LUDWIG
CANCER
RESEARCH

2019 RESEARCH HIGHLIGHTS

LIFE-CHANGING SCIENCE

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WELCOME

Welcome to the Ludwig 2019 Research Highlights Report.

As we do every year, we've selected a handful of recent discoveries and advances made by researchers affiliated with Ludwig Cancer Research and woven the stories behind each into profiles of the scientists responsible for them.

The intention is to illustrate the depth and breadth of Ludwig's life-changing science. But the stories told in this report also speak to the human side of science—the aspirations and fascinations that drive scientific research, the relationships that fuel its progress—as much as the fundamental challenges of cancer biology and care. Beyond that, they illustrate the truly global scope of the scientific effort to defeat cancer.

You will thus read here about the improbable journey of a boy living hand-to-mouth in a coastal Chinese village to a U.S. university and on to the frontiers of medical nanotechnology, where his inventions, now in clinical trials, are putting a new twist on chemo- and radiotherapy. You'll learn about how a bright young girl from California moved to aid children with Down syndrome grew into an inspired clinical neurologist whose research is bringing new hope to kids diagnosed with an intractable brain cancer and people afflicted by the cognitive decline caused by chemotherapy. Another profile charts the path taken by a British nephrologist to a landmark discovery of how cells sense and respond to oxygen—a system of profound significance in cancer—and his recent identification of a second, evolutionarily ancient cellular oxygen sensor. A fourth recounts how a young immigrant to the U.S. from Taiwan, drawn to dermatology and genomics in his postgraduate years, deciphered the body's cellular GPS, discovered a sprawling family of gene-regulating RNA molecules and went on to illuminate the vast, dark and vitally active expanse of the noncoding genome across 23 types of tumors.

Also included are stories of vibrant collaborations that have spawned exciting new technologies for cancer diagnosis and care. One such profile tells of an ongoing partnership between two globetrotting researchers that has spanned a decade and two continents, culminating most recently in a remarkable clinical trial of an individualized vaccine for the treatment of ovarian cancer. Another charts an even longer collaboration between a researcher of Greek origin and his American mentor that has transformed our understanding of the cancer genome and is now yielding breathtaking advances in the development of noninvasive, "liquid biopsies" for the detection of multiple types of cancer. A final story describes how the collaborative framework of one Ludwig Center has enabled an ambitious effort to generate high-dimensional maps of tumors.

We trust you will enjoy this sampling of Ludwig's contributions to cancer research.

Sincerely,

Edward A. McDermott Jr.

Chi Van Dang

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THE NANODESIGNER

Defying all odds, Wenbin Lin made his way from a coastal village in China to a U.S. graduate school and on to the frontiers of medical nanotechnology, where his inventions promise to supercharge cancer therapy.

Within a year of Wilhelm Roentgen's discovery of X-rays, the ionizing radiation was already being deployed in the clinic to destroy tumors. In the 123 years since, technologies for the sourcing, targeting and detection of X-rays have improved dramatically. But the one thing nobody has yet done is boost the primary effects of the radiation—the generation of violently reactive ions known as free radicals that induce the destruction of tumors—without also amplifying the treatment's toxicity. This, Ludwig Chicago's Wenbin Lin will tell you, is what he and his team have accomplished.

In 2018, Lin and his colleagues reported in *Nature Biomedical Engineering* and *Nature Communications* their design and preclinical evaluation of a nanotechnology to boost the effects of radiotherapy when delivered into tumors. The studies, done in collaboration with Ludwig Chicago Co-director Ralph Weichselbaum, demonstrated that, when combined with checkpoint blockade, the treatment not only destroyed the targeted tumors but also led to the regression of untreated tumors in mouse models of breast and colorectal cancers, neither of which is typically responsive to immunotherapy.

Proletarian antecedents

Lin was born in 1966 in the Fujian Province of China, just as Mao Zedong's decade-long Great Proletarian Cultural Revolution was getting underway. But its upheavals—the suspension of education, the dismantling of the urban intelligentsia—were not much of a factor in Lin's early life. He was, after all, a peasant child, growing up in a small coastal town, where his parents subsisted off a small patch of land and fished off the coast in the Taiwan Strait to feed their growing family. "To say that I come from a humble background would be an understatement," says Lin.

He was, he recalls, expected to pull his weight on field and sea. Schooling was a secondary consideration. There was certainly no time for homework; hobbies and interests were luxuries beyond contemplation. "Surviving was my interest," Lin says, laughing. "Not dying from hunger was *really* my interest."

WENBIN LIN

LUDWIG CHICAGO

Photo by Anne Ryan

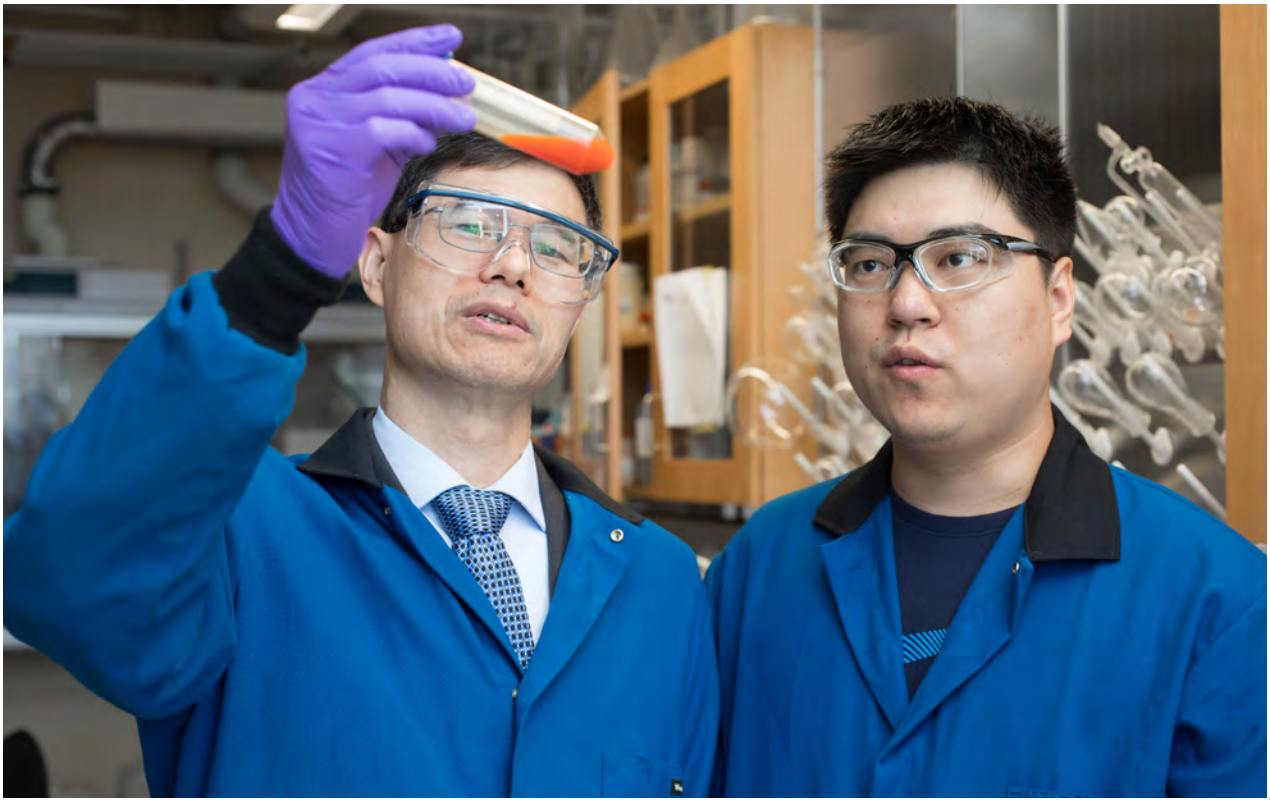


Photo by Anne Ryan

Lin in his Chicago lab with graduate student Guangxu Lan.

Still, in 1980, Lin's parents scraped together enough money to send Lin, the eldest of their four boys, to a proper school in a nearby city to complete the last three years of his secondary schooling.

Entering eighth grade, Lin became a full-time student for the first time in his life. "Even though we were dirt poor, my parents supported me," says Lin. "It wasn't much but they had to pay, and not having me working in the field was a cost as well." Lin turned out to be a gifted student and was soon dreaming about college.

After the Cultural Revolution ended in 1976, the government reopened universities and instituted national exams to select students for entry. The measure had an equalizing effect. "We knew that if you studied hard and did well, you could escape your circumstances," says Lin. "That was enough motivation for me to study hard." Lin made the cut for the University of Science and Technology of China, then the most prestigious institution of higher learning in the country. One of his younger siblings dropped out of school to help his parents make up for the lost labor. None of his brothers attended college.

Like most people in his generation, Lin says, he had no idea what he should study. The general feeling, however, was that math, physics and chemistry—in that order—would most likely help you get ahead in life. Lin decided he'd study chemical physics, a relatively theoretical take on chemistry. "We didn't know what it entailed, but the name sounded really cool," says Lin. He would eventually switch his focus to the decidedly stodgier field of inorganic chemical synthesis and enroll for an additional year, in 1988, for a master's degree. But then, halfway through

that year, Lin won a fellowship to join a PhD program at the University of Illinois, Urbana-Champaign.

That he should go was beyond question. Trouble was he needed a passport, and that was going to be difficult. The rules of China's Hukou system of household registration required that Lin apply for the passport from his hometown, so he quit university and went home. "After six years of higher education," he says wryly, "I was a peasant again."

A path forward

Obtaining the passport took time, but Lin felt lucky to have received one, even if he arrived in Urbana-Champaign three weeks late for his PhD program in 1989. But there were other problems. The professor he hoped to work with had no opening for a graduate student in his chosen area—bioinorganic chemistry. To make matters worse, his first meeting with the man was a disaster. "Instead of asking, 'what's your name?', he asked me, 'what do you want to be called?'" Lin recounts. "I had no idea what he was asking, even after he repeated it three times. It was a rough start: I had become this potential problem student because I could not speak English."

Fortunately, an organometallic and materials chemist named Gregory Girolami did have an opening in his lab—and an eye for talent that saw past the language barrier. "Working for him was the turning point of my scientific career," says Lin. "He is one the best scientists I've ever met and one of the nicest people you could ever meet."

Lin spent more than two years making and characterizing unusual molecules in Girolami's lab before venturing into chemical vapor deposition, a branch of chemistry essential to microchip design. He soon discovered an unusual reaction that permitted the exchange of metal ions after vapor deposition, and Girolami sent him over to the laboratory of his friend and colleague, Ralph Nuzzo, to figure out how it worked. "Having two advisors who were so different and focused on such different areas of chemistry was a very educational experience," says Lin.

> "We knew that if you studied hard and did well, you could escape your circumstances. That was enough motivation for me to study hard."

After receiving his PhD in 1994, Lin moved to Evanston, Illinois, to work with the prominent organometallic and materials chemist Tobin Marks at Northwestern University. His luck kicked in again: He managed to obtain permanent residency in the U.S. With green card in hand, he qualified for a postdoctoral fellowship from the National Science Foundation. Lin used the funding to explore nonlinear optical materials, which are useful for creating lasers in the blue light range—a capability of some interest to the U.S. Department of Defense.

With a strong suite of publications from that work, Lin was hired as an assistant professor at Brandeis University in 1997. But the startup funding at Brandeis was relatively small, so he began looking for ways to continue his research on the cheap. Coordination polymer chemistry fit the bill.

A class of large molecular structures that

"I have a very practical mind. I want to solve problems. I give myself a hard time, asking, 'Ok, what can this do?' "

include the better known metal-organic frameworks (MOFs), coordination polymers are built by linking a variety of metal atoms to complex organic molecules. The resulting molecular frameworks have geometries and chemical properties that are endlessly tunable and of dizzying diversity and utility. Lin initially applied the chemistry to grow crystals for nonlinear optics but was soon exploring them as useful materials in their own right. "Coordination polymer chemistry was something that could be done in a small place with modest resources. I got into the field because of my upbringing," he says, laughing. "You've got to be practical, you've got to survive!"

Into the nanosphere

In 2001, Lin moved his operation over to the University of North Carolina, Chapel Hill, where he was appointed professor of chemistry and quickly broadened his exploration of MOFs. Their frameworks served as exceptional platforms for fundamental research into such things as the mechanisms by which catalysts accelerate chemical reactions, and Lin continues to pursue such studies. But it was their practical utility that fascinated Lin most. "Sometimes I think I should be a CEO, not a professor," he says. "I have a very practical mind. I want to solve problems. I give myself a hard time, asking, 'Ok, what can this do?' "

Applying an acute chemical intuition, Lin has mixed, matched and fiddled with the molecular constituents of MOFs, generating multifariously structured frameworks to perform tasks ranging from capturing toxic gases to collecting uranium from seawater to stably storing hydrogen to harvesting solar energy. "It's like building a puzzle," he says. "You get the different pieces of the MOF to work cooperatively to give you the best effect you're after."

Work on the energy and environmental applications of MOFs continue in the lab today. But Lin has in recent years increasingly favored their use in medical nanotechnology. "I'm very critical about what we do because, with my background, I feel we cannot afford to waste any resource," says Lin. "If you want to make an impact in the energy landscape, you need to invest billions of dollars to make a difference. That's a difficult thing for an academic entrepreneur to do. But in the biomedical sciences, there's an established, stepwise approach to translate your discoveries into products for clinical trials."

Lin took the first of those steps a few years after he arrived at Chapel Hill. The National Cancer Institute launched its Alliance for Nanotechnology in Cancer in 2004, and Lin applied for a grant to develop an iron oxide nanoparticle for theranostic applications, which combine diagnostic and therapeutic functions in a single agent. But he never started that project. Instead, he convinced the program director to allow him to pioneer nanoscale versions of MOFs for similar purposes.

Initially, Lin and his team focused on developing nanoscale MOFs (or nMOFs) to improve the images generated by magnetic resonance imaging and computed

Lin and postdoc Tasha Drake.

Photo by Anne Ryan

tomography, publishing several papers that put the lab on the map as a pioneer in the field. Within a few years, they were designing nMOFs as vehicles for drug delivery—packaging chemotherapies, RNA therapies and other drugs into their capacious interiors to treat cancer. By 2011, his team had reported the development of coated nMOFs that could be preferentially delivered to tumors to minimize toxicity.

A year after moving to the University of Chicago in 2013, Lin and his colleagues published another paper describing a self-assembling nanoscale coordination polymer (NCP) that could carry two types of chemotherapy (for example, cisplatin and paclitaxel)—one water soluble, the other hydrophobic—at once to a tumor. Once inside, the NCP was triggered to release both drugs at the same time. The researchers also showed in mouse models of pancreatic, lung and colon cancers that their NCP system was likely to be not only less toxic but far more efficacious. In 2015, Lin established a company named Coordination Pharmaceuticals to commercialize the drug delivery system. Two NCP formulations are currently in a Phase 1 clinical trial to assess their safety and most effective dosage.

Radical improvements

From his early days developing contrast agents for CT imaging, Lin had been intrigued

by the interactions between nMOFs and X-rays. After moving to Chicago and meeting Ralph Weichselbaum—an authority on radiotherapy—he began exploring how those interactions might be harnessed for therapy. "Ralph is big on research, and he has accumulated technology, facilities and resources at the Ludwig Center that are very valuable and accessible to researchers," says Lin. Most significantly, Weichselbaum provided Lin with access to X-ray irradiators and a wellspring of clinical and immunologic insight.

In 2014, Lin and his colleagues published a paper in which they examined how the energy of X-rays is transferred within the framework of one of their MOFs. Their nMOF—clusters of the metals hafnium or zirconium linked by an organic molecule known as anthracene—was an X-ray scintillator. The metal clusters at the corners of the pyramidal structure served as antennae for the X-rays, capturing and transferring that energy to multiple anthracene bridges, which then emitted a flash of visible light.

It didn't take long for Lin to realize that this system of energy transfer could be adapted to enhance radiotherapy. Lin and his group soon came up with an nMOF made of hafnium clusters linked by the organic molecule porphyrin—schematically resembling a cage bejeweled with metallic flowers—that amplified the effects of radiation in tissues. "We didn't realize it at the time, but we had discovered an entirely new mechanism for enhancing radiotherapy," says Lin. They named it radiotherapy-radiodynamic therapy, or RT-RDT.

When X-rays hit tissues, they break apart water molecules to generate free radicals. The damage free radicals cause prompts cells to commit suicide, which in turn induces immune responses that dismantle the targeted tumor. But X-rays interact with water molecules only rarely, limiting the generation of free radicals. The hafnium clusters in Lin's nMOFs, on the other hand, sponge up the X-rays. So energized, they not only split water to generate hydroxyl free radicals, but transfer excess energy to the nMOF's porphyrin linkers as well. The excited linkers then shoot off energized oxygen (also called singlet oxygen) to cause even more cellular mayhem. This explosion of reactive oxygen species makes RT-RDT about ten times more efficient than ordinary irradiation.

A platinum-based chemotherapy, cisplatin, is already used with X-rays to enhance the destruction of some head and neck tumors. But its effects are merely additive, and the combination of the two therapies can be highly toxic; Lin's nMOFs are, on the other hand, synergistic in effect and appear to be biodegradable and nontoxic.

In 2015, Lin started another company named RiMO Therapeutics to commercialize the nMOFs as radio-enhancers. That year, Lin and Weichselbaum obtained a grant from the National Cancer Institute to develop nMOFs for radiotherapy and for photodynamic therapy, in which near-infrared light is used on superficial tumors to generate free radicals and kill cancerous cells. Over the next couple of years, the pair led studies establishing the proof of these concepts in animal models. They also showed that the cell-killing accomplished by RT-RDT and nMOF-boosted photodynamic therapy created an environment highly conducive to anti-tumor immune responses.

In the 2018 study reported in *Nature Biomedical Engineering*, Lin, Weichselbaum and their colleagues filled the cage-like nMOF with an IDO inhibitor—an apparent booster of immune responses in animal studies and early clinical trials that recently failed in larger trials—and injected it into a single tumor in a mouse. The tumor was then irradiated, and the mouse bearing it given a round of checkpoint blockade.

Remarkably, this treatment completely eliminated untreated tumors in models of

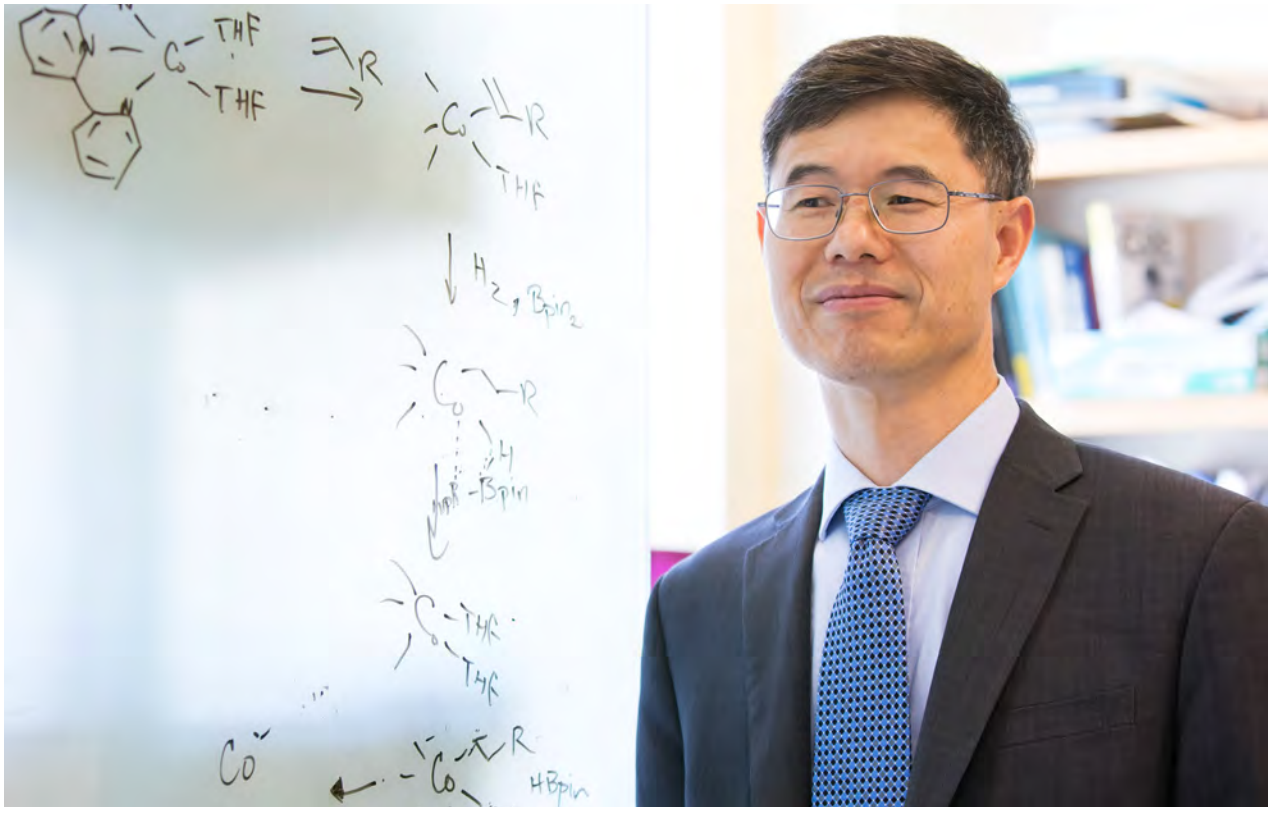


Photo by Anne Ryan

breast and colorectal cancer, both of which are typically resistant to immunotherapy. It also served as proof of concept that the drug delivery capacity of Lin's nMOFs might be combined with RT-RDT to more efficiently conquer tumors.

Their subsequent study in *Nature Communications* dispensed with the IDO inhibitor. But it too showed that the nMOF-enabled combination of radiotherapy and checkpoint blockade caused impressively broad regressions of untreated tumors—or abscopal effects—in a mouse model of colorectal cancer. The results suggest nMOFs might ultimately help expand the variety of cancers amenable to immunotherapy. But, for now, Lin is testing his nMOF only as a booster of radiotherapy: RiMO Therapeutics is already enrolling patients into a clinical trial to establish a safe and effective dosage of nMOFs for RT-RDT in head and neck cancers.

Meanwhile, Lin—who folded RiMO Therapeutics into Coordination Pharmaceuticals in 2018 to streamline operations—continues to innovate. His team separately reported in 2018 in the *Journal of the American Chemical Society* an iron-based nanoparticle that similarly drives abscopal effects in mice with colorectal cancer when combined with checkpoint blockade and photodynamic therapy. Most notably, it overcomes the limitations imposed on such therapies by the oxygen-poor environment at the heart of tumors. In another 2018 *Nature Communications* publication, his lab described an nMOF that could be targeted to mitochondria—the power stations of cells—to more efficiently induce cell death by RT-RDT.

"We hope that these technology platforms lead to things that are so new and so different that we'll really help patients in the clinic," says Lin.

It's odds-on that something he invents ultimately will.

THE PERSONAL VACCINOLOGIST

Lana Kandalaft's scientific journey, which began in Jordan, led to an ongoing collaboration in translational medicine with a leading immuno-oncologist and their creation of a personalized cancer vaccine.

The plan was that Lana Kandalaft would get her PhD and then return to Jordan. That, at least, was what she'd told her dad, a surgeon and her role model, when she first informed him she wanted to study abroad. But as she wrapped up her doctoral studies in pharmaceutical cell biology and drug delivery in the UK in 2003, Kandalaft realized this wasn't going to happen. She wasn't entirely sure what she'd do next, but she was sure about a couple of things. One was that a young scientist in a hurry wasn't likely to hurry anywhere professionally back home. The other was that whatever she wound up doing, she wanted to see her science in action. "I was so attracted to applying what I had learned," she says, "not just doing drug delivery for the sake of delivery, but actually curing a disease."

In 2018, Ludwig Lausanne's Kandalaft passed a milestone on the road to realizing that dream. A study she led with George Coukos, director of the Lausanne Branch of the Ludwig Institute for Cancer Research, and her colleague there Alexandre Harari, showed that an entirely new type of personalized cancer vaccine she and Coukos developed over the course of a decade induces clinically effective immune responses in patients receiving a combination of standard therapies for recurrent and advanced ovarian cancer. Reported in *Science Translational Medicine*, the study revealed that the vaccine—made from a processed sample of a patients' tumors and delivered via their own immune cells—is well tolerated and elicits therapeutically effective immune responses when delivered in combination with a pair of drugs currently used to treat ovarian cancer.

A pathway to science

Kandalaft was born in Germany, where her mother, a dentist, and father both got their postgraduate training. Soon after her

LANA KANDALAFT

LUDWIG LAUSANNE

Photo by Eric Déroze

Alexandre Harari, left, Lana Kandalaft and George Coukos.

parents returned to Lebanon, however, civil war broke out and the family emigrated to Jordan. An all-round athlete and excellent student, Kandalaft got a scholarship in 1995, at the age of 16, to join the University of Jordan's School of Pharmacy. After graduating in 2000, Kandalaft enrolled in a PhD program at the Welsh School of Pharmacy at Cardiff University, where she focused on drug delivery.

In 2004, Kandalaft started a three-year postdoctoral stint at The National Cancer Institute, in Bethesda, Maryland, studying cancer therapeutics, and then continued for an additional year as a senior research fellow. She also met her future husband, a fellow globetrotting Lebanese émigré who worked in private equity and who was, like her, an avid runner.

As her postdoctoral studies drew to a close in 2008, Kandalaft came across an advertisement from the University of Pennsylvania (UPenn) for a coordinator for translational science and clinical development at a new Ovarian Cancer Research Center. The job was posted between Coukos, the founding director of the Center, and Carl June, who had

Photo by Felix Imhof

pioneered a promising immunotherapy for cancer known as chimeric antigen-receptor T cell (CAR-T) therapy. After more than a dozen interviews, Kandalaft was hired.

Toward translation

Carl June had already built a translational research capacity in developing his CAR-T therapies, and Coukos wanted to do the same for his new Center. That would be Kandalaft's primary responsibility. Her first task, however, was to develop a clinical trial protocol for a CAR-T therapy for ovarian cancer and obtain approval for its clinical evaluation from the U.S. Food and Drug Administration. "It was totally different from my background in drug delivery, but it was a new challenge and I was very excited to work on it," says Kandalaft.

Next, Kandalaft worked with Coukos on a clinical trial applying a novel cancer vaccine developed by a biotechnology company in combination with a targeted therapy, bevacizumab, designed to inhibit new blood vessels in tumors. Coukos was interested in the vaccine's potential to treat recurrent ovarian cancer. "It was George's vision to bring immunotherapy to ovarian cancer patients," says Kandalaft.

Cancer vaccines, like other inoculations, teach the immune system's T and B cells to recognize small fragments of proteins, known as antigens, whose molecular aberrations betray disease. A few cancer antigens are shared within and across cancer types, but the majority are generated by random mutations and are frequently unique to individual patients. These are known as "neoantigens." Ovarian cancer has relatively few such mutations and had long resisted immunotherapy. Coukos, however, suspected the tumors could be coaxed to respond under the right conditions.

The experimental vaccine they initially tested was based on dendritic cells. These are immune cells that patrol the body for suspicious biological detritus, which they gobble up and process, "presenting" antigens to T cells to inform them about a looming threat. The dendritic cells of the vaccine were exposed to the cellular contents of each patient's tumors, or "whole tumor lysate," before being given to the patients. While managing this trial, which produced modestly positive results, Kandalaft enrolled in a master's degree program in translational medicine at UPenn. She also started working with Coukos to

"The CTE is the infrastructure for taking these innovative lab projects from Ludwig laboratories to patients."

develop their own dendritic cell vaccine for ovarian cancer.

Kandalaft was by then supervising a graduate student, Cheryl Chiang, who would play an integral role in the coming vaccine development. Chiang's previous studies had demonstrated that treating tumor lysates with hypochlorous acid made them better provocateurs of immune responses. Working with Coukos, June and their UPenn colleagues Daniel Powell Jr. and Bruce Levine, Kandalaft and Chiang began preclinical studies to develop a more potent version of the vaccine.

The next few years were extraordinarily busy. Kandalaft had her first son in 2011, when the trial of her dendritic cell vaccine was well underway. She also learned from Coukos that he was in discussions to move to Lausanne , where he hoped to establish a new translational research center dedicated to developing personalized immunotherapies for cancer. He wanted her to come along to oversee this center. Fortunately, her husband, whose clients live all over the world, didn't mind where they went so long as he had easy access to an airport. As opportunities go, Kandalaft realized, this one was tailor-made for her.

A tailored vaccine

The pilot study of the dendritic cell vaccine continued apace as Kandalaft and her family moved to Lausanne in 2013. Her first job there was to set up a Center of Experimental Therapeutics (CTE), a collaboration between the University of Lausanne, the Lausanne University Hospital and Ludwig Lausanne. "The CTE is the infrastructure for taking these innovative lab projects from Ludwig laboratories to patients," says Kandalaft, who is its director, overseeing some 130 staff and participating researchers.

Kandalaft, Coukos and their colleagues had already reported in *Clinical Cancer Research* in 2013 that the acid-treated tumor lysate vaccine induced potent antitumor immune responses in mice and even in patients. The study published in *Science Translational Medicine* in 2018 evaluated the same type of vaccine for recurrent ovarian cancer. But the clinical protocol of its delivery was designed to maximize the vaccine's immunologic kick.

Many tumors evade immune attack by barring entry to killer T cells. They also selectively recruit and retain regulatory T cells (Tregs), which suppress any killer T cells that slip through those barriers. Coukos and his colleagues had previously shown that VEGF-A, a factor secreted by tumor cells to stimulate the growth of blood vessels, also keeps killer T cells from infiltrating the tumor; others had found that the same factor suppresses dendritic cell maturation.

Bevacizumab blocks VEGF-A activity. Another standard of ovarian cancer care—the chemotherapy cyclophosphamide—

Photo by Eric Déroze

Alexandre Harari

Tapping TILs

Among the biggest challenges of truly personalized immunotherapies for cancer lies in developing standardized processes to reliably and swiftly identify the best immune cells to grow or genetically manipulate for subsequent therapy. At Ludwig Lausanne that responsibility is shared by the Human Integrated Tumor Immunotherapy Discovery & Development Engine (Hi-TIDE) and the Immune Monitoring Core of the Center for Experimental Therapeutics (CTE).

If there was ever any doubt that Ludwig Lausanne is up to the challenge, a study published in 2018 in *Nature Communications* has probably put it to rest. In the paper, a team led by Ludwig Lausanne's Alexandre Harari and Director George Coukos reported their development of a process to isolate cancer cell-killing T cells from tumors and optimize them for use in personalized, cell-based immunotherapies. In the months since, their method has been scaled up and standardized for application in clinical studies of personalized immunotherapy that will be carried out at the CTE with support from the Hi-TIDE.

"Our development of this method illustrates the advantages of coordinating basic and clinical research from the outset to solve difficult problems in medicine," says Coukos. "We are excited to put this new T cell therapy to the test in patients—and very hopeful that it will be to their benefit."

As cancer cells accumulate mutations across their genomes, they express aberrant proteins—or antigens—that reveal the malignancy to the immune system's cells. Some of these antigens are common to various cancers, but the majority are

randomly generated, so such antigens vary wildly from patient to patient, even within the same type of cancer. Killer T cells recognize mutated bits of these antigens that are known as neoepitopes, destroying the cells that bear them.

Many researchers have developed sophisticated methods to isolate, grow and infuse T cells into patients for therapy. But the cells used for such treatments are typically isolated from the bloodstream, and the proportion of T cells that recognize neoepitopes tends to decline significantly when circulating T cells are expanded in culture.

The method developed by Harari, Coukos and colleagues selectively expands the most reactive tumor-infiltrating lymphocytes (TILs) for individualized immunotherapy. Their analysis also demonstrated that even ovarian tumors—which tend not to be heavily mutated and have long resisted immunotherapies—harbor TILs that react vigorously to neoepitopes and can be harnessed for therapy. This suggests that other tumors with low mutational burdens may also be similarly infiltrated.

Comparing TILs with T cells from each patient's blood stream, the researchers showed in their study that TILs grown using their method are much better at recognizing neoepitopes than are circulating T cells. "We could even compare T cells from the two compartments that target the exact same mutation and show that the TILs were more functional than the T cells we collected from the peripheral bloodstream," says Harari, who is a team leader at Hi-TIDE and director of the CTE's Immune Monitoring Core.

Such selectively grown and optimized TILs have become a key asset in Ludwig Lausanne's plans to develop and standardize the production of tailormade cell therapies for cancer, efforts in which the Hi-TIDE and the CTE are playing a central role.

Tumor samples from patients at the Swiss Cancer Center-Léman, which houses the CTE, will be handled primarily by two groups at the Hi-TIDE. The first is led by Michal Bassani-Sternberg, who has combined cutting edge genomics-related technologies to predict the neoepitopes generated by cancer genomes that are likely to be recognized by killer T cells. The selected neoantigens are then moved on to Harari's team, which has developed assays to validate the predictions and prioritize the neoantigens that provoke the most potent T cell responses.

Harari and his colleagues at the Hi-TIDE and the Immune Monitoring Core then isolate those T cells from patients and grow them in a manner that gets the most out of them using methods described in the *Nature Communications* paper. These optimized T cells can then either be used for experimental therapies at the CTE or be sent to Melita Irving, whose Hi-TIDE team can engineer them to further boost their anti-tumor activity. "What George has established in the Hi-TIDE is a network of subgroups with distinct but extremely complementary expertise," says Harari.

While much of the scientific tinkering goes on at the Hi-TIDE, the clinical manufacturing, regulatory coordination and the trials themselves are done by the CTE. But the work flows freely between the two units. "It's dynamic," says Harari. "We have people going from one side to the other every day."

The CTE now has two facilities qualified by Swiss authorities to make cellular products for immunotherapy. One is already operational, and the other—which will expand the number of patients who can be treated with individualized cell therapies tenfold—is currently validating its instruments and will open its doors in 2019. Their capabilities will soon be put to the test in a planned trial of T cell therapy for multiple tumor types that is based on Harari and Coukos' new method for isolating and growing therapeutic TILs.

Photo by Felix Imhof

had been previously shown to suppress Tregs when given in low, repetitive doses. Kandalaft and Coukos wanted to use both these therapies to boost their vaccine's effects.

To make the vaccine, the researchers gently separated out the cancer cells in tumor samples obtained from patients with recurrent ovarian cancer and treated them with hypochlorous acid before breaking them open to collect their contents. Next, they isolated precursors of dendritic cells from patients and coaxed them to mature in a dish. They then pulsed each patient's dendritic cells with her tumor lysate to generate a personalized vaccine.

The vaccine was delivered directly into selected lymph nodes in patients. "The lymph nodes," Kandalaft explains, "are the headquarters where dendritic cells meet T cells." One cohort of patients received just the personalized vaccine. A second received

Studies of killer T cells isolated from patients showed that the immune responses elicited by the regimen were vigorous and targeted known cancer antigens as well as a broad variety of neoantigens.

vaccine along with bevacizumab. The third got, in addition to bevacizumab, low doses of cyclophosphamide.

Though not a randomized, placebo-controlled trial, the study's results were compelling. One year after receiving the vaccine, all patients who received all three treatments were still alive, as compared to 60% historical survival rates for patients receiving just bevacizumab and cyclophosphamide. "The regimen used for the third cohort really made a difference—first in eliciting an immune response in patients who received it, and then in the progression-free survival and the overall survival of those patients a year and even two years after receiving the therapy," says Kandalaft. One woman with stage IV ovarian cancer remained cancer-free five years after completing the regimen.

The immune analysis of the vaccine's effects, led by Kandalaft and Alexandre Harari, was just as encouraging, and it validated the clinical protocol.

"This was our first mission, our first challenge," says Harari, who directs the immune monitoring core of the CTE and co-directs the antigen discovery unit of the Hi-TIDE (for Human Integrated Tumor Immunotherapy Discovery & Development Engine) at Ludwig Lausanne (see page 19). "We were still establishing the assays we'd need to analyze patient T cell responses, so it was a bit tricky. But in the end, it all came together, and the main observations were in line with the most ambitious hypotheses George and Lana had formulated for this trial."

Studies of killer T cells isolated from patients showed that the immune responses elicited by the regimen were vigorous and targeted known cancer antigens as well as a broad variety of neoantigens. Intriguingly, following immunization, the T cells not only recognized a broad spectrum of pre-existing neoantigens but new ones as well. The T cells were also far more sensitive to lower levels of those antigens, and more fiercely activated when exposed to them.

Looking ahead

Work on the dendritic cell vaccine

continues. Coukos, Kandalaft and their colleagues at UPenn completed in 2018 the clinical phase of another small trial built out of the cohorts of the first. Its three cohorts are evaluating the effects of the individualized vaccine in combination with aspirin, with the therapeutic immune factor interleukin-2 and when bevacizumab is given prior to vaccination with the intent of boosting T cell infiltration into tumors. Analysis of the samples from that trial should be completed at Ludwig Lausanne in 2019.

Aside from directing the CTE, Kandalaft is also building on her vaccine research in her own lab at Lausanne, addressing follow-up questions from the *Science Translational Medicine* study. She is working with Ludwig Lausanne's Michal Bassani-Sternberg, a protein chemist who directs the antigen discovery unit with Harari, to determine how whole tumor lysate vaccines compare with synthetic vaccines based on computationally predicted neoantigens. Is one better than the other? Or would they work best in sequential combination? Kandalaft is also trying to engineer dendritic cells as agents of vaccination. "We have the translational facilities here to take these cells to their maximum potential," she says.

Those translational facilities are already being deployed in a clinical trial examining the selective reinfusion of tumor-targeting T cells as a treatment for melanoma. Other immunotherapies translating Ludwig Lausanne science are being planned as well.

This is, in other words, Kandalaft's dream job. "Being in the middle of it is very rewarding because you really get to see all the innovations scientists come up with to get to the clinic, and then see it in the patients, and get to change some lives," she says. "Maybe not as many as we want yet, but we're getting there."

Photo by Eric Déroze

"Being in the middle of it is very rewarding because you really get to see all the innovations scientists come up with to get to the clinic."

THE DARK GENOME'S EXPLORER

Howard Chang's discovery of the body's cellular GPS drew him into the vast, dark expanse of the noncoding genome, exploring its control of gene expression and how its dysfunctions fuel multiple cancers.

In 2001, Howard Chang was planning a series of experiments to examine how aging alters gene expression in skin cells known as fibroblasts when he noticed that an important control was missing. Skin scientists, it seemed, had implicitly assumed that one fibroblast—which churns out fibrous proteins and other macromolecules that build the scaffolding of tissues—is pretty much like any other. Chang, who had recently started a postdoctoral fellowship in Patrick Brown's laboratory at Stanford University, realized this assumption needed checking: If he was going to compare gene expression in cultured fibroblasts with that of their counterparts from more aged and different anatomical sites, he needed to know how similar they were to begin with. "It turned out," Chang recalls, "that fibroblasts from different parts of body were as different from each other as different types of white blood cells."

In subsequent studies conducted in Brown's and, later, his own lab, Chang discovered a key cause of those differences: the humble fibroblast, it turned out, is a vital component of the body's global positioning system. "Fibroblasts have positional memory and gene expression programs that are distinct based on where they come from in the body," says Chang, who has since 2017 been the Virginia and D.K. Ludwig Professor of Cancer Genomics at Stanford. "They retain that information and then share it through signaling to other surrounding cell types." In exploring the genomic source of this anatomical GPS, Chang would wander into the darkest regions of the genome—that 98% of the whole that encodes no proteins but controls which genes in the remaining 2% are expressed. Along the way, he and his team devised powerful new technologies to probe the dark genome, detailing how it controls programs of gene expression in health and disease.

HOWARD CHANG

LUDWIG STANFORD

Photo by Mark Tuschman

"If we're saying that different parts of the skin have beautifully laid out address codes, a cancer cell going from one part of the body to another clearly has to deal with those address codes."

In 2018, Chang and his Stanford colleague William Greenleaf reported in *Nature Medicine* their use of the latest of those technologies, ATAC-Seq, to characterize the subtly different states assumed by seemingly identical T cells of the immune system. The technique, they showed, could also be used as an important diagnostic tool in treating a T cell leukemia that manifests in the skin. But that was just for starters. Later in the year, in partnership with researchers from multiple institutions, they reported in *Science* a granular map of the dark genome's regions that are open for business in 23 types of tumors, revealing how mutations alter the landscape of the genome and patterns of gene expression in each to activate cancer-driving genes, and why subtle variations in noncoding DNA sequences predispose people to various cancers.

Apprenticeships

Chang was born in Taipei, Taiwan, and emigrated to the U.S. when he was 12 years old. Though his was always an academic-minded home—his father was a physician—Chang says he became interested in science only after arriving in the U.S., when he and his friends proposed projects for the science fair that required instruments not typically found in a high school laboratory. His biology teacher introduced him to a friend at the University of California, Irvine, whose lab focused on transplantation biology. It was there that Chang got his first taste of scientific research.

After his freshman year at Harvard University, Chang did a summer research stint in the laboratory of the enzymologist and former Ludwig scientific advisor Christopher Walsh exploring the mechanism of action of the transplant rejection drug cyclosporin A. "Over the course of that summer, I realized that what people had thought about how this drug works was about to be transformed," Chang recalls. "That was tremendously exciting. It was one of the reasons I became interested in fundamental research." The interest solidified into a plan after he spent two formative summers in the Undergraduate Research Program at Cold Spring Harbor Laboratory, where, along with a rigorous training in laboratory practice, he was exposed to the camaraderie and intellectual spark of the scientists who gathered there from around the world. "This was," he says, "an attractive aspect of being a scientist."

And so, in 1994, Chang enrolled in the MD-PhD program of Harvard and MIT. After two years of medical school, he joined the laboratory of the Nobel laureate David Baltimore. In his doctoral studies, Chang explored the signaling cascades and biochemical mechanisms by which cells are chopped up from the inside during a type of programed cell death known as apoptosis, completing his PhD in just two years. After finishing medical school at Harvard in 2000, Chang returned to California for an internship at the Santa Clara Valley Medical Center followed by a residency in clinical dermatology at Stanford.

Toward the genome

Eager to enter the burgeoning field of

Chang with scientist Tansu Bagdatli.

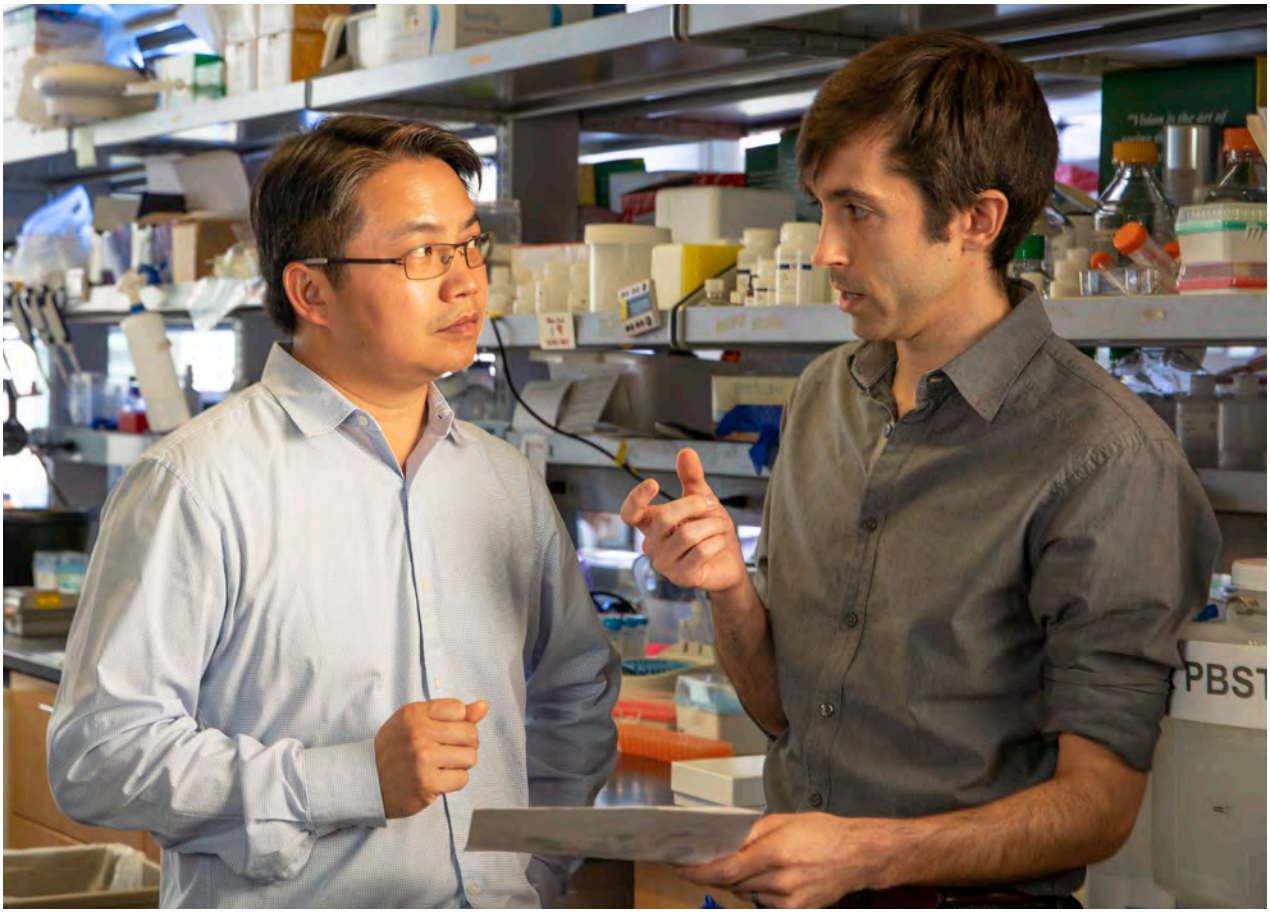
Photo by Mark Tuschman

genomics, Chang joined Brown's Stanford lab as a postdoctoral fellow in 2001, where he would make his pioneering contributions to our understanding of how cells know where they are in the body. Using DNA microarrays, which fish out the transcripts of expressed genes, Chang detailed how distinct gene expression patterns in fibroblasts reflect their locations in relation to the various axes of the body. Aside from defining the outlines of the organismal GPS, the work opened a new window into the deadliest outcome of malignancy—metastasis.

"If we're saying that different parts of the skin have beautifully laid out address codes," he explains, "a cancer cell going from one part of the body to another clearly has to deal with those address codes. I was able to characterize the gene expression profiles associated with cancer cells that have different rates and proclivities for metastasis, which turned out to be pretty useful."

Chang now became increasingly curious about the means by which so many distinct gene signatures are generated in cells. "With a few exceptions," says Chang, "cells of the body have the same DNA. But they make different choices about which genes to turn on and off. So the next question was, how does that happen?"

The first map of the human genome, completed just before Chang began his postdoctoral fellowship, had surprised everyone by its paucity of protein-coding genes, which numbered in the range of 20,000. Researchers had expected it would encode five times as many. "We were doing all these experiments probing just 2% of



Chang and postdoc Ryan Corces.

Photo by Mark Tuschman

the genome's output," says Chang. "A major theme of my work became understanding the hidden information in the noncoding genome. Subsequent work has shown that most of the variation associated with human disease resides there."

Into the dark

Chang's lab began by adapting a version of the microarray called a tiling array to look not just for mRNA transcripts of genes but for all RNAs read out of the genome. Contrary to their (and the field's) expectations, he and his colleagues saw scads of RNA transcripts emerging from regions known to be devoid of protein coding genes. These molecules, they found, belonged to a sprawling family of RNAs—now known to be some 60,000-strong—that have many of the properties of mRNAs yet do not encode proteins.

The molecules, subsequently named long noncoding RNAs (lncRNAs), turned out to be variegated in form, selectively expressed in tissues and deployed across the entire protein-coding genome. In 2007, Chang and his colleagues described in *Cell* how one lncRNA, which they later named HOTAIR, suppresses the expression of HOX genes, which dictate the body plan during development and—they discovered—the assignment of positional identity in fibroblasts.

Since discovering lncRNAs, Chang's lab has developed groundbreaking methods to harness them for the study of the genome's architecture and expression. His group has meticulously mapped lncRNA association with the genome and delineated the principles guiding those interactions. Other

studies have explored the functional role of lncRNAs, revealing how they participate in everything from embryonic development to stem cell biology to cancer. Chang and his colleagues found, for example, that HOTAIR and another lncRNA, HOTTIP, serve as scaffolds for protein complexes that chemically modify DNA and its protein packaging—collectively referred to as "chromatin"—to control HOX expression. Such "epigenetic" modification determines which genes in a given cell are switched on or off.

Stretched out, the DNA in a cell would be about two meters long. Yet it is, remarkably, crammed into a nucleus just 10 microns across. To fit, DNA is tightly spooled and packed into fractal chromatin structures that sequester most of its information from the cell's gene-reading machinery. Only DNA that must be read for a cell to survive and perform its unique function is unraveled and made available to the protein machines that control and execute gene expression.

Which segments of the genome are so favored is determined in large measure by epigenetic modifications, and these modifications are almost universally disordered in cancers. Chang's work has shown that lncRNAs are intimately involved in these processes. He and his colleagues discovered, for example, that HOTAIR reprograms chromatin to drive cancer and its metastasis. They also have mapped the lncRNAs expressed in various cancers along with the gene expression profiles associated with each.

Mapping access

By 2012, Chang's ambitions had grown to encompass the mapping and characterization of all accessible regions of the dark genome. These stretches would also include enhancers and suppressors, which are DNA sequences that produce no RNA of any kind but guide proteins to mute or amplify the expression of distant genes. To that end, Chang began collaborating with Stanford biophysicist William Greenleaf and a gifted graduate student, Jason Buenrostro, who now has his own lab at Harvard University, to develop the required methods.

> "A major theme of my work became understanding the hidden information in the noncoding genome."

The two-step method they reported in *Nature Methods* in 2013, dubbed ATAC-seq, profiled the accessible genome with a million times greater sensitivity than comparable techniques, which would take days to furnish results. They showed that ATAC-Seq could, by contrast, profile the accessible chromatin of T cells overnight and from a standard clinical blood-draw. "Turning it into a daily blood test was pretty cool, we thought," says Chang.

By 2015, the researchers reported in *Nature* the development of an ATAC-Seq to profile individual cells. The study revealed that even immune cells that appear to belong to the same subclass display enormous diversity in their genomic expression, an insight of material relevance to immunotherapy. To test the method's clinical utility, the researchers picked a cancer Chang treats as a dermatologist—cutaneous T cell leukemia (CTCL), which presents in the skin and is treated with a drug that inhibits an epigenetic modification.

"Only a subset of patients benefit from this

"Almost half of the DNA elements that we found in cancer were not known to be active before in the atlas of normal tissues."

drug and we have no way of knowing who's benefiting until they've gone through multiple rounds of therapy," says Chang. "We asked, can we take blood samples from patients as they go through this treatment and use our method to watch the chromatin in real time to see what's happening?"

The researchers reported in *Cancer Cell* in 2017 that only patients whose chromatin was altered during treatment benefited from the therapy. "Those whose chromatin didn't change did not benefit," says Chang. "Their cancer cell counts did not drop." With more vetting in clinical trials, the technology could give clinicians an early warning that other treatments might be in order for a given CTCL patient.

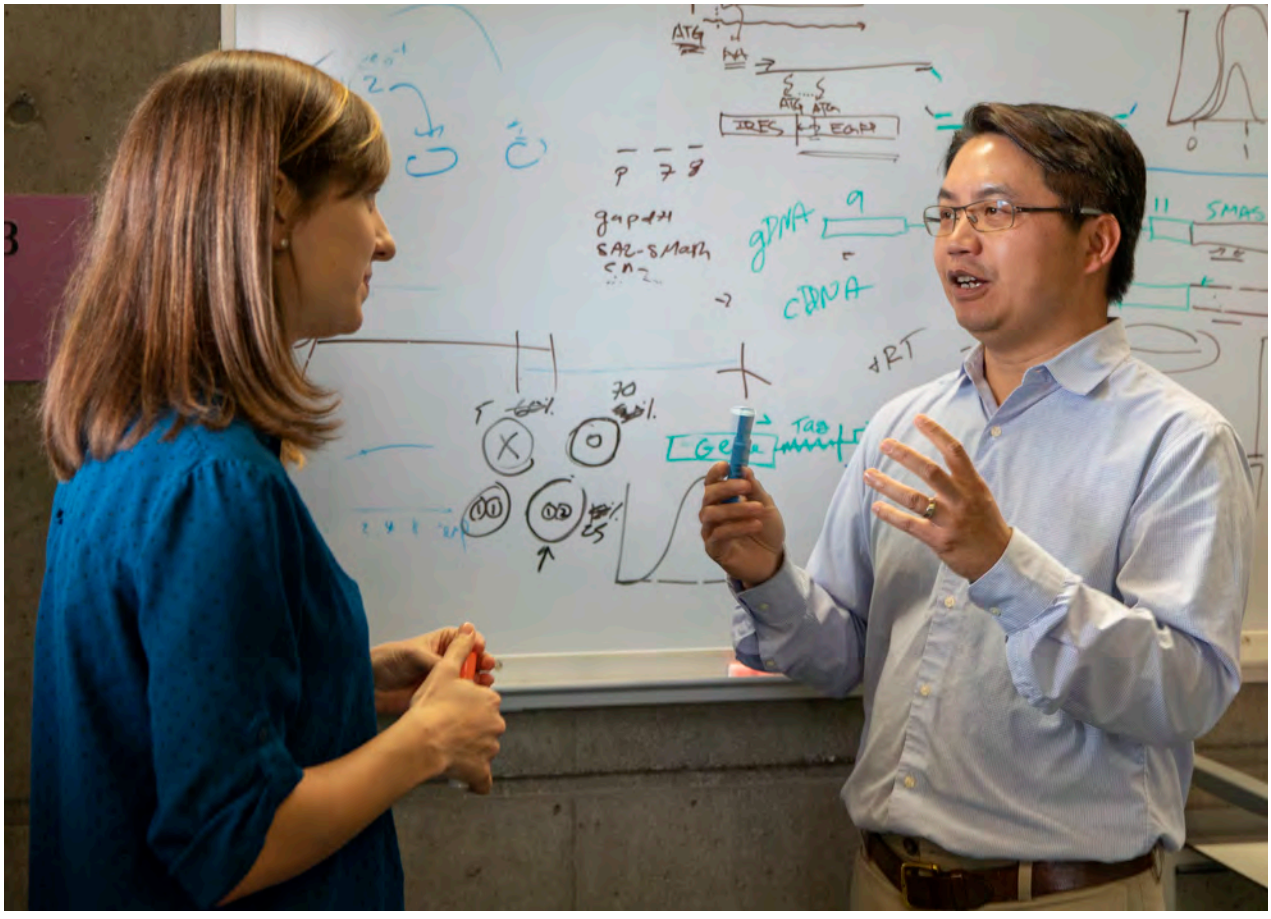
Inspired by that success, Chang and Greenleaf decided next to similarly apply ATAC-Seq to a broad range of cancers. Chang's new effort coincided with his appointment to the Ludwig Professorship, which provided him with the resources in part to pursue this ambitious goal. "The wonderful gift of the Ludwig Institute is that we are able to quickly pursue new and exciting ideas, including high risk ideas that have the potential for big rewards," says Chang.

To examine the accessible genome across cancers, Chang and his colleagues modified ATAC-Seq so that it could be used on archival samples of tumors. This would permit the analysis of human tumor samples stored in The Cancer Genome Atlas (TCGA), a vast collection that dates back more than a decade, is annotated with clinical information and has been exhaustively analyzed in other types of genomic studies.

"We'd know who got better, who had a worse outcome, how they responded to different drugs," says Chang. "If we could only work with fresh samples, we'd have to wait another ten years for something to happen prospectively. The TCGA samples represented this ability to go to the samples that had the most information, apply cutting edge genomic technologies and learn something new."

Their study, published in *Science* at the end of 2018, surveyed the accessibility of genomes in 410 tumor samples representing 23 types of cancer to map DNA sequences that regulate gene expression in the malignancies. By integrating these results—which identified 562,709 such "cis-regulatory elements"—with other genomic, clinical and biochemical information about the same tumors, the researchers identified such things as new molecular subtypes of cancers and their relationship to patient prognoses. Notably, the findings also shed light on how inherited variations in DNA sequence in noncoding DNA can predispose people to cancer—illuminating a poorly understood aspect of cancer risk.

Analysis of the data revealed how mutations in noncoding sequences thousands of bases away from a gene can alter chromatin to create a newly accessible stretch of DNA that promotes the aberrant expression of that gene. In a bladder tumor, for example, a mutation generates a new binding site for a protein that regulates gene expression, driving the expression of a neighboring gene that influences cell size, motility and shape—all key factors in cancer metastasis. The findings indicate that unique suites of such mutations may drive different types of cancer.



Chang and graduate student Kathryn Yost.

Photo by Mark Tuschman

By layering their chromatin accessibility map over the gene expression data for various cancers, the researchers also identified tens of thousands of likely interactions between regulatory elements of DNA and genes known to play an important role in cancer and the ability of tumors to evade immune attack. This is invaluable information: Mutations to genes have consequences on proteins that can be detected and functionally analyzed. But mutations and variations in noncoding DNA sequences do not produce such readily measurable readouts, and most sequence variations associated with disease reside in just such stretches of the genome.

"Using the chromatin accessibility map, you could actually get a sense of which mutations had a biochemical consequence on the DNA element, making it more accessible or less so," says Chang. "I hope that will prove to be a useful lens for distinguishing passenger mutations that have no biochemical consequence from mutations that actually change chromatin accessibility in human cancers."

The findings, he notes, also demonstrate that the genome is every bit as complex as you'd expect it to be. "Almost half of the DNA elements that we found in cancer were not known to be active before in the atlas of normal tissues," observes Chang. "They're only accessed in the pathology of cancer, which suggests there's a lot left to be learned about the genome."

Fortunately, Chang is looking into the matter.

THE INSPIRED PHYSIOLOGIST

Peter Ratcliffe's landmark discovery of how cells sense and respond to the availability of oxygen has transformed our understanding of cancer and other diseases—and he's far from done with the discovering.

Doctors pick their specialties for all sorts of reasons. Peter Ratcliffe, for his part, suspects he might have been flattered into his.

While a house officer—or resident—at a London hospital in the late 1970s, Ratcliffe worked for a time under the supervision of a respected nephrologist. While on rounds one day, he recalls, the senior doctor complimented him on his grasp of nephrology and suggested he specialize in the field. "He was an inspiring person, and I believed him," says Ratcliffe. Other senior colleagues, however, were less sanguine. The UK National Health Service was as short on cash as ever and funding for expensive renal specialists was unlikely to ever be placed high on the list of priorities. "They said, 'Good luck,'" Ratcliffe recalls, "'you'll have to distinguish yourself'."

Ratcliffe evidently took that suggestion as well. By the early 90s—having moved to Oxford to study renal medicine—Ratcliffe was among the leaders in a trans-Atlantic race to find the molecular sensor by which animal cells respond to oxygen starvation, or hypoxia. His efforts contributed not only to the discovery of that crucial sensor but to the illumination of an entirely new mechanism of intracellular signaling as well. For these discoveries and their contributions to our understanding and potential treatment of disorders ranging from anemia to heart disease and cancer, Ratcliffe was knighted in 2014 and shared with U.S. researchers William Kaelin and Gregg Semenza the prestigious 2016 Albert Lasker Basic Medical Research Award.

Ratcliffe, meanwhile, has dug deeper into the cell's oxygen sensing systems at his lab in Ludwig Oxford. In 2018, he and his colleagues detailed in *EMBO Reports* the interactions of two controlling elements of that system—hypoxia inducible factor-

PETER RATCLIFFE

LUDWIG OXFORD

Photo by Paul Wilkinson

Ratcliffe in his Oxford lab with postdoc Norma Masson.

Photo by Paul Wilkinson

1α (HIF-1α) and HIF-2α—across the entire genome. Most notably, he and his team also put the finishing touches on a study, published in 2019 in *Science*, describing an entirely new system of oxygen sensing so fundamental to cell biology that it is shared by plants and animals.

"Like many things, that we actually did this work owed a lot to serendipity," says Ratcliffe. "But part of that serendipity was the support I received from the Ludwig Institute to do something different. This was one of those things."

Stumbling into a calling

Ratcliffe grew up in a small railway town in Lancashire named Carnforth, where his father was a lawyer and his mother a homemaker. When he was close to graduating from Lancaster Royal Grammar School, intent on someday becoming an industrial chemist, the head master—an austere, begowned sort—wandered into his chemistry lab. Calling him aside, he said, "'Ratcliffe, I think you should study medicine'," Ratcliffe recalls. "To this day, I have no idea why he said that, but he was not the sort of guy you challenged so I immediately said, 'yes, sir,' and changed my university application from chemistry to medicine."

Ratcliffe won a scholarship in 1972 to study medicine at Gonville & Caius College,

Cambridge, and St. Bartholomew's Hospital in London, from where he graduated with distinction in 1978. Following a series of house jobs at London hospitals, he won a fellowship from the UK National Medical Council in 1984 to study renal medicine at the Nuffield Department of Medicine at the University of Oxford. In 1987, Ratcliffe was hired as a clinical lecturer in the department.

Having published a handful of case studies, he was now eager dive deeper into scientific research. After a false start or two, he decided to explore the body's ability to sense and respond to subtle changes in oxygen levels, a capability in which the kidneys were thought to play a central role. Ratcliffe began by exploring the organ's production of erythropoietin (EPO), a hormone (first cloned by Ludwig researchers) that stimulates the production of oxygen-carrying red blood cells.

EPO production is exquisitely attuned to oxygen levels in the body, so it was widely believed that some factor X that regulates the expression of the EPO gene would be the body's oxygen sensor. To find it, Ratcliffe and many other researchers, including Gregg Semenza, were looking for a DNA sequence—a regulatory element—that boosts EPO production when switched on by the putative sensor.

Ratcliffe and a trainee nephrologist in his lab, Chris Pugh, described in 1991 a short DNA sequence near the EPO gene that did just that. But it soon became clear that their premise needed reexamining. Ratcliffe, Pugh and another nephrologist trainee in the lab, Patrick Maxwell, soon discovered that the hypoxia-responsive DNA element was active in all sorts of mammalian cells, not just those that produce EPO.

"We were so prejudiced that the oxygen sensor was specific for EPO that we were looking to identify the process by transferring it from an EPO-producing cell, which we thought would have it, to a non-EPO producing cell we believed would not," says Ratcliffe. "To our astonishment, we found the property wasn't private to the EPO producing cells. It was general. That experiment transformed my life. It brought me into contact with cancer research and other types of biology."

> "Like many things, that we actually did this work owed a lot to serendipity. But part of that serendipity was the support I received from the Ludwig Institute to do something different."

Around the same time, Semenza reported his discovery of HIF-1α, a master regulator of gene expression that drives the hypoxia response of cells. He subsequently showed that its product combines with a standard-issue nuclear factor, HIF-1β, to switch on the gene expression that drives adaptations to hypoxia. By 1994, Ratcliffe and his colleagues had identified the first of the hundreds of non-EPO genes regulated by HIF-1α, and they turned out to encode metabolic enzymes—particularly those known to play a critical role in cancer metabolism, a finding confirmed by Semenza's group.

The discovery of the oxygen sensing system in cells would enable new approaches to treating cancer—and many other ailments in which hypoxia plays a major role, from anemia to heart disease to wound healing.

Ratcliffe's team reported three years later that tumors engineered to be defective in HIF-1β had trouble growing in a mouse model, cementing the importance of hypoxic pathways in cancer. That this should be the case was not exactly a surprise. It was well known that the cores of tumors are often starved of oxygen and that hypoxia can drive drug resistance and metastasis.

The big breakthrough

The race was now on to find the factor that regulates HIF-1α—the primary oxygen sensor that would give every cell in the body the ability to respond directly and swiftly to that indispensable resource, oxygen.

A clue came from the Harvard laboratory of William Kaelin, who was studying von Hippel-Lindau syndrome, an inherited propensity for cancer that often manifests in the kidneys. Kaelin reported in 1996 that pVHL, a tumor suppressor protein mutated in the cancer, normally suppresses many hypoxia-related genes. The field had, meanwhile, identified three regions of HIF-1α crucial to the protein's function in hypoxia. These domains received some signal transmitted by the unknown oxygen-detector in cells.

Ratcliffe and his colleagues showed that the signal itself was atypical—that is, not conveyed by enzymes known as kinases that add a phosphate group to specific amino acids on proteins. They also began exploring what exactly pVHL was doing in the oxygen-sensing business, and reported in *Nature* in 1999 that when oxygen is abundant, the tumor suppressor interacts directly with HIF to target it for degradation.

Two years later, they reported in *Science* and the *EMBO* journal that pVHL recognizes two specific amino acids—proline residues—in HIF-1α that are independently chemically modified by the addition of an oxygen atom to create hydroxyproline. Kaelin and his colleagues simultaneously published similar findings. That same year, Ratcliffe and his colleagues, now including a collaboration with an Oxford chemist, Christopher Schofield, reported in *Cell* the identification of the enzymes that are responsible for these hydroxylations. These enzymes are dioxygenases, which absolutely require molecular oxygen (O_2) to function.

These were the long-sought oxygen sensors that link oxygen levels to hypoxic responses. When oxygen is abundant, the enzymes—known in humans as PHD-1,2 and 3—hydroxylate the HIFs, setting them up for pVHL binding and their subsequent degradation. When oxygen is scarce, they fail to hydroxylate the amino acids and the

Photo by Paul Wilkinson

HIFs are permitted to linger on and trigger the necessary cellular adaptations.

The use of hydroxylation to control these responses also represented a new mechanism of signaling within the cell. "Hydroxylation wasn't an unprecedented modification," of proteins, says Ratcliffe. "But as a signaling mechanism it was at the time unprecedented." The discovery of the oxygen sensing system in cells would enable new approaches to treating cancer—and many other ailments in which hypoxia plays a major role, from anemia to heart disease to wound healing.

Cancer's pathways

Over the next several years, Ratcliffe explored the biochemistry of HIF regulators and, with his colleague Christopher Schofield, began designing inhibitors of the family of enzymes that inhibit HIF as potential therapies. With others, his lab also showed that HIF-2, specifically, was a driver of clear cell renal carcinoma. This discovery led to the ongoing development of HIF-2 targeting drugs for that cancer by scientists at the University of Texas South Western and a biotechnology company.

In 2018, Ratcliffe—who is also director of the Target Discovery Institute at the University of Oxford, and clinical research director at The Francis Crick Institute in London—published with his colleagues a study in *EMBO Reports* mapping HIF binding across the genome.

Photo by Paul Wilkinson

Although the two HIFs recognize the same sequence of DNA, they showed that each activates distinct suites of genes in every cell type examined and cannot compensate for the loss of the other. This implies that each of the HIFs may be independently targeted to induce distinct therapeutic effects, much as HIF-2 is being specifically targeted in kidney cancer.

Yet how hypoxia pathways drive cancer progression, says Ratcliffe, remains mechanistically unclear. The hypoxia response alters almost every aspect of the cell's internal life, sparking—as their study showed—the expression of hundreds of genes and the activation of countless biochemical pathways. Further, experimental evidence suggests that some of those pathways drive malignancy, while others work in the opposite direction. In fact, HIF activation is inhibitory in some cancers. It thus seems likely that the cells of tumors in which HIF is activated need to modulate, or tune, the pathway, and that the cells which drive cancer are the products of an evolution that ultimately favors the pro-cancerous pathways while muting suppressive ones.

"Only when the mutations are right, pathways are right, the tissue context of the cell is right, and previous mutations have occurred that help set the stage—only then can that pathway switch be tolerated and promote cancer," says Ratcliffe. "I think this is a central principle restraining tumor development and a central issue that we have to understand if we're going to understand cancer." Ratcliffe is preparing now to examine his hypothesis using hypoxic signaling in renal cancer as a model.

Back to basics

The oxygen-sensing system discovered by Ratcliffe in 2001—in which oxygen levels are directly linked to the degradation or retention of proteins governing the hypoxic response—was initially thought to be unique to animal cells. But over the years parallel mechanisms of sensing and responding to oxygen levels were discovered in all the other kingdoms of life as well.

In plants, the sensing is executed by a family of enzymes known as plant cysteine oxidases (PCOs), which prime proteins for destruction in a different way. The existence of these and other such mechanisms of oxygen sensing got Ratcliffe wondering whether human cells might harbor alternative oxygen sensors. Evolution, after all, tends to favor redundancy in mission-critical processes, and oxygen sensing certainly falls into that category.

In 2016, while attending a meeting in Rome,

Ratcliffe got into a discussion on the matter with Francesco Licausi, a plant physiologist at the University of Pisa. They wondered whether the plant system of oxygen sensing might also be present in human cells and what would happen if plant oxygen sensors, known as PCOs, were inserted into human cells. Would these plant sensors still be able to regulate hypoxic responses in their new homes, exposing an unknown mechanism of cellular oxygen sensing? The pair decided to find out when they got back to their labs.

The researchers began by constructing a readout for the proposed experiment: a fusion protein built from the oxygen-sensitive part of a PCO target named RAP2.12 and a fluorescent protein. They then engineered cancer cells to stably express the fusion protein, and exposed them to hypoxic conditions. To their surprise, the hypoxic cancer cells glowed considerably longer than their oxygenated counterparts, even though they hadn't yet been engineered to express PCOs.

"That told us that something in the human cell was working on the artificial plant protein," Ratcliffe explains. A search of the genome revealed that the enzyme, cysteamine (2-aminoethanethiol) dioxygenase, or ADO, was one of two proteins in human cells that resembles PCOs and would fit the bill. Notably, the similarities between ADO and the PCOs indicate that this mechanism of oxygen sensing arose several hundred million years ago in some primitive, cellular progenitor of both the plant and animal kingdoms. Remarkably, the researchers showed that PCOs would substitute for ADO in human cells and insertion of human ADO would revive plants that were deficient in PCOs.

They also identified three of ADO's protein targets and showed that the ADO system and the HIF system work on different timescales. Since ADO can alter other signaling proteins directly, the sensor exerts its effects in the range of minutes to hours. HIFs, by contrast, exert their effects over hours to days because they drive the expression of genes whose products then drive hypoxic signaling cascades.

The similarities between ADO and the PCOs indicate that this mechanism of oxygen sensing arose several hundred million years ago in some primitive,cellular progenitor of both the plant and animal kingdoms.

This is physiologically relevant. "For example, the constriction of blood vessels in response to hypoxia has to occur very rapidly," says Ratcliffe, "whereas acclimating the body to reduced oxygen at higher altitudes can occur more slowly." Given the centrality of oxygen to biological processes, the newly discovered system of oxygen sensing, like that of the HIFs, is likely to play a role in diseases like cancer as well.

Ratcliffe suspects there are more oxygen sensing systems to be found, including a type that exerts its effects in a matter of seconds. If so, it's probably fair to say he's qualified to find them.

THE CONSUMMATE NEURO-ONCOLOGIST

Michelle Monje's teenage project to aid the disabled led her to neurology and a research career that's bringing new hope for the treatment of childhood brain cancers and the mind-fog caused by chemotherapy.

Competitive figure skating was once a big part of Michelle Monje's life. By the time she was in middle school in the Bay Area of San Francisco, Monje was squeezing in as many as 35 hours of practice every week at the rink. Then her mother, who'd started at IBM in the late 60's as a computer programmer and worked her way up to the executive ranks, had a little chat with her. "She pointed out that dedicating that much time to a sport was great," Monje recalls, "but perhaps I should also think about how I'm going to be productive and contribute to the rest of the world." Just 13 at the time, Monje mulled the matter for a spell and came up with a precociously fitting answer: She created a figure skating program for children with Down syndrome.

The experience left Monje, who is today a researcher at the Ludwig Center at Stanford and a pediatric neuro-oncologist at Stanford University's School of Medicine, with an abiding interest in neurology. In keeping with her mother's advice, Monje has over the past quarter century made significant contributions to our understanding of the brain's postnatal plasticity and the neurological disorders caused by cancer therapies. She has also led the charge against a swiftly lethal childhood cancer of the brainstem known as diffuse intrinsic pontine glioma (DIPG), charting new approaches to the treatment of the long-neglected cancer and other high-grade gliomas that she is now—or soon will be—evaluating in clinical trials.

In 2018, Monje and her colleagues reported in *Cell* their dissection of the cellular interactions underlying an enduring fogging of the mind often caused by chemotherapy and identified a potential treatment for its mitigation. In another study, done in collaboration with Ludwig Stanford researcher Crystal Mackall and published in

MICHELLE MONJE

LUDWIG STANFORD

Photo by Flynn Larsen

"Doing neurology and pediatric oncology in the clinic, I was really compelled by the patients I saw who were suffering from the long-term neurological side effects of cancer therapy."

Nature Medicine, Monje and her colleagues applied an engineered immune cell therapy that, for the first time, almost eliminated DIPG in a mouse model of the incurable cancer.

"Ludwig funding has been really critical for my research program, as the flexible nature of the funding allows us to test new hypotheses and leads in real time, rather then needing to first write a specific grant proposal and then wait for the funding to do the work," says Monje. "This has allowed our research relevant to cancer stem cell biology to move forward more quickly than would otherwise have been possible."

Brainy pursuits

After completing high school in Danville, California, Monje went to Vassar College, where she initially planned to major in English. Her freshman advisor, neuroscientist Kathleen Susman, revived Monje's early interest in biology and neurology, beginning a long and cherished mentorship. Monje enrolled in many of Susman's courses and conducted research in her lab, even authoring a paper under her supervision. "Kate was enormously influential in my life," says Monje. "I really fell in love with the nervous system at Vassar."

In her first year of medical school at Stanford in 1998, Monje applied to the university's Neuroscience PhD program but deferred enrollment until she had completed her clinical rotations to ensure she still wanted to focus on neuroscience. The answer, she discovered, was yes. "Doing neurology and pediatric oncology in the clinic," Monje recalls, "I was really compelled by the patients I saw who were suffering from the long-term neurological side effects of cancer therapy."

Studies suggested that the cognitive decline associated with cranial radiotherapy, in particular, stemmed from neural dysfunction in the hippocampus, a region of the brain involved in emotion and memory. When she joined the Stanford PhD program, Monje asked Theo Palmer, who was studying neural stem cells in the hippocampus, if she could do her doctoral research in his lab exploring the phenomenon.

The project was a runaway success. Monje, Palmer and their colleagues reported in *Nature Medicine* in 2002 that the neural dysfunction was caused by changes in the hippocampal microenvironment induced by radiation. X-rays, they discovered, activated the brain's resident immune cells, or microglia, and the factors they secreted compromised the ability of stem cells to generate neurons. In 2003, Monje, Palmer and colleagues reported in *Science* that the anti-inflammatory drug indomethacin could restore hippocampal neurogenesis in mice after irradiation. Those findings laid the groundwork for clinical studies done by others that have since altered the delivery of cranial radiotherapy.

The initial discovery also challenged dogma.

It demonstrated that microglia aren't just defenders against microbial invasion but modulators of neural function as well—a novel idea at the time. "That was a really compelling concept for me," says Monje, "one that I have continued to study throughout my career: the way that different cells in the brain communicate and influence each other's ability to do their jobs."

Another experience in those years would shape Monje's career. While completing her continuity clinic requirement at Stanford under the supervision of pediatric neuro-oncologist Paul Fisher, she saw her first patient with DIPG, a nine-year-old girl. "It was astounding to me that we had no effective therapy for this cancer, that we knew so little about it even though it's one of the leading causes of childhood cancer-related death," she recalls. Monje decided that when she started her own lab someday she would work on the cancer.

Photo by Monty Rakusen

Glial groundwork

Getting her medical degree and PhD in 2004, and completing her internship the following year, Monje began her residency in neurology at a combined program of the Massachusetts General Hospital, the Brigham and Women's Hospital and Harvard Medical School. "I had wonderful mentors in neuro-oncology, and I considered staying there," says Monje. But Stanford's pull was stronger. Her husband, the prominent neuropsychiatrist Karl Deisseroth, whom she'd met in medical school, had accepted a job at their alma mater. Further, pediatric high-grade cancers that arise from glial cells—the collective term for cells that support, nourish and defend neurons—continued to fascinate Monje, and she was eager to learn more about their treatment under the guidance of her mentor Paul Fisher.

Monje returned to Stanford in 2008 to begin a fellowship in pediatric neuro-oncology and a postdoc with Phillip Beachy, who is also a member of the Ludwig Center. Beachy was investigating a cellular signaling pathway of importance to both neural development and the genesis of gliomas. Monje began addressing one of the biggest barriers to studying DIPG at the time: the lack of experimental model systems for the cancer. "There was very little tissue in the world to study," says Monje. "There were no cell cultures, no mouse models, and we knew nothing at the time about the genomic landscape of the cancer. It was really a black box."

Given its location, in a region of the brainstem known as the pons that controls several vital body functions, including breathing, the tumor was rarely biopsied. The tumor itself posed problems as well. "This isn't a golf ball in the middle of the brain," says Monje. "It is a diffuse, infiltrative disease that is intermingled with normal tissues." Monje's PhD advisor, Palmer, had

Photo by Flynn Larsen

pioneered techniques to culture normal stem cells taken from the brain a few hours after death, and Monje started by adopting those protocols for the generation of DIPG cultures.

She soon got a chance to put her procedures to the test. Among the first patients Monje encountered in her fellowship was a five-year-old boy with DIPG. As he neared death—most children diagnosed with DIPG die within the year—his family asked about donating his organs to medicine. Monje told them his corneas could be donated, and then asked if they might also consider donating his brain to science. They said yes. "As a parent, think about what that means," says Monje, who is herself a mother of four. "You're giving your child's brain to a researcher to study to help other children who get this disease in the future. It's amazing."

Monje established the first ever DIPG culture from that patient's cells in 2009 and immediately began sharing it with the small community of DIPG researchers. (Drug screens on DIPG cultures done by a consortium of researchers a few years later led to the identification in 2014 of a drug—panobinostat—that may slow DIPG growth. Monje, who led that study, is now overseeing a clinical trial testing the possibility.)

Examining pons tissue from a variety of noncancerous samples, Monje had noticed that a cell type known as the oligodendroglial precursor cell (OPC) was present in increased numbers exactly when these tumors tended

to emerge, most noticeably around age six. OPCs give rise to oligodendrocytes, which make the myelin that insulates neurons and gives the brain's white matter its color. That myelination continues well into the third decade of life, in distinctly timed and located waves. The cresting of those waves, Monje found, mapped neatly against the times and locations at which high grade gliomas arise in children. This suggested to her that DIPG arises from dysfunctional OPCs—a hunch now supported by multiple lines of evidence.

"That was an important thing to recognize at the start of my career," says Monje, "because I wanted to study not only the postnatal developmental processes that go wrong in glial malignancies, but also the normal developmental processes that might be central to the long-term effects of cancer therapy. Myelin biology appears to be central to both."

> "There was very little tissue in the world to study. There were no cell cultures, no mouse models, and we knew nothing at the time about the genomic landscape of the cancer. It was really a black box."

Illuminating results

When Monje started her own lab in 2011, there was some debate about whether neurons regulate the myelination of their own axons—the thread-like projections that conduct signals between the cells. To find out, Monje applied a technique pioneered by her husband known as optogenetics, in which light is used to control the firing of specific neurons, causing minimal confounding damage to brain tissue. She and her colleagues reported in *Science* in 2014 that light-induced neural firing sent a brisk proliferative signal to OPCs, causing them to replicate and then differentiate into oligodendrocytes. These cells then remodeled the myelin structure in the active neural circuit—in this case the motor circuit of a mouse brain. Done repeatedly, this remodeling improved function of the limb controlled by that circuit, suggesting that the plasticity of myelination is essential to neurological function. That itself was a landmark discovery.

Next, Monje and her team repeated their experiment in mice bearing high-grade human gliomas in the same neural circuit. They reported in *Cell* in 2015 that neural firing drove the growth of a variety of glial malignancies, including DIPG. One of the key messengers of that proliferative signal, they found, is a version of a neural protein known as neuroligin-3.

In 2017, Monje and her colleagues reported in *Nature* that, upon neural firing, Neuroligin-3 is snipped by an enzyme in neurons named ADAM10, generating a secreted protein fragment that induces cancer cell proliferation. "We found that if we blocked that enzyme pharmacologically using drugs that are already in the clinical pipeline, we could robustly slow tumor growth in mice," says Monje. She is now working with two brain cancer consortia to test the potential

"I didn't believe it the first time because I'd never seen anything do that. I think it was the sixth time my poor graduate student did this experiment that I really believed the results. It was just night and day. It was incredible."

therapy in clinical trials for all childhood high-grade gliomas and for glioblastoma in adults.

Demolishing DIPG

Monje has continued to refine her lab's protocols for obtaining DIPG cultures. As part of that effort, her graduate student examined whether some molecule on DIPG cells might be grasped with antibodies to improve their isolation from the sticky, myelin-rich tissue in which they grow. An antibody panel unearthed a complex sugar chain named GD2. The sugar, it turned out, is found at uncommonly high levels in the 80% of DIPG tumors driven by a mutation known as H3K27M.

As it happened, Crystal Mackall, a leader in the field of cell-based immunotherapies, had just arrived at Stanford (where she too is a member of the Ludwig Center). Mackall's lab had recently engineered immune cells—chimeric antigen-receptor T cells (CAR-T)—to target and destroy GD2-bearing cells, which are found on other tumors as well. Mackall immediately agreed to test her anti-GD2 CAR-T cells on Monje's DIPG models.

The results, reported in *Nature Medicine* in 2018, were unprecedented. Given systemically to the mice, the CAR-T cells traveled to the brain and tore into the DIPG tissue, leaving only a few dozen cancer cells in their wake. "I didn't believe it the first time because I'd never seen anything do that," says Monje. "I think it was the sixth time my poor graduate student and a postdoc in Mackall's lab did this experiment that I really believed the results. It was just night and day. It was incredible."

There are risks, of course. The ferocity of the CAR-T attack in the cramped precincts of the brain stem caused inflammation that killed some of the mice. Further, the survival of cells that do not express GD2 suggests even the CAR-T therapy may not promise a complete cure. But Monje and Mackall think the brain inflammation is clinically manageable and are now preparing a trial to evaluate the therapy for children with high-grade gliomas rich in GD2.

Clearing a fog

On Fridays Monje sees patients, primarily survivors of childhood cancer and kids with high-grade gliomas enrolling in one of her clinical trials. The former often have long term neurological issues caused by chemotherapy—anxiety, impaired attention and memory dysfunction. These sequalae remain a central focus of Monje's research.

After starting her lab in 2011, Monje had examined brain tissue from patients who had been treated with the chemotherapy methotrexate and noticed in them an abnormal depletion of OPCs. To study the phenomenon, she began constructing a

Photo by Flynn Larsen

mouse model to mimic the exposure and effects of the chemotherapy on the human brain.

Studies using that model revealed that methotrexate shifts microglia into an activated state for six months or more after its administration. This chronic activation, Monje and her colleagues reported in *Cell* in 2018, muddles the brain's microenvironment enough to compromise cells known as astrocytes, which help nourish and link neurons and generally keep things in balance. It also disrupts the ability of OPCs to replenish themselves and to differentiate into oligodendrocytes. "The cells were getting stuck between the precursor cell state and the mature, oligodendrocyte cell state," explains Monje. All this disrupted the myelination of neurons, compromising their function—which is why mice treated with methotrexate displayed many of the same behavioral symptoms seen in patients.

To see if the pathological cascade could be reversed, Monje and her colleagues gave the mice a CSF-1R inhibitor, a drug that selectively depletes microglia. The effects were striking: OPCs, astrocytes and the myelination of neurons all normalized, and the neurological symptoms dissipated in the mice. "This is very exciting because CSF-1R inhibitors are already in clinical trials, already being used in humans," says Monje. More preclinical work must be done before the treatment can be tested in patients undergoing chemotherapy, says Monje. On the other hand, she is excited to finally have a strategy to develop. In her hands, that strategy is more than likely to yield results.

THE CANCER HUNTER

Nickolas Papadopoulos' early fascination with molecular genetics fueled a career-long adventure mapping cancer genomes, unearthing cancer genes and devising tests for the minimally invasive detection of cancer.

Nickolas Papadopoulos was about as surprised as he was relieved to hear the voice on the other end of the line when he answered his lab phone one afternoon in the summer of 1992. The voice belonged to Bert Vogelstein, a man he had never met before and to whom he had sent his one and only application for a postdoctoral position. If he didn't get it, Papadopoulos was ready to fly home to his native Greece. He had labored and fussed over his letter to the up-and-coming cancer geneticist, but the fact was he had no experience, let alone publications, in the field. Fortunately, Vogelstein had ignored that deficiency and was now inviting him over for an interview. Just come over, he said. No need to prepare a presentation.

The interview, touching on everything from Papadopoulos's musical skills to technical queries to the big questions of cancer research, went well. When it was over, Vogelstein asked a graduate student to take Papadopoulos out to lunch while, it turned out, he prepared another surprise. "When I came back from lunch, Bert had gathered a few people in a little room outside his office that we called the kitchen—a refrigerator, small tables and a whiteboard," Papadopoulos recalls. "He said, 'OK, now you're going to present your work to us.' " Vogelstein brushed off his protests, saying it wouldn't be a problem if he knew his stuff. After the presentation and a little Q&A, he had someone show Papadopoulos around the lab. When he returned, he found Vogelstein hammering out a letter on his electric typewriter. The job would start in a few months, it said; Papadopoulos had to promise he wouldn't even interview elsewhere.

Papadopoulos signed, and so joined a scientific adventure that has transformed the fields of cancer genetics and diagnostics. Working with a who's-who of scientists led by Vogelstein and Kenneth Kinzler—co-directors of the Ludwig Center at Johns Hopkins—Papadopoulos has had a hand in the discovery of several novel cancer genes and the mapping of scores of cancer genomes. He and his colleagues

NICKOLAS PAPADOPOULOS

LUDWIG JOHNS HOPKINS

Photo by Flynn Larsen

Working with a who's-who of scientists, Papadopoulos has had a hand in the discovery of several novel cancer genes and the mapping of scores of cancer genomes.

have in recent years also scoured that vast repository of genetic information to develop increasingly precise and minimally invasive DNA tests—or liquid biopsies—for cancers. A study Papadopoulos led with Vogelstein, Kinzler and Hopkins colleagues reported in 2018 in *Science Translational Medicine* the initial, retrospective evaluation of such a test for the early detection of ovarian and endometrial cancers, which are typically detected only in their advanced stages, when a cure is usually impossible. Another paper similarly reported in *Science* a single blood test that screens for eight common types of cancer. The malignancies detected by these liquid biopsies, which require further clinical development, account for more than 60% of cancer deaths in the U.S.

Chasing fascinations

Papadopoulos was born and raised in the historic city of Thessaloniki, in Greece, where his mother was a homemaker and his father a salesman for the Dutch multinational Philips. "He was traveling all over Greece making sure every house had a TV," says Papadopoulos. His mother, who had helped make ends meet as a seamstress during the Second World War and felt lucky to have attended school, was adamant that her children complete college. Not that Papadopoulos needed much encouragement. He had always wanted to be a biologist.

After getting his bachelor's degree from the University of Thessaloniki, Papadopoulos considered going to the UK for his postgraduate education. But his brother fell seriously ill, and Papadopoulos accompanied him and their mother to the U.S. for his treatment at a hospital in Houston. He used his spare time to take a course in English at the University of Houston and then applied to a master's program at the school. During his first year, his advisor—with whom he was investigating how muscle innervation influences the expression of myosin, a constituent of muscle fibers—moved to the University of Texas, Houston. He asked Papadopoulos to stay on as a PhD candidate in a joint program of UT Houston and the MD Anderson Cancer Center.

Papadopoulos took full advantage of the move, taking courses in molecular genetics and cancer biology. "I started having second thoughts about what I wanted to be when I grow up," he says. It was in one of those cancer biology courses at MD Anderson that Papadopoulos first heard of Bert Vogelstein and his work exploring mutations in colon cancer.

In 1992, Papadopoulos moved with his advisor to Baltimore, to a National Institutes of Health lab in the Francis Scott Key Medical Center, where he wrapped up his doctoral studies and sent his letter to Vogelstein.

Running start

Starting at Vogelstein's lab in late 1992, Papadopoulos entered a race to find the

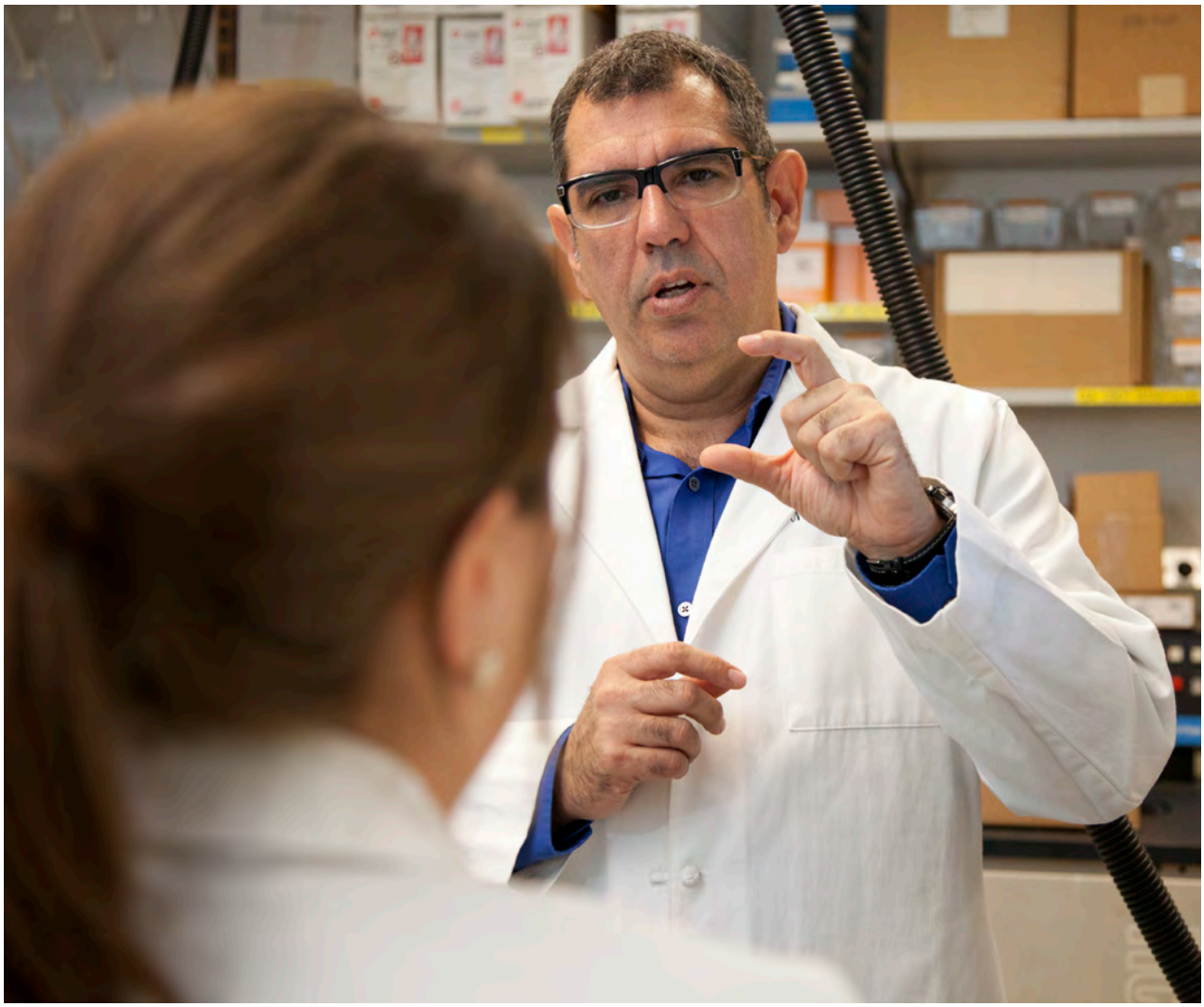


Photo by Flynn Larsen

genes responsible for an inherited propensity for cancer known as Lynch syndrome. In December 1993, the researchers reported in *Science* that a gene encoding an enzyme involved in the repair of mismatched DNA bases drives HNPCC, a colorectal cancer associated with Lynch syndrome. Over the next few months, Papadopoulos became a primary contributor to the discovery of other mismatch repair (MMR) genes, reporting the discoveries in *Science, Nature* and *Cell*. The discoveries led not only to the identification of a novel family of human DNA repair enzymes, but also to the development of clinical tests for Lynch syndrome.

After his postdoc, Papadopoulos was hired by Columbia University in 1997, where he opened his own lab. But in 2000 a biotech named GMP Genetics recruited him as its chief scientific officer. Papadopoulos wore many hats at the startup, learning translational research on the fly even as he tended to such mundane matters as laboratory floorplans and equipment procurement. After five years at the company, he'd had enough.

Following a stint as a consultant, Papadopoulos got in touch with Vogelstein to discuss how he could get back into academia: Aside from the intellectual adventure of academic research, he missed training young scientists, something he

A big part of what Papadopoulos and everybody else at Ludwig Johns Hopkins wanted to do was design DNA tests that could be routinely used to detect tumors early, monitor responses to cancer therapy and catch relapses swiftly.

says he still finds to be among the most rewarding aspects of his daily work—not least due to consistently high caliber of trainees in the Hopkins group.

"I sent Bert my proposals and he said, well, that's what we want to do too," Papadopoulos recalls. "Why don't you come here, and we can do it together."

Exomic landscapes

Papadopoulos rejoined the group just as the Ludwig Center at Johns Hopkins opened its doors in 2006. The group was then busy mapping the full spectrum of expressed genes—or exomes—in various cancers, working with a company to get the DNA sequencing done. Papadopoulos applied his industry experience to set up a next-generation sequencing facility at the Center. Over the next two years, the Ludwig Johns Hopkins team published the first maps of the breast, colon and pancreatic cancer exomes, as well as that of the brain cancer glioblastoma. The maps contained a trove of new information on the mechanisms of carcinogenesis and clues to the development of diagnostics and therapies. Subsequently, the Ludwig team would map 88 of the first 100 cancer exomes, exposing many novel oncogenes.

Papadopoulos, a key part of those efforts, hypothesized that the exomes of relatively rare cancers would reveal novel mechanisms of malignancy, and led the mapping of two of them: a type of pancreatic neuroendocrine tumor and ovarian clear cell carcinoma. He was correct. Papadopoulos and his colleagues reported in *Science* in 2010 one of the first examples of mutations to a chromatin remodeling protein—which manipulates the stuff of chromosomes to make genes available for expression—in cancer. The pancreatic tumor's exome, described in *Science* the following year, revealed mutations in three genes that are predictive of patient survival. One of the mutations also explained a known aberration in the chromosomes of these cancer cells and, it turned out, those of a brain cancer—for which it is today used as a diagnostic marker.

Fishing for cancer

A big part of what Papadopoulos and everybody else at Ludwig Johns Hopkins wanted to do was design DNA tests that could be routinely used to detect tumors early, monitor responses to cancer therapy and catch relapses swiftly. To do that, the team needed a way to find in body fluids the vanishingly rare fragments of DNA shed by tumors. "Now that we had these genomic

Photo by Flynn Larsen

landscapes of cancers, we felt we had enough information to detect tumor DNA in body fluids if we could develop technologies sensitive enough to detect the mutations," says Papadopoulos.

While he was away, Kinzler and Vogelstein had developed just such a technology, digital PCR, that permitted the capture, expansion and detection of DNA shed by colon tumors. (The process has long since been automated, and the work itself led to the development of the first home test for colon cancer.) "We kept maturing the technology to the point that we developed something we called the safe sequencing system," says Papadopoulos. Co-developed by Kinzler, Vogelstein, Papