikaël Pittet was about 15 years old, his mother, a nurse by profession, took him to Epalinges, a suburb of his hometown of Lausanne. Someone she knew knew someone known to be a renowned scientist and, having noticed her son's incipient fascination with science, she had asked-and the scientist had graciously arranged-to have young Pittet visit his laboratory. The scientist was immunologist Jean-Charles Cerottini, then director of the Lausanne Branch of the Ludwig Institute for Cancer Research and a pioneer in the study of the immune system's T cells. Pittet wound up visiting Ludwig Lausanne for a full week. "And so my very first exposure to science, when I was still a kid, was at the Ludwig Institute," says Pittet. "I was impressed."

And, apparently, inspired. Not only would Pittet turn to immunology for his graduate studies at the University of Lausanne, but he would also conduct his doctoral research and his postdoctoral fellowship—at Ludwig Lausanne under the mentorship of none other than Cerottini and the immunologists Pedro Romero and Daniel Speiser before leaving

Photo by Felix Imhof



for the U.S. in 2003. "I was fascinated by the vision that Jean-Charles had for how the immune system could be harnessed to fight cancer," says Pittet. "This was obviously long before it was fashionable."

In the years since, Pittet has only burrowed deeper into the complexities of tumor immunology, beginning as a professor at Harvard Medical School and, since 2020, at the University of Geneva, holding the ISREC Foundation chair of immuno-oncology. His laboratory, now at the AGORA Cancer Research Cluster in Lausanne, has become an epicenter for the study of myeloid cellsincluding neutrophils, dendritic cells and macrophages—associated with tumors. Pittet has teased apart unique states assumed by these frontline soldiers of the innate immune response and detailed how they can support tumor growth and survival, interfere with immunotherapies or boost anti-tumor immunity, depending on their functional flavors.

In 2021, Pittet began his third stint at Ludwig Lausanne, this time as a full Member of the Ludwig Institute.

#### **GETTING HOOKED**

For a fledgling scientist, few experiences could have been more inspiring than joining the team at Ludwig Lausanne in 1998. For one thing, Pittet got to travel with his mentors to meetings of Ludwig researchers from around the world hosted in New York by the late Lloyd Old, former Ludwig CEO and scientific director.

"Being able to interact with this giant of cancer research was amazing," recalls Pittet, who was no less dazzled by the intellectual firepower Old convened at the New York meetings. "It was just crazy as a young scientist to meet people whose names I'd seen on papers," he says. "It gave me the additional motivation to do the best I could to also contribute a little bit to the science. I was hooked." And then there was, of course, the work itself. Ludwig Lausanne researchers had adapted and developed a technology, known as tetramer assays, to rapidly detect antitumor T cells isolated from patients. For his doctoral research and his subsequent postdoctoral fellowship in Romero's lab, Pittet used the technology to identify tumorreactive T cells isolated from melanoma patients and analyze responses to a cancer antigen known as Melan-A. Aside from their intrinsic significance to tumor immunology, these studies entailed the development of methods that have since been widely adopted to assess T cell responses to immunotherapy.

Most significant for Pittet, however, were the larger lessons garnered from his studies. His findings helped establish that anti-tumor T cell responses were indeed occurring naturally in melanoma patients—that they weren't just artifacts of experimentation. Yet, notably, these killer T cells also seemed to be largely ineffectual within the tumor. Intriguingly, when those same, lethargic T cells were put in a dish and fed certain stimulatory immune factors known as cytokines, they revived their cancer killing function within a day.

"So there was this notion that there would be some reversibility in the T cell suppression, or anergy, as we called it at the time," says Pittet. "We now know how important this was, considering the capacity to activate or reactivate T cells with immunotherapeutic agents."

#### THE IMPORTANCE OF LOOKING

It was this suppression of the anti-tumor response and its reversal that Pittet decided he would explore next. For this, he reasoned, he'd need to pursue both imaging in live animals and mechanistic studies. "I visited labs that were best known for their imaging capabilities and ended up going to the Center for Molecular Imaging Research at Massachusetts General Hospital, which was



Photo by Felix Imhof

led by Ralph Weissleder, who became a great mentor," says Pittet. "I've worked closely with him for 17 exciting years."

In Boston, Pittet began exploring how killer T cells are suppressed in the tumors of mice. His real-time imaging studies-done with Weissleder, Thorsten Mempel and Ulrich von Andrian at Harvard Medical School and Harald von Boehmer and Khashayarsha Khazaie at the Dana Farber Cancer Institute-showed that immunosuppressive regulatory T cells (Tregs) in tumors monkey-wrench the engagement of the killer T cell's weaponry, specifically, the release of cytotoxic granules into target cells. Their mechanistic analyses showed the effect to be dependent on a factor known as TGF- $\beta$ . Reported in 2005 and 2006, these studies were among the first to capture how precisely Tregs repress killer T cell function. Also notable was the discovery

that the killer T cells themselves retained their cytotoxic capabilities and that their suppression could be reversed.

#### **THE ORIGINS OF THINGS**

Setting up his new laboratory at Massachusetts General Hospital in 2006, Pittet decided he was ready to move on from T cell immunology. "I realized that tumors are complex entities, and while focusing on T cells was important, they represent just one part of an intricate ecosystem," he explains. Of all the noncancerous cell types in tumors, Pittet chose myeloid cells as a worthwhile focus because they tend to be so numerous in tumors and because, being an immunologist, they felt somewhat closer to him.

But he first a took a detour, turning his attention for a spell to the role of these

"When we give a drug to a patient or a mouse, we still know little about why it sometimes works and sometimes fails. We want to address this black box."



Photo by Felix Imhof

cells in chronic and acute inflammation in mouse models of myocardial infarction and atherosclerosis. These studies demonstrated that subsets of macrophages change dramatically in inflammatory conditions related to cardiovascular disease. They also identified myeloid cells—like monocytes, and the macrophages derived from them—causally involved in such things as atherosclerosis and the healing of heart muscle. Perhaps the splashiest discovery Pittet's lab made in this arena was the identification in 2009 of the much-neglected spleen as a vital reservoir of monocytes that heal cardiac muscle after a heart attack.

That fascination with the origins of myeloid cells persisted when Pittet turned once again to cancer research. Macrophages, including those that support tumor growth, derive in part from monocytes in the blood, which in turn are generated by hematopoietic stem cells in the bone marrow. "We wondered, could it be that hematopoietic [bloodforming] stem cells are regulated by cancer?" says Pittet. "Is there long-range communication between cancer and other locations in the body? And that became very important for my lab—seeing cancer as a systemic disease, one that can have an impact far away from its location."

In 2012, Pittet's lab showed in a mouse model of lung cancer that the spleen also serves as a site for the production of macrophage and neutrophil precursors, which are then sent to tumors where they promote malignant growth. A year later, he and his colleagues reported that tumors can manipulate a hormonal circuit controlled by the blood pressure-regulating hormone angiotensin-II to remotely drive the production of macrophage and neutrophil precursors. Another study, published in 2017, showed in both mouse models and cancer patients that lung tumors—even before they've metastasized to bone—can remotely influence certain marrow cells to drive the production of a subtype of neutrophils that strongly promote cancer growth.

These studies continue today. "They represent one facet, or one half, of the work in my lab—these fundamental studies connect cancer to the immune system in the entire organism, not just in the tumor," says Pittet.

The other half relies on sophisticated realtime imaging in living animals to figure out how drugs work.

#### THE THIEVING MACROPHAGE

These pharmacokinetic and pharmacodynamic studies—combined with the analysis of global gene expression in individual cells—have opened a unique window into the influence tumor-associated myeloid cells have on therapy.

"When we give a drug to a patient or a mouse, we still know little about why it sometimes works and sometimes fails," explains Pittet. "We want to address this black box, to literally illuminate it using fluorescence imaging and microscopy. We want to be able to see the drugs, tumor cells and key immune cells and molecules involved in anti-tumor immunity, and we want to see all this in real time."

Seeing—not merely deducing—what goes on inside a tumor can make all the difference. Tracking anti-PD-1 antibodies in real time in a mouse model of cancer, for example, Pittet and his colleagues found that the drugs were binding their targets on killer T cells within tumors, presumably disengaging the brakes on their anti-tumor activity. But what they saw next surprised them. When the T cells bearing the antibodies bumped into a tumorassociated macrophage, the latter stole the antibody off the surface of the T cell. "This is not a good thing because the T cell can no longer be fully activated, which prevents the treatment from being fully effective," says Pittet.

The more such macrophages there were in tumors, the more likely the drug would be stolen away. Pittet and his colleagues reported these findings in 2017, revealing that a receptor normally expressed by macrophages was snaring the antibodies. Further, blockade of that receptor could boost the efficacy of anti-PD-1 immunotherapy in mouse models, suggesting a novel strategy to enhance checkpoint blockade.

#### **A WELCOMING COMMITTEE**

Pittet's lab has also explored the determinants of success for checkpoint blockade. Imaging studies using intravital microscopy revealed that treatment with anti-PD-1 antibodies caused a massive activation within tumors of a population of dendritic cells—which direct and stimulate the T cell attack. Further analysis indicated that this newly identified state of intratumoral dendritic cells is relatively rare but absolutely essential for effective checkpoint blockade.

When killer T cells are activated by anti-PD-1 antibodies, they produce a protein factor known as interferon (IFN)- $\gamma$ . Mechanistic analysis revealed that IFN- $\gamma$  prompts this state of dendritic cells to produce an immune factor, interleukin-12, that is sensed by the T cells. "The production of IL-12 inside the tumor tells the T cells that they can go kill their target," explains Pittet. In addition, these dendritic cells tend to congregate around the blood vessels feeding tumors. "When a T cell arrives in the tumor, the first cells it sees are likely to be these dendritic cells, which are strategically positioned like a welcoming committee."

> "When a T cell arrives in the tumor, the first cells it sees are likely to be these dendritic cells, which are strategically positioned—like a welcoming committee," says Pittet.

Since publishing these findings in 2018, Pittet's lab has also reported that the dendritic cells produce a factor that retains T cells in their niche and another, interleukin-15, that is known to promote T cell survival. Since their discovery of these dendritic cells, other labs have independently identified the same cells. What they should be named, however, remains up in the air. "The field is still in its infancy," says Pittet.

#### THE NEGLECTED NEUTROPHIL

Similar studies done on the types of neutrophils within tumors, meanwhile, have revealed one state that promotes tumor growth and another that has antitumor activity. "A few years ago, neutrophils were mainly considered a homogeneous population whose role in cancer was not clear," says Pittet.

Analyzing the gene expression patterns of

individual neutrophils—in work done in collaboration with Harvard researcher Allon Klein—his lab found that there are multiple states of neutrophils associated with lung tumors. Further, as they reported in 2019, the various states recur across patients and species, suggesting that interventions to manipulate specific neutrophil states in mice are likely to hold in humans and also to be of general benefit to lung cancer patients.

Dissecting one of these neutrophil states, Pittet and his colleagues identified a population whose presence in the tumor microenvironment is consistently associated with poor patient outcomes. This neutrophil state tends to be very long lived. "We believe these cells could be an important immunotherapeutic target because they have all the characteristics of a tumor-promoting cell, are present in many patients, sometimes in very high frequencies, and are largely ignored from a therapeutic perspective," says Pittet.

#### LOOKING AHEAD

As a tumor ecologist of sorts, Pittet finds himself in excellent company at Ludwig Lausanne. Other researchers at the Branch, most notably in the laboratories of Member Johanna Joyce and Associate Member Ping-Chih-Ho, are interested in different aspects of the role played by myeloid cells in cancer.

Pittet is also collaborating with Branch Director George Coukos to examine the promise of a novel approach to cancer therapy, FLASH radiotherapy, being developed at the Lausanne University Hospital (CHUV). The approach employs novel technology to target tumors with extremely high doses of radiotherapy while sparing healthy tissues. He and Coukos will be examining how the immune system might be recruited to



Photo by Felix Imhof

destroy tumors during FLASH radiotherapy. Beyond that, Pittet notes, he has already worked or interacted with several researchers affiliated with Ludwig Centers at Harvard and MIT and looks forward to collaborating with other Ludwig researchers in the years ahead.

The potential for local collaborations also excites Pittet. The AGORA Cluster, which houses most of Ludwig Lausanne, is itself a petri dish for a larger experiment in interinstitutional partnership: Pittet's lab represents the University of Geneva there. "The idea is not to worry about institutional boundaries," he says. "We are all in the same boat, on the same team: we want to fight against cancer and understand the disease. I am a big fan of that experiment."

Ludwig is as well. 🖿



## JOSHUA RABINOWITZ

# The metabolic portraitist

By the mid-2000s, word had spread in certain circles that Joshua Rabinowitz was a man with a solution. Since starting his laboratory at Princeton University in 2004, he had pioneered a unique approach to the comprehensive measurement and analysis of metabolites—or metabolomics—and was applying his technologies to make important discoveries in yeast biology and virology. Scientists confronting metabolic puzzles in their studies were calling him with growing frequency. Among them was Craig Thompson, a pioneer of modern cancer metabolism research.

Thompson, then head of the University of Pennsylvania Cancer Center and now president of Memorial Sloan Kettering, paid a personal visit to Princeton to see Rabinowitz, then a young assistant professor. After a tour of Rabinowitz's lab and technologies, Thompson pitched to Rabinowitz some of the problems in cancer research that he thought they might crack together. "A couple of minutes into Craig's talk, my brain started buzzing," says Rabinowitz. "I was sold on the intellectual challenge and life-improving potential of investigating these connections."

The meeting would draw Rabinowitz into a series of collaborations with cancer researchers, beginning with a pair of major studies that contributed to the development of a cancer therapy targeting the mutated metabolic enzyme IDH by the drug company Agios. A decade later, Rabinowitz's increasingly sophisticated exploration of the chemistry of life is



Photo by Flynn Larsen

transforming our understanding of systemic and tumor metabolism, opening exciting new possibilities for the prevention and treatment of cancer. On the strength of that research, Rabinowitz was in 2021 named founding director of a new Ludwig Branch based at Princeton University and dedicated to the study and disruption of cancer metabolism.

#### **PANNING FOR NUGGETS**

A relatively cautious child, Rabinowitz enjoyed an idyllic upbringing in Chapel Hill, North Carolina, enjoying lazy days at the pool in summer and college basketball games (some featuring Michael Jordan) in winter. His parents, both political scientists, were pioneering quantitative methods for studying voter behavior. Home life was peppered with talk of both politics and mathematical models, and Rabinowitz showed an early talent for mathematics that he cultivated with the encouragement of his father. In high school, beyond the standard challenges of being a teenager, Rabinowitz spent a year in Norway with his parents, who were there on sabbatical. After returning, his quest for a summer job landed him in a cancer research internship at nearby Duke University, getting his first taste of scientific research in the laboratory of William Peters, where he was charged with measuring cytokine levels in samples obtained from breast cancer patients. He stayed in Chapel Hill for college, and after graduating with degrees in chemistry and mathematics, enrolled in the MD/PhD program at Stanford University, where he joined the laboratory of the physical chemist Harden McConnell.

For his doctoral studies, Rabinowitz elucidated how naturally occurring antagonists of the immune system's T cells exert their effects. The experience, he says, was similar to what he does now—a mix of wet lab work and writing equations—and it convinced him he wanted to be a researcher rather than a doctor. "Being a great doctor means making mistakes as rarely as possible, and that's not my natural mindset," he says. "I want to push the frontiers, to challenge dogma and do things differently. Lab science is really good for me that way. I love that feeling of coming in every day, dreaming up a new experiment or calculation and hoping that it will pan out and yield a nugget of scientific gold. Such nuggets are rare. But the search is thrilling."

After receiving his PhD in 1999, and while completing his medical studies, Rabinowitz began looking around for a biotech startup to join. Aware of his search, McConnell called him into his office one day. "He had this very scratchy, deep voice," Rabinowitz recalls. "He said, 'Josh, if you want to do startups, look at this thing.' And he pulled this little metal canister out of his pocket and said, 'My friend, a great entrepreneur, has a dream of using this to deliver pharmaceuticals. If you're interested, call this number.'"

That number belonged to the legendary Silicon Valley biotechnologist Alejandro Zaffaroni. A leading contributor to the invention of the birth control pill, the nicotine patch and other slow-release drug delivery systems, Zaffaroni now saw great potential in rapid drug delivery and wanted to develop an inhaled delivery device. Rabinowitz began in 2000 working out of Zaffaroni's family office and, six months later, convinced his employer to launch a biotech company. It was named Alexza, and it started out in the basement of another biotech, Surromed.

Leading the new company's product discovery efforts, Rabinowitz oversaw the growth of a more than 50-person R&D team, eventually leaving in 2004 as the company grew into a clinical development phase. Within a year of his departure, Alexza had four inhaled drug delivery systems in trials, one of which is now in the market for the treatment of acute agitation in psychiatric disorders. "Being a great doctor means making mistakes as rarely as possible, and that's not my natural mindset. I want to push the frontiers, to challenge dogma and do things differently."

#### **COOL RESULTS**

Ready to reenter research, Rabinowitz headed back east, where his future wife Emily Pronin—a research psychologist he first met at an open house, when the pair were graduate students apartment hunting in Stanford—had been recruited as an assistant professor in the psychology department at Princeton University.

As it turned out, David Botstein, the new director of the Lewis-Sigler Institute for Integrative Genomics at Princeton, was looking for a chemist who could teach chemistry from an interdisciplinary perspective and, on the research side, develop systems for the quantitative analysis of metabolites. Rabinowitz, whose PhD research had involved some of that invention and analysis, fit the bill. He was hired in 2004 as an assistant professor at the Institute, where he started a metabolomics group. "I started at ground zero," says Rabinowitz. "I'd "We were doing a lot of collaborations because, all of a sudden, people were like, 'Oh, there's this new thing we can measure that seems like it can give cool results.'"

never even touched a mass spectrometer before."

Used to identify and quantify molecules in a sample, the mass spectrometer is the workhorse of metabolomics. Rabinowitz began with a relatively simple machine and, rather than look for as many metabolites as possible, focused on about 100 that were well known and of fundamental importance to biology. "It was a very different approach from what others were taking," he says. He also picked a simple problem for starters, beginning with an analysis of nitrogen metabolism in the bacterium *E. coli*, before moving on to increasingly sophisticated analyses of yeast metabolism in collaboration with Botstein and others.

"We were doing a lot of collaborations because, all of a sudden, people were like, 'Oh, there's this new thing we can measure that seems like it can give cool results'," says Rabinowitz. One of those people was the virologist Thomas Shenk, with whom Rabinowitz launched the field of viral metabolism when the pair reported in 2006 that, to replicate, the cytomegalovirus—a common human pathogen—alters glucose metabolism in its host cells to drive the synthesis of fats.

#### **INTO CANCER RESEARCH**

Another, of course, was Craig Thompson. Cancer cells are often forced to rewire their metabolism to generate the raw energy and molecular building blocks required for their ceaseless proliferation. Rabinowitz's technologies offered a more comprehensive picture of the adaptations that make this possible, and he was soon collaborating with a growing list of leading cancer researchers, developing new metabolomic methods, technologies and analytical systems that extended well beyond the measurement of just the fundamental 100 metabolites with which he'd started.

Further, his analyses were now distinguished by their focus not only on the comprehensive identification and measurement of metabolites, but on their flux as well. "Flux is the most important output of metabolism," says Rabinowitz. "If we see a metabolite that's gone up or down, we need to know if it's up because consumption decreased or production increased. If you want to target a pathway to treat cancer, you want to target the one that's hyperactive to produce the required metabolite, not where the metabolite builds up because its consumption is gone."

In 2008, Rabinowitz was included in a proposed Stand Up 2 Cancer (SU2C) Dream Team seeking to explore the metabolic disruption of pancreatic cancer for therapy. That was how he met Ludwig Scientific Director Chi Van Dang, a slated teammate then at Johns Hopkins University who had offered to critique his SU2C grant proposal. "I remember Chi pointing to one part of my proposal and saying, 'you have to explain why someone should care about this, not just what measurement you're going to make'," says Rabinowitz. "It was really good advice,



Photo by Flynn Larsen

which impacted how I wrote all my grants going forward. And it was critical advice because getting that SU2C grant transformed my career due to all that I learned from my grant teammates."

Closer to home, Rabinowitz also began working with Princeton colleague Yibin Kang, and Eileen White of the Rutgers Cancer Institute of New Jersey, both now founding Members of the Ludwig Princeton Branch (see accompanying profiles, pages 22 and 30). He and Kang first collaborated on a small but pioneering study, on metabolic changes in metastatic cancer cells, which was published in 2010. Around the same time, White got in touch with him to discuss how his expertise might aid her in exploring autophagy, a process of self-cannibalization that White had discovered many cancer cells depend on for survival and growth. cancers driven by the oncogene Ras rely on autophagy for core metabolic processes essential to energy generation. Over the next several years, he and White detailed the role of autophagy in the maintenance and progression of Ras-driven lung tumors, confirming its candidacy as a metabolic dependency that might be disrupted for cancer therapy.

"Autophagy is important for nutrient supply, for eliminating antigens and, at a wholebody level, in some complicated way, setting immune tone," says Rabinowitz. "From the metabolism perspective, it's one noncanonical way of getting nutrients."

Another, Rabinowitz would show in collaborations with colleagues on the SU2C dream team, is a process known as macropinocytosis. "This is the cancer reaching out arms and doing autophagy to stuff outside the cell," says Rabinowitz. "It

In 2011, Rabinowitz and White described how

grabs whatever surrounds it, takes it in and chops it up." Rabinowitz and his colleagues on the SU2C team subsequently demonstrated its importance in Ras-driven tumors and pancreatic cancer, suggesting another metabolic dependency to explore for cancer therapy.

#### METABOLIC FUNDAMENTALS

Meanwhile, Rabinowitz and his team were also developing new experimental and computational methods to track the flux of molecules of fundamental importance to metabolic processes. One of them was NADPH, which is second in importance only to the molecule ATP as a cellular currency of energy. In 2014, they reported that a previously unknown source of this exhaustively studied energy molecule is folate, a vital nutrient and co-factor in many metabolic reactions—and, as it happens, a target of the oldest of chemotherapies.

Over the next year, Sean Morrison of the University of Texas Southwestern Medical Center built on these findings to show that this mode of NADPH generation is of functional importance to metastatic cancer cells. Rabinowitz and his team have since further elucidated links between folate metabolism and NADPH. More recently, they've been generating and evaluating targeted small molecule inhibitors of mitochondrial folate metabolism—which is hyperactive in multiple cancers—as potential cancer therapies.

By the middle of the last decade, Rabinowitz was also moving beyond cell cultures and examining systemic metabolic flux in living animals. In collaboration with White's lab and others, his team examined the fate of a circulating metabolite known as lactate in both healthy mice and those with pancreatic and lung cancers.

Lactate is produced by the breakdown of the sugar glucose, a molecular building block of carbohydrates, through a pathway known as glycolysis. This pathway either produces pyruvate—which can be shuttled though a series of reactions known as the TCA cycle to produce energy—or lactate, which is secreted into the circulation. Produced by cells starved of oxygen, like over-exerted muscle or cancer cells at the heart of a tumor, lactate was long believed to be a waste product primarily cleaned up by the liver.

Rabinowitz and his team reported in 2017 that lactate is in fact a major source of fuel for cells throughout the body. "Different parts of the body work in concert to metabolize carbohydrates," Rabinowitz explains. "This occurs in two main steps: conversion of glucose to circulating lactate and burning of lactate. The second step, of lactate burning, is a generic process that happens everywhere in the body, while the first step is a special process that happens preferentially in the certain types of muscle fibers, the brain, activated immune cells, and cancer. The universal role of lactate as a fuel means that the whole body, not just liver, will clean up any 'extra' lactate made by tumors. At the same time, lactate in the tumor microenvironment is a fuel available to both cancer cells and immune cells."

Rabinowitz has also continued collaborating with Kang, an adept developer of mouse models for the study of metastasis. The pair reported in 2016, for example, that an amino acid known as serine is the source of single carbon units that are used to generate the bases of DNA in proliferating cells, and that two different enzymes can perform the same key reaction in that process using folate. In a more recent study co-authored with Kang, Rabinowitz's team showed that an enzyme ordinarily essential to maintaining NADPH levels in cells can be circumvented in breast and lung cancers driven by the K-Ras oncogene. In other tumor types, such as lung cancers driven with KEAP1 mutation, this same enzyme is essential and a promising drug target.



Photo by Flynn Larsen

Work on pancreatic cancer, meanwhile, has pulled Rabinowitz closer to the clinic. He is already involved in a clinical trial examining the effect of a ketogenic diet—high fat, moderate protein and very low carbohydrate on triple chemotherapy for pancreatic cancer. "If we succeed in completing this trial, it'll be the first adequately powered, randomized trial of dietary intervention to augment cancer therapy," says Rabinowitz. At the Rutgers Cancer Institute, where Rabinowitz directs a metabolomics center, his team is also applying its technologies to study the metabolism of glucose in pancreatic cancer patients.

Ludwig Princeton's partnership with the Institute will play a big part in its work, especially as discoveries made at the Branch are translated into clinical applications. One challenge Rabinowitz has for the Branch, for example, is to explore cachexia, the deadly wasting that accompanies advanced cancer, with an eye to developing preventive treatments. "Ludwig support is enabling my lab both to push frontiers of metabolism measurement technology, and to engage more intensively with clinicians to understand the metabolic vulnerabilities of human cancer and the metabolic needs of cancer patients," says Rabinowitz.

What really captures Rabinowitz's imagination is the establishment of a scientific foundation for dietary interventions to prevent and treat cancer. "It's amazing how many people—since hearing about the Ludwig Princeton Branch—have told me about their struggles with cancer, and they just didn't know what to eat and wanted to make wise choices, but didn't have the guidance," says Rabinowitz. "I want to fix that."

Few are better suited to the challenge. L





# The cancer cell cuisinologist

Eileen White wasn't quite sure what she wanted to do for her postdoctoral research as she wrapped up her graduate studies at the Stony Brook campus of the State University of New York. But she was sure about a couple of things: she wanted to take on something really difficult, and she wanted to make it count. As she mulled over how to do that, White learned of an opening in Bruce Stillman's group at the famed Cold Spring Harbor Laboratory nearby on Long Island. Stillman was interested in how a set of oncogenes identified in the recently sequenced genome of the adenovirus caused cancer. That focus-at the intersection of cancer research and virology-perfectly fit her criteria, and White joined Stillman's group in 1983.

It was a fortuitous choice and a perfect fit for a young researcher with a yen for big problems. "Bruce said, 'This is the virus, this is the oncogene, figure out how it causes cancer'," recalls White, who is today a distinguished professor of molecular biology and biochemistry at Rutgers University in New Jersey and associate director of Ludwig Princeton. "To me, that was a gift. What a great project for a postdoc!"

Her work on that adenoviral oncogene played a key role in establishing the importance of apoptosis, or programmed cell death, in suppressing cancer, and helped launch a field

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24

White's work on the adenoviral oncogene played a key role in establishing the importance of apoptosis, or programmed cell death, in suppressing cancer.

> of cancer research that has already yielded new therapies targeting apoptosis blockers and promises to generate many more. That research, in turn, led to White's landmark identification of autophagy—in which cells cannibalize themselves to recycle nutrients as an important survival mechanism of cancer cells, launching yet another subfield of cancer research with notable promise for the development of new therapies. It has also drawn White deep into the study of cancer metabolism, the focus of the Ludwig Princeton Branch.

#### WAIT A MINUTE!

The daughter of a lawyer and an elementary school teacher, White grew up in a small town on Long Island, New York. Her father had as a teenager hoped to become a physicist and retained a fascination with science. His influence contributed to White's interest in biology, which she majored in as an undergraduate at the Rensselaer Polytechnic Institute in New York, before pursuing graduate studies in Eugene Katz's laboratory at the State University of New York, Stony Brook, studying the genetics of development. Not quite cancer research, but viral oncology was then in its heyday, and Stony Brook's Department of Microbiology was at the forefront of the field. Arnold Levine, the departmental chair, had recently isolated in complex with a viral antigen the cellular p53 tumor suppressor protein, whose function is now known to be disrupted in more than half of all cancers. The department also counted among its faculty at the time Ludwig Harvard Co-director Joan Brugge, who had previously discovered and characterized a viral oncogene named Src and shown that the human genome encodes its homologue-a milestone of modern cancer research. Both were mentors to White, who received her PhD in 1983.

At the time, it was almost an article of faith that viral oncogenes function exclusively by promoting cell proliferation, the fundamental, unifying characteristic of cancers. Another principle taking shape in the field—one that has survived the test of time—was that multiple oncogenic mutations are required to initiate cancer. White's work would help confirm the latter concept, while debunking the former.

The adenoviral gene Stillman assigned to White was named E1B 19K. Its oncogenic partner in the viral genome was named E1A. White was told that only when inserted into a healthy cell together—not individually—would the gene pair transform it into a cancer cell. But in repeating those experiments White noticed that when E1A alone was inserted into cells, colonies of proliferative cells would indeed form. Puzzled, she reported this observation to more experienced lab members.

"They said to me, and this is a direct quote, 'Don't worry, those colonies will go away'," White recalls. "I said, 'what do you mean, they'll go away?' But it was true: the colonies formed, and then the cells died. Then, when I put E1A and E1B in together, the colonies didn't die. That's when the light bulb switched



Photo by Flynn Larsen

on in my head. This suggested to me that E1A was the one driving proliferation, and E1B was allowing the colonies to survive. This was conceptually novel at the time."

White suspected that E1B was supporting survival by preventing apoptosis, or programed cell death, a phenomenon that was poorly understood at the time. As it happened, the Harvard scientist Stanley Korsmeyer had discovered that an oncogene in cells, Bcl-2, drove cancer by preventing apoptosis. White believed E1B was the viral equivalent of Bcl-2, so she contacted Korsmeyer and proposed that they collaborate to test that hypothesis. In studies that continued after White had set up her own lab at Rutgers in 1990, the pair demonstrated that this was indeed the case. suppressed apoptosis is mediated by the p53 protein and contributed key discoveries on how p53 induces cell suicide. They and other researchers would also demonstrate that the Bcl-2 family includes proteins that either drive or suppress apoptosis. Whether or not a cell commits suicide depends on the balance of these opposed proteins. "What we—the whole field—wanted to do ultimately was get a therapy that inhibited the anti-apoptotic proteins to unleash the proapoptotic proteins for cancer therapy," White explains.

They succeeded: new drugs that tip the balance in favor of apoptosis are now approved or in the late stages of development for cancer therapy. White's contributions also helped establish the suppression of apoptosis as a hallmark of cancer (see profile on Douglas Hanahan, page 44) and

White's group subsequently reported that the



Photo by Robert Essel

underscored p53's critically important role as a tumor suppressor.

"In the beginning, when your cells died, you threw them in the garbage," White says. "I was one of those people saying, wait a minute...!"

All this while, White had also remained busy on the administrative front. Soon after she arrived at Rutgers, the university had recruited William Hait from Yale University to set up and direct a new cancer center. "Bill had an enormous task at hand," says White. "He had to build a cancer center from scratch. I mean, there was nothing." her help obtained a Comprehensive Cancer Center designation from the National Cancer Institute. Today, White is the chief scientific officer and deputy director of the Rutgers Cancer Institute of New Jersey. The Ludwig Princeton Branch will be conducting many of its translational clinical studies in partnership with the Institute.

#### **STUBBORN SURVIVORS**

By the late 1990s, White's lab was brimming with cancer cell lines engineered to be defective in apoptosis.

Hait asked White to join the effort, and with

"We were doing all sorts of things to cells to find out what it meant to be unkillable by apoptosis, and there were things we couldn't understand," says White. "You put them in buffer and went home for the weekend, and they'd still be alive. It didn't make any sense. Making a cell unable to kill itself should not impart a miraculous ability to survive starvation."

Hoping to discover their secret, White and her colleagues put the cells under an electron microscope and noticed they were packed to the proverbial gills with autophagosomes. These are small membranous sacks typically containing the cell's defunct proteins and old organelles, destined for dismantling and recycling. Though mammalian autophagy was not well understood at the time, a vast body of work had established its genetics and metabolic chemistry in yeast, which switch on autophagy during starvation. White and her team thought that their never-say-die cancer cells might be doing the same thing.

It turned out, however, that the cancer cells gobbled up their innards even when they were swimming in nutrients. White's subsequent studies examining this unexpected discovery demonstrated for the first time that autophagy is a key mechanism of survival for solid tumor cells.

"Autophagy is important when you're starved or stressed—your cells turn it on, stuff gets recycled, and you're fine," says White. "But what we were seeing was that cancer cells were usurping that pathway. Anytime you find a survival pathway that cancer cells are using, you have to block it. No one in my lab wanted to work on anything else."

The White lab's experiments established, first, that autophagy is a survival mechanism: knocking out the genes essential to autophagy killed cancer cells, especially when they were simultaneously starved or subjected to other types of stresses.

"The other function of autophagy is quality control, getting rid of the garbage—bad "In the beginning, when your cells died, you threw them in the garbage. I was one of those people saying, wait a minute...!"

organelles, bad protein aggregates, things that are toxic," White explains. "So the two fundamental properties that it impinged on were metabolism and inflammation, because when you don't throw out the garbage, that triggers inflammation." Inflammation, meanwhile, is an invitation to immune attack, which tumors take great pains to avoid.

#### WADING INTO METABOLISM

By the late 2000s, White's lab began working extensively with genetically engineered mouse models of cancer to examine how autophagy influences tumor metabolism. To get a better handle on the new discipline, White began a close and continuing collaboration with Josh Rabinowitz, who helped pioneer the field of metabolomics the large-scale, quantitative analysis of metabolites—and is now director of Ludwig Princeton (see accompanying profile, page 14).

"Josh is down the road from us at Princeton, and is the master of all things metabolism," says White. "It's really been wonderful because when we first began working together, he did not know very much about cancer and I did not know much about metabolism. We've learned a lot from each other. It is a perfect example of scientific synergy."



Photo by Flynn Larsen

## "This is the perfect time to target metabolism in cancer ... "

Over the next decade and a half, White's lab would demonstrate in mouse models the importance of autophagy to lung, prostate, breast and melanoma tumors. Using lung cancer models, for example, they showed that disabling autophagy at the point of cancer initiation results in tumors with lower proliferation and malignancy and a higher level of apoptosis compared to controls.

They also discovered, in an early collaboration with Rabinowitz's group, that in cancer cells autophagy is critical to ensuring a steady supply of amino acids to mitochondria—the bean-like organelles that generate cellular energy. That supply ensures the optimal function of the TCA cycle, a core series of metabolic reactions essential to mitochondrial energy generation. TCA cycle dysfunction depletes reservoirs of molecules required to make DNA and other cellular components required for cell proliferation. "Knock out autophagy in these cells," says White, "and they suffer a metabolic crisis."

In another series of studies, White and Rabinowitz compared in an animal model of lung cancer how autophagy loss in cancer cells alone affects tumor growth compared to its systemic loss in the mouse. They found that cancer cells die far more quickly than healthy ones following the loss of autophagy, suggesting that the strategy might well be safely and effectively employed for therapy. In fact, tumors regressed far more dramatically when autophagy was inhibited systemically and didn't grow at all if it was already absent.

This suggested that the autophagy conducted by healthy cells also supports tumor growth. Their studies revealed that mice deficient in autophagy had low circulating levels of an amino acid, arginine, that drives tumor cell proliferation. It turned out that the livers of autophagy-deficient mice were secreting an enzyme that breaks down arginine, an essential tumor nutrient; dietary arginine could, they showed, partially revive tumor growth.

#### INTO TUMOR IMMUNOLOGY

But that wasn't the only reason tumors weren't thriving in autophagy-deficient mice. Previous studies done by White, Rabinowitz and their colleagues had shown that autophagy reshapes the global expression of proteins in cancer cells to eliminate proteins that drive inflammation and can provoke a lethal immune response. White's group had also found that in mice deficient in autophagy, antibodies that removed T cells which kill cancerous and infected cells could restore tumor growth to some degree.

In 2020, White led a study in collaboration with several other labs showing that systemic autophagy suppresses the anticancer immune response. In its absence, cancer cells activate the frontline cells of the innate immune system, initiating a cascade of molecular events that culminates in the recruitment and activation of antitumor T cells.

"By inactivating autophagy, we were unleashing inflammation in a way that activates T cells to kill tumor cells," says White. "A whole flurry of papers that came out around the same time found that autophagy profoundly suppresses the antitumor immune response." White and her colleagues also discovered that autophagy in the liver is most responsible for that inhibition. This opens up the possibility that preferentially targeting autophagy in the liver in combination with other treatments could improve outcomes of cancer therapies and immunotherapies. White's lab is now conducting studies to better understand the phenomenon.

With cancer metabolism and immunology now major areas of study in her lab, it's little surprise she was excited to join Ludwig Princeton. "It's not like Josh had to twist my arm," White says, recalling how she reacted to being recruited to the Branch. "It was an amazing opportunity, everything all wrapped up together. It was perfect."

White's membership in Ludwig connects her to an accomplished community of tumor immunologists and an institution that helped launch the modern era of cancer immunotherapy. "Being a part of the Ludwig Princeton Branch will allow Josh and me to expand our collaboration to new areas to achieve even greater impact. We are very excited to tackle mechanisms of cancer metastasis with Ludwig Princeton's Yibin Kang (see accompanying profile, page 30) and to build on success in cancer immunology with other Ludwig researchers."

Immunotherapies, White says, are among the most remarkable achievements of cancer research, but they need to be made applicable to a far broader range of cancers. She suspects the manipulation of cancer metabolism could be key to that effort.

"This is the perfect time to target metabolism in cancer," says White. "There are already anti-metabolites in our armamentarium of cancer drugs, but now we can understand metabolism at a level we never previously could. We can now more effectively take advantage of seeing cancer as a metabolic disease."



# Disruptor of metastasis

Yibin Kang's ambitions were once nearly thwarted by a pebble.

Growing up in the 1980s in the small coastal town of Longhai on the southern edge of China's Fujian Province, the young Kang had decided by middle school that he wanted to be a scientist. In 10th grade, in pursuit of that goal, he participated in a national chemistry competition organized by the Educational Council of China to find and cultivate the nation's most talented young scientists. On the day of the exam, however, Kang found himself in a quandary. Playing basketball barefoot on a clay court a couple of weeks before, he had landed hard on a sharp pebble and the wound, impervious to treatment, was now festering. "My head was spinning, and I hadn't had much sleep due to the pain, but I decided to take the test anyway," he says. With his chemistry teacher pushing him on a bicycle in a downpour and his father holding an umbrella over his head, Kang made it

to the test and, winning first place in his school, got a seat in a specialized secondary school science program at Peking University High School in Beijing.

With that first hobbled step, Kang began a journey that would take him from the shores of the Taiwan Strait to the U.S. and the cutting edge of research on cancer metastasis, by far the single deadliest consequence of malignant disease. Now the Warner-Lambert/ Parke-Davis Professor of Molecular Biology at Princeton and a founding Member of the Ludwig Princeton Branch, Kang has over the past two decades illuminated key mechanisms of breast cancer metastasis to the bone and described the molecular biology and residential niches of rare breast cancer stem cells capable of seeding new tumors. His research has also explored the complex molecular signaling that underlies the transformation of settled cancer cells into mobile agents of metastasis and their subsequent





Photo by Flynn Larsen

reversion to anchored, tumor-seeding cells in distant organs, processes known respectively as epithelial-mesenchymal transition (EMT) and MET. Aside from their scientific contributions, his studies have generated five experimental drugs—and counting—for treating metastatic cancer.

#### **GROWING UP AND OUT**

Born in the mid-1970s, Kang spent his early years running wild and playing along the shore. "I almost drowned once, trying to catch fish," Kang recalls. "But it was a great way to explore nature. It made us observant and adventurous." His father, a marine biochemist whose budding career had been derailed in the late 1960s by the Cultural Revolution, taught chemistry in Longhai, and Kang joined him there when he was six years old, followed a few years later by his mother, who was a primary school teacher. His father often brought chemistry demo sets back home between classes, and Kang was experimenting with them by the time he was in 6th grade. "In the end, I had my own little laboratory, where I designed my own little chemistry experiments and made specimens out of animals I'd caught," says Kang. "I feel very fortunate to be doing today what I always wanted to do."

Completing the college level natural sciences course at Peking University High School, Kang went on to Fudan University in Shanghai, joining the Department of Genetics headed by the C.C. Tan (a.k.a. Tan Jiazhen), who had brought molecular biology to China. Finding classwork a breeze, Kang spent a good deal of time in the lab learning gene mapping and cloning, and became fast friends with a masters-level student, Yong Wei, who he looked up to as a prototype scientist. "What a crazy scientist!" says Kang. "He lived in the lab and only went to the dorm to tidy himself up when his girlfriend was visiting. He lived and breathed science." Wei is today a staff scientist and manager of Kang's Ludwig Princeton lab. "He's a lifelong friend and a great mentor to my students."

After initially enrolling in a graduate program in Michigan, Kang transferred to Duke University in 1996 to earn his PhD in the laboratory of virologist Bryan Cullen, where he studied the processing and nuclear export of viral gene transcripts. "He was very insightful, always right to the point and blunt," Kang says of his mentor. "He would come to my bench and blast me with his ideas in that British accent, and initially I'd get maybe 30% of what he'd said." The remaining 70% was often salvaged in long discussions with Hal Bogerd, then a technician in the lab and today an accomplished research scientist, who was something of an extracurricular mentor to Kang and remains a close friend today.

With a PhD in hand, and eager to move out north, where he would be closer to his future wife, Kang applied in 2000 for a postdoctoral position in Joan Massagué's laboratory at Memorial Sloan Kettering Cancer Center. After the interview, he and Massagué decided over a few beers that Kang would work on two projects related to cancer metastasis. One concerned a signaling pathway involved in metastasis that is controlled by a protein named TGF $\beta$ . But it was the other one that most excited Kang: an effort to capture the genes essential to bone metastasis.

#### **INTO METASTASIS**

With the sequencing of the human genome and invention of DNA microarrays (gene chips), the tools required to conduct an open-ended search of such genes were, if expensive, now available. And Massagué, it turned out, had the resources and the confidence in Kang to let him give it a try. "I almost drowned once, trying to catch fish. But it was a great way to explore nature. It made us observant and adventurous."

After learning how to work with animals, Kang developed a mouse model in partnership with Massagué and Theresa Guise at the University of Texas, San Antonio, to uncover the genes required for breast cancer metastasis to the bone. Cancer cells derived from breast cancer patients were placed in the mice and assessed for their ability to colonize the bone. Gene expression profiling of the avidly bone-metastatic cells revealed a trove of highly expressed genes, which could then be subjected to functional analysis to identify true drivers of metastasis.

In 2003, Kang, Massagué and their colleagues reported a suite of overexpressed genes that enable breast cancer bone metastasis. They also described how a few of them help carve out a niche in bone to initiate a metastatic tumor. Conceptually, their study supported the hypothesis that only a small subset of cells in a primary tumor are capable of metastasis, and that distinct suites of genes such cells overexpress determine where they wind up. In practical terms, they had developed and tested a model system for the identification of cellular factors that control metastasis to various organs, enabling assessment of their possible blockade for therapy. They went on to conduct similar analyses for breast cancer metastasis to the lung and brain.

#### PHARMA TAKES NOTE

In 2004, Kang joined Princeton as an assistant professor. Partly as a training exercise for graduate students rotating through his lab, he began a project to add an imaging capability to his mouse model to observe signaling events and tumor growth in living animals. "It was a crazy way to do a project," says Kang. But it worked. In 2009, Kang's lab reported the use of that model to show that TGF $\beta$  signaling is associated with the destruction of bone, which occurs in the creation of a metastatic niche, and that its blockade is most effective in suppressing tumor growth in the early stages of metastasis.

The study snagged the attention of scientists at the drug firm Merck, who suggested a collaboration to examine possible links between the TGF $\beta$  signaling pathway and another such pathway controlled by Notch, a protein involved in stem cell maintenance and embryonic development. Led by Kang and Nilay Sethi, then an MD/PhD student in Kang's lab, the researchers showed in 2011 how TGF- $\beta$  stimulates a vicious cycle that fuels metastasis. Tumor cells respond to TGF $\beta$ by expressing a protein named Jagged1, which activates Notch in bone cells to drive further bone destruction while prompting the release of a factor, IL-6, that stimulates tumor progression.

This time around, the results caught the eye of researchers at the biotech Amgen. "They contacted me and said, 'We have a Jagged1 antibody, which we developed for our antiangiogenesis program, but it didn't work at all, so how about we test it in your model?" Kang recalls. Working with the Amgen team, Kang reported in 2017 that the Jagged1 antibody inhibits bone metastasis of breast cancer and makes existing metastases highly sensitive to chemotherapy. Amgen is now developing it for possible use in patients.

#### **BRANCHING OUT**

Developing increasingly sophisticated mouse models, Kang continued to broaden the ambit of his research through the 2010s. His investigations eventually encompassed the similarities and differences between normal and cancer stem cells in the breast and bone, their respective interactions with other cells in their niches and the cellular transformations that accompany the migration and resettlement of metastatic cells—or EMT and MET.

EMT promotes stem cell-like states in cancer cells destined to form new colonies. MET would presumably reverse that process as the cells settle down at a new location. Yet this presented a paradox, as the migrant cell would need to retain its "stemness" to establish a new tumor even as it underwent MET. Kang and his colleagues discovered that metastatic breast cancer cells engage a protein named E-selectin in the bone to undergo MET and settle down while still maintaining their stem-like properties. Partly on the strength of this work, a drug that inhibits E-selectin developed by the firm GlycoMimetics is now in clinical trials for treating breast and prostate cancer metastases.

In other work, Kang and his team discovered how certain small RNA molecules microRNAs—that regulate gene expression support cancer stem cells and metastasis. One such RNA, miR 199a, they showed, helps maintain the stemness of healthy breast stem cells but is coopted by cancer stem cells to escape immune suppression. They also showed how members of another family of microRNAs (miR 200s) suppress EMT



Photo by Flynn Larsen

yet drive metastasis: by simultaneously blocking the cancer cell's secretion of a factor, Tinagl1, that inhibits metastasis. Kang and his colleagues demonstrated that supplementing Tinagl1 undermined tumor progression and metastasis in mouse models of triple-negative breast cancer. This technology has been licensed to a startup trying to translate the discovery into a therapy.

In parallel with the studies modeling metastasis, Kang and his team began searching more generally for genetic factors that contribute to poor outcomes in cancer patients, developing new techniques for the analysis of cancer genomes to that end. The effort yielded an obscure gene encoding a protein named metadherin whose expression was linked to aggressive metastasis and drug resistance in breast cancer. "There were maybe six papers out about this protein when we started working on it," recalls Kang.

Kang's subsequent studies showed that metadherin promotes cancer progression by supporting tumor cells under various stresses such as chemotherapy, and by suppressing the recognition of tumor cells by cancer-targeting T cells. His team demonstrated in mouse models that its inhibition suppresses the growth and metastasis of breast, lung and colorectal cancers, and that mice lacking the gene seem to suffer no ill effects. The protein, "Cancer cells are under constant stress ... The reason they can survive and progress is due to the fitness pathways that allow them to cope with that stress."

> it appears, is not essential to healthy cells in animals—except perhaps under certain stressful conditions—and could therefore be safe to target for therapy. Kang and his colleagues have launched two biotechnology companies to develop drugs targeting metadherin and other cancer fitness genes for therapy.

#### **NEW FRONTIERS**

For the longest time, Kang notes, scientists have considered the genes that gain and lose function to initiate cancer as a set separate from those that enable metastasis. Based on his own studies, Kang considers this view inaccurate, noting that Jagged1 is essential for bone metastasis but also plays a key role in the establishment of primary tumors. Ditto for metadherin, he says, whose loss in engineered mice compromises the formation of primary tumors as well.

"The concept steadily evolved in my lab that cancer is a continuous process and, in fact, many of the so-called oncogenes also play a role in metastasis, and so-called pure metastasis genes are essential for the formation of primary tumors as well," says Kang. "The reason they behave like metastasis genes is that they allow the cells to survive under stressful conditions. Cancer cells are under constant stress mitotic stress, metabolic stress, immune cell attack, genomic instability, to name some. The reason they can survive and progress is due to the fitness pathways that allow them to cope with that stress, so if you target those pathways, they become very vulnerable to therapy. A lot of our research now is focusing on these fitness pathways."

Kang's interrogation of metastasis has also drawn him into cancer metabolism-the focus of Ludwig Princeton. Branch Director Josh Rabinowitz (see accompanying profile, page 14) joined Princeton the same year Kang did, and the two reported the results of their first collaboration in 2010. Employing Rabinowitz's sophisticated technologies for the large-scale analysis of metabolism, the researchers analyzed metabolic changes associated with the metastasis of breast cancer cells. Though a relatively small paper published in the Journal of Biological Chemistry, says Kang, it remains one of his most highly cited studies, since it was among the first to attempt such an analysis.

The pair have since collaborated on other studies of cancer cell metabolism, examining the reliance of cancer cells on various energy-generating and biosynthetic pathways. "We are very complementary to each other," says Kang. "Josh's lab is very strong in metabolomics and chemistry, and we have all the resources in mouse modeling."

Kang has also worked with Eileen White, associate director of the Princeton Branch (see accompanying profile, page 22), in a study on the metabolism of cancers driven by the oncogene Ras. He is, further, associate director for consortium research at the Rutgers Cancer Institute of New Jersey, where White is chief scientific officer.

Kang's focus on cancer metabolism seems



Photo by Flynn Larsen

set to grow at Ludwig Princeton. He is, he says, very interested in exploring the role of exercise, diet and other lifestyle factors that influence the risk and treatment response of metastasis. (It bears noting here that, despite the pebble injury, Kang remains an accomplished athlete: he completed a half IRONMAN contest this summer and then a full IRONMAN in Arizona on November 21st a triathlon that includes a 2.4-mile swim, a 112-mile bike ride and a 26.2-mile run.)

Kang is particularly excited about the many opportunities created by the establishment of the Ludwig Princeton Branch. "Cancer research used to be a relatively niche research area at Princeton, since we do not have a medical school on campus," he says. "Now we are part of a large global family of Ludwig researchers, many of whom work on areas that synergize with our main research interests: metastasis, tumor environment, cancer immunology, epigenetics, cancer stem cells, just to name a few. This will elevate our research to a whole new level."

On the therapeutic front, Kang's team is in collaboration with the Rabinowitz lab now developing candidate drugs targeting a family of metabolic enzymes for the treatment of cancer, stemming from research that will soon be published. Another area of interest to him in cancer metabolism, says Kang, is the role of diet in cancer metastasis to the liver.

"My ultimate goal," says Kang, "is to make a medicine that really helps cancer patients."

He seems well on his way already. L



# The epigenetic explorer

Yang Shi has always associated science with a sense of freedom. This is one reason he enjoys his job so much.

"I'm happy to go into work every day," says Shi, who joined the Oxford Branch of the Ludwig Institute for Cancer Research in the summer of 2020. "You can pursue things that you find interesting. You could be listening to a talk when something just clicks, and you come up with an idea and think to yourself, 'Oh, I have a unique perspective on this. I could try it.' Your discovery might have an impact on human health, or answer a very important fundamental question in biology."

Shi knows this better than most. In 2004, he and his colleagues identified and characterized an enzyme, LSD1, that erases methyl marks from histones, the bead-like proteins DNA spools around in the nucleus of the cell. The discovery by Shi's team upended a 40-year-old dogma that considered this particular kind of epigenetic modification-as the chemical tagging of DNA and histones is known-to be irreversible. It forced a reconsideration of existing models of genomic regulation, since epigenetic marks help determine which stretches of the genome are available for reading by the gene expression machinery of the cell-and that, in turn, controls every aspect of a cell's identity and function. As might be expected, aberrations in the distributions of genetic marks are common drivers of disease, especially cancer.

Shi's laboratory went on to identify many other histone demethylating enzymes with roles in a wide array of biological processes. More recently, he and his colleagues have





Photo by Flynn Larsen

discovered several enzymes that methylate RNA and possibly influence RNA splicing and the translation of gene transcripts into proteins, another level of regulation in the expression of the genome. On the translational front, Shi's work contributed to the development of LSD1 inhibitors for cancer therapy, and these drugs are already in clinical trials for the treatment of cancer and neurological disorders. Meanwhile, Shi's laboratory continues to contribute studies to that end-most notably on the potential use of such drugs to enable and improve the efficacy of immune checkpoint blockade (ICB) therapy, and to achieve sustained reinvigoration of T cells for ICB. His team is also exploring the pharmacological targeting of epigenetic modifiers for the treatment

of pediatric brain cancers and the blood cancer acute myeloid leukemia, both of which are especially characterized by epigenetic dysfunction.

#### **AN UNLIKELY START**

Shi's journey into cancer research was an unlikely one. In high school, Shi was interested in many different topics, including science.

He ultimately opted to indulge his interest in biology, joining the graduate program at New York University, where he explored the regulation of a multi-gene family in mice for his graduate studies in Eva Derman's laboratory, earning his PhD in 1987. The following year, he began a postdoctoral fellowship in the laboratory of Princeton University researcher Thomas Shenk.

Working out of Shenk's lab in 1991, Shi discovered YY1, a mammalian transcription factor (a regulator of gene expression) that can, rather uncommonly for proteins of its ilk, both activate and repress transcription (the reading of a gene for its expression). YY1 was discovered by three independent groups around the same time, but Shi's name for it, short for Yin Yang 1, was broadly adopted because his work captured the functional quirk of the protein as both an activator and repressor.

Work done by multiple labs has since shown that YY1 helps regulate many important biological processes, including cell proliferation, DNA repair and programmed cell death, or apoptosis—all of which can play major roles in the genesis of cancer. Recent evidence suggests that, perhaps due to its dual function, YY1 can operate as either a promoter or suppressor of cancer.

#### LSD1

Following his postdoctoral fellowship, Shi joined the faculty of Harvard Medical School in 1991 as an assistant professor. He was granted tenure and made a full professor in 2004.

That same year, Shi's group reported the discovery for which it is best known: histone demethylation. Owing to its chemical stability and the failure of researchers to find an enzyme capable of stripping it from histones, the methyl mark had long been considered irreversible. "People had always thought methylation was a static modification, and therefore not as interesting as phosphorylation, which is reversible and plays a very important regulatory role because it's dynamic," says Shi.

The discovery of LSD1 was somewhat unexpected. Shi and postdoctoral fellow

"There are so many different types of RNAs and they are methylated by different enzymes. ... These enzymes ultimately will be tied to human diseases, I'm sure of it."

Yujiang Shi were studying the role of metabolic enzymes and their cofactors in epigenetic regulation when they grew curious about how the homolog of a metabolic enzyme, nPAO—which they had discovered in a scrum of proteins involved in transcribing genes—might function in such processes.

They hypothesized that nPAO regulates chromatin structure either through a reaction called polyamine oxidation or demethylation of histone. But months of experimentation failed to detect polyamine oxidase activity. It was only when they switched the substrate in their experiments from polyamine to one of the histones—H3—that they discovered nPAO's ability to strip specific methyl groups from histone proteins, and gave their enzyme the name that stuck: lysine-specific histone demethylase 1, or LSD1.

When Shi and his colleagues reported their landmark discovery in 2004, the response from the scientific world was immediate. "I got phone calls before the paper was officially out from people who had heard rumors about the discovery and wanted to learn more," Shi recalls. "The thought did cross my mind that if I got this wrong, my career would be over."

He was, of course, far from wrong. His



Photo by Flynn Larsen

discovery prompted a profound rethink of existing models of genomic structure and regulation, since epigenetic modifications alter the packaging of DNA, either unfurling it for reading, or tucking it away, inaccessible to the cell's transcriptional machinery. Further, the specificity of the LSD1 enzyme immediately suggested the existence of other demethylases with different specificities, spurring a broad search for those enzymes. Shi was a major contributor to that search as well, identifying other histone demethylating enzymes with roles in a diverse array of biological processes.

Today, more than 20 histone demethylases are known that catalyze the demethylation of almost all major histone lysine methylation sites in the histone proteins. "People soon realized that these enzymes could be a very interesting area to pursue for drug development," Shi says. Work by his group led to the development of LSD1 inhibitors that are currently in clinical trials for the treatment of cancer.

More recently, Shi and his colleagues have explored the potential role of LSD1 in antitumor immunity. They reported in 2018 that the ablation-or removal-of LSD1 in cancer cells leads to the accumulation of double-stranded RNA within the cells. This, they found, induces the activation of an immune factor known as type I interferon, which stimulates anti-tumor immunity mediated by T cells of the immune system. Depleting LSD1 also led to increased infiltration of tumors by T cells, and inhibiting the enzyme made a mouse model of melanoma that otherwise resists immunotherapy susceptible to checkpoint blockade.

In a more recent study examining the effects of LSD1 inhibition on checkpoint blockade therapy, Shi and his colleagues showed in mouse models that the intervention indeed led to an infiltration of T cells into tumors. This desirable effect was, however, countered by the increased production of TGF- $\beta$ , a signaling protein that suppresses the ability of infiltrating T cells to kill cancer cells. They demonstrated that a combination therapy that depletes both LSD1 and TGF- $\beta$  during anti-PD-1 checkpoint blockade immunotherapy results in a significant increase in immune cell infiltration, the killing of cancer cells and elimination of tumors in syngeneic mouse models.

On an entirely different tack, Shi and his colleagues have also been investigating methylation regulation on RNA. In 2017 they discovered a biological role for the methylation of RNA-a molecular transcript of DNA-at a specific spot on the base adenosine. The researchers reported that this methylation event, known as m6A, plays an important and specific role in the cell's repair response to DNA damaged by ultraviolet light. That discovery opened a whole new area of research in the Shi laboratory. Harnessing new technologies to identify nucleic acid modifications, Shi's team has identified several RNA methylating enzymes and is now engaged in the exciting endeavor of describing their biology.

#### **AT LUDWIG**

At Ludwig Oxford, Shi continues to explore how epigenetic modifications to chromatin impact cancers. One of his main goals is to more broadly examine the role of epigenetic regulators in anti-tumor immunity. Through these studies, he and his colleagues hope to uncover effective means for turning socalled "cold" tumors, which are not inflamed, or infiltrated with cancer-targeting immune cells, into "hot" ones that are, and are thus more likely to respond to immunotherapy. His lab is also focusing on the epigenetic aspects of two cancers-diffuse intrinsic pontine glioma (DIPG), an aggressive pediatric brain cancer, and the blood cancer acute myeloid leukemia (AML)where epigenetics has been shown to play a crucial role. An overarching goal is to identify epigenetic regulators whose perturbation can lead to differentiation of tumor cells that can be clinically beneficial. In June, for example, his team reported in a study done in collaboration with Ludwig Harvard investigators that a combination therapy targeting metabolic pathways in combination with LSD1 inhibition might one day serve as a new AML treatment approach. In 2019, he and his colleagues reported evidence that the dual inhibition of LSD1 and another epigenetic enzyme, histone deacetylase, holds some promise for the treatment of DIPG.

Work on RNA methylation too proceeds apace and, in some ways, resembles the early days of research on histonemodifying enzymes—a landscape wide open for discovery. "There are so many different types of RNAs and they are methylated by different enzymes," Shi says. "What do these modifications do? These enzymes ultimately will be tied to human diseases, I'm sure of it."

Finding out if that's true will be made considerably easier with Ludwig support. Shi is excited by the core funding provided by Ludwig, which frees him of some of the burden of grant solicitation, giving him more time to think through scientific problems and their solutions. "Ludwig not only provides the necessary financial resources, but has also created an exciting intellectual environment where like-minded investigators with diverse backgrounds and skill sets come together to tackle cancer, one of the greatest medical threats that humans face," Shi says. "I feel very fortunate to be a member of the Ludwig family." 📕



## DOUGLAS HANAHAN

# Modeler of malignancy

One of the most influential papers in modern cancer biology might never have been written if Doug Hanahan had not issued an open invitation to a few colleagues to explore a dormant Hawaiian volcano.

In 1998, Hanahan, then a professor at the University of California, San Francisco (UCSF), was attending a cancer conference on the island of Maui. "I had decided that I was going to play hooky one day from the meeting," says Hanahan, who in January 2020 became a distinguished scholar at the Lausanne Branch of the Ludwig Institute for Cancer Research. "I announced to a small group of scientists in the bar after the afternoon session that I had rented a car and was planning to drive up to Haleakalā because l think it's one of the most amazing places on the planet, and that if anyone wanted to join me, they were welcome."

Only one person took Hanahan up on his offer: Robert Weinberg, who is now the director of the Ludwig Center at MIT. The next day, during the long drive there and back, and while strolling across the moonscape of the crater rim, the conversation turned to one of their mutual interests. "Rather than talking about our families or whatever, we just got into a conversation about the complexities of cancer," Hanahan says.

Over the next several hours, an idea began to crystalize between them: that underlying the bewildering variety of cancers that afflict people are a set of common capabilities that cancer cells and tumors must acquire before they can become deadly. Differing cells in differing tissues might acquire them in different orders, but all tumors must acquire these core biological functions-what Hanahan and Weinberg would call the "hallmarks of cancer"-to become malignant. "We compared it to climbing Mount Everest," Hanahan says. "There are many routes you can take to climb the mountain, but all of them must

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pass through the same heights to reach the top."

Hanahan and Weinberg refined this idea over the next two years, ultimately publishing their meditations on the hallmarks of cancer as a perspective in the millennium issue of the journal Cell in January 2000. In that first essay, the pair identified six capabilities acquired by incipient tumors as they develop, step by step, into full-blown malignancies. These included sustaining proliferative signaling, evading growth suppression, resisting cell death, enabling replicative immortality, activating invasion and metastasis and inducing angiogenesis, or blood vessel formation. While each of these phenomena were already known at the time, no one had ever arranged them so coherently. Their synthesis served as an unparalleled conceptual framework for understanding the cellular and molecular underpinnings of cancer.

"Neither of us had any great sense of destiny or thought that this was going to somehow be very impactful. We just thought this was kind of a cool idea and maybe we should throw it out there," Hanahan says. "But within a couple years, it became clear that it was being widely cited. People were really resonating with it."

"I've often wondered whether the hypoxia at

10,000 feet that day had anything to do with it," Hanahan jokes.

In 2011, the pair published an updated version that included two new hallmarksthe reprograming of energy metabolism and evading the immune response. They also further emphasized in that update the importance of the "tumor microenvironment," a unique ecosystem of noncancerous cells and molecular factors that contribute critically to the acquisition of hallmark capabilities. Aside from their outsize influence on the sprawling field of cancer research, the hallmarks described in the two papers also established an enduring infrastructure for Hanahan's program of research as his laboratory moved from UCSF to the EPFL in Lausanne and then to the Lausanne Branch of the Ludwig Institute for Cancer Research. As for the papers themselves-citations of the sequel have now far outpaced even the impressive numbers of the first, which long held the title of Cell's most highly cited paper of all time.

#### **COLD SPRING HARBOR**

It probably helped that by the time the Hallmarks of Cancer was published, Hanahan was already well known as a pioneering molecular biologist with several



Photo by Felix Imhof

seminal discoveries in cancer biology and immunology to his name.

While still a Harvard graduate student working at the Cold Spring Harbor Laboratory in New York in the 1970s, Hanahan developed new and better methods for gene cloning and bacterial genetic engineering, which were very new biological technologies at the time. "I ended up having a nomadic PhD career, where I went back and forth between Harvard in Cambridge and Cold Spring Harbor in Long Island," Hanahan says.

After Hanahan earned his PhD, he had a life-changing conversation with Cold Spring Harbor Laboratory's Director, Nobel laureate James Watson, who asked him what he wanted to do with his career. "I told him I wanted to study gene regulation by introducing genes into mice. There were a few studies suggesting you could introduce DNA into the mouse germline by injecting fertilized mouse eggs," Hanahan recalled. "Jim said, 'It's really tough. Other people have tried and failed. Why do you think you'd succeed?' I just said I thought I could do it, and he said, 'Well, I want to get this technology working here, so if you want to move down here and make a commitment, I'll support you.'"

Succeed Hanahan did. By the mid-1980s, he had engineered one of the first transgenic mouse models to express oncogenes and develop tumors, which he described in a single-author *Nature* paper. The mice would prove to be an extraordinary asset to the study of not only cancer but immunology, and Hanahan immediately set about using



Photo by Felix Imhof

the mice to explore such phenomena as autoimmunity and the induction of immune tolerance. In fact, Hanahan says, his initial interest in cancer was limited to how oncogenes could be exploited to study differentiated cells and to induce cells to undergo proliferative expansion. "You could get access to more cells to study this way," he explains. "Those were the original reasons for my interest in oncogenes."

But when his transgenic mice began developing tumors, he became increasingly curious about the mechanisms of cancer. "One of the cell types I had chosen to focus on was the islet cells that make insulin in the pancreas," Hanahan says. "It became very clear that the oncogene was not instantaneously transforming every cell that expressed it into a tumor."

Rather, he noticed, there was a latent period, and that out of hundreds of islet cells, only a few developed into solid tumors. "As I read more about cancer, it became apparent that what I was observing in the mice fit with the epidemiology and histopathology of the human cancer, in that it was a multi step process that takes time and requires a sequential progression through differing premalignant stages," Hanahan says. "It began to dawn on me that maybe our mouse model is very interesting for studying cancer."

Working with Judah Folkman, who died in 2008, Hanahan used the model to first report in 1989 that the transition from precancerous growth to malignancy is preceded by the assumption of angiogenic potential by a subset of abnormally proliferating cells. When enough tumor cells become angiogenic, the tumor can grow and eventually metastasize. The pair described an "angiogenic switch" that allows blood vessels required for solid tumor growth to form-showing that about a quarter of the rapidly proliferating cells developed this capability. They went on to identify the protein factors that trigger the transformation and later explored the pharmacological inhibition of the switch and its effect at distinct stages of tumorigenesis, contributing critically to the development of anti-angiogenic drugs for cancer therapy.

Hanahan and his colleagues also employed transgenic mice to explore tumor immunology. They showed in the late 1990s, for example, that antigens expressed in solid tumors are poor stimulators of T cell attack, and that the tumor microenvironment suppresses immune responses. In various other studies, they showed how immune cells contribute to angiogenesis and other events essential to tumor growth and survival. And so, by 2000, Hanahan's sprawling exploration of multi-stage tumorigenesis using mouse models developed in his laboratory was already eliciting routine invitations to speak at cancer conferences one of which took place in Maui, where he and Weinberg would have their fateful conversation atop Haleakalā.

#### **MOTIVATING TEACHERS**

Hanahan credits inspirational college mentors for putting him onto a scientific track. It wasn't biology that excited him at first, however, but physics.

"During my sophomore year at the University of Washington, I had a physics teacher, Lowell Brown, who was terrific. It was a really hard course, but he was inspirational," Hanahan says. When Brown encouraged Hanahan to transfer to another university with a better physics department, Hanahan set his sights on MIT and was accepted.

At MIT, Hanahan took a general biology course, where he encountered another inspirational teacher, Nobel laureate Salvador Luria. "He started the course talking about how the single cell fertilized egg of a human, through the selective expression of the information encoded in the genome, divides and differentiates and evolves into an organism with all the amazing characteristics that we have," Hanahan recalls. "It was something I hadn't thought about before and which I found very intriguing."

Luria also told his students a revolution was afoot in biology involving recombinant DNA and cloning. This was in 1975, the same year as the famous Asilomar Conference in California, where 140 professionals—including biologists, lawyers and physicians—convened to draw up guidelines for ensuring the safety of recombinant DNA technology. "The revolution that was happening, and the controversy and debate around it, stimulated a transition into biology for me," "Amazingly enough, that same mouse model of cancer that I first created in 1984 continues to teach us interesting lessons about biology."

Hanahan says. "I wanted to get involved in this revolution and to do molecular biology and clone genes."

After graduating from MIT, Hanahan entered the Biophysics PhD program at Harvard. "There is a grand tradition of physicists who've moved into biology and have made epic contributions, so this program has long had the view that bringing in people with a background in physics was a good thing," Hanahan says. "The main lesson from physics that I brought into my PhD research was the notion of problem solving, because throughout your physics training, you're given very difficult problems and you have to figure out how to solve them. Often, you try one kind of a strategy, it doesn't work, so you go back and try another and you keep persisting, now having some sense that the problem had to be solvable. I really brought with me that sense of determination to solve problems and to try different angles."

At Harvard, Hanahan joined the lab of Paul Doty and worked on cloning collagen genes. By that time, the Asilomar conference had prompted a moratorium in Cambridge on the cloning of mammalian genes. Fortunately, Doty was able to arrange for Hanahan to continue his work at Cold Spring Harbor,



Photo by Felix Imhof

one of the few places on the East Coast possessing so-called biosafety-3 level facilities where such research was allowed at the time.

"When I was down there working, I met a bunch of the scientists and got really excited about the notion of cloning tumor associated genes," Hanahan says.

#### **GET OUT, MOVE AROUND**

The genetically engineered cancer mouse models Hanahan first developed at Cold Spring Harbor would remain a staple of his research, even as he became a professor of biochemistry and biophysics at UCSF in 1988, and then director of the Swiss Institute for Experimental Cancer Research at EPFL, in Lausanne, starting in 2009. The mouse models became platforms for elucidating the mechanisms underlying each stage of tumorigenesis and the acquisition of hallmarks of cancer. In the early 2000s, for example-led in part by Ludwig Lausanne's Johanna Joyce, who completed her postdoc in Hanahan's UCSF laboratory-Hanahan's lab described in a series of studies how specific protein-snipping enzymes known as cathepsins contribute to distinct stages of tumor growth and metastasis. In 2009, Hanahan's team used a mouse model to identify sets of microRNAs—which regulate gene expression-that contribute to each step of tumorigenesis and the acquisition of specific hallmark traits. His lab today continues to explore the biology of some of those microRNAs.

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model of cancer that I first created in 1984 continues to teach us interesting lessons about biology," Hanahan says.

Earlier this year, for example, a team led by Hanahan published a study identifying a previously unrecognized mechanism by which pancreatic cancer cells methodically retrace their developmental pathway to an immature state of cellular development to spawn highly aggressive tumors. The discovery provided concrete evidence that such cellular de-differentiation, widely observed across cancer types, is not merely a random occurrence but rather an independently regulated and separable step in tumorigenesis.

In addition to pancreatic cancer, his team is also studying mouse models of melanoma and glioblastoma, cervical cancer and breast cancer. "We've got a broad based set of models of different forms in human cancer that we're interrogating in different ways," Hanahan says. His team is today especially interested in how the tumor microenvironment collaborates with cancer cells to manifest malignant disease and resist therapy.

He and his colleagues are exploring new technologies-in part as participants in Ludwig's multi-center Tumor Atlas Projectto interrogate tumors in greater depth. In another collaboration with Ludwig Lausanne colleagues, Hanahan's team is studying how the tumor microenvironment contributes to drug resistance, with a particular focus on its role in thwarting anti-tumor immunity. In addition, the lab is studying mechanisms of adaptive resistance to therapies targeting hallmark capabilities and exploring ways to circumvent such drug resistance through the use of combination therapies that simultaneously target distinct hallmark capabilities.

"My engagement with the Lausanne Branch of the Ludwig Institute, and increasingly with the Ludwig community at large, is stimulating collegial interactions that are spawning new collaborative opportunities based on complementary expertise and insights, both for me and my research team," says Hanahan. "This will enrich our agenda and foster our progress in cancer science."

#### **THINGS ARE HAPPENING**

In their 2000 Hallmarks of Cancer essay, Hanahan and Weinberg predicted that in 25 years, cancer research would develop "into a logical science, where the complexities of the disease described in the laboratory and clinic will become understandable in terms of a small number of underlying principles."

Hanahan doesn't think his field has yet achieved this goal, but he remains optimistic that one day, perhaps sooner than anyone expects, it will. "Things are happening," he says. "There has been an explosion in enabling technologies to interrogate tumors, particularly at the single cell level. I think you can foresee that the field is becoming more logical and we're starting to understand more than ever about cancer."

Reflecting on his career, Hanahan says he thinks a hallmark of his own life has been a willingness to go out and experience new environments. "Whether it was moving from Seattle to MIT, MIT to Harvard, Harvard to Cold Spring Harbor, Cold Spring Harbor to San Francisco, or now most dramatically from the US to Europe, I was happy and successful in each place I lived and worked," Hanahan says. "I was never obligated to move but, rather, was inspired to move. I tell my students that you shouldn't think that you work in the same institution until you're ready to retire. You should instead make strategic moves, getting out of your comfort zone, taking on new challenges with no guarantee of success, but with exciting opportunities to make an impact." L

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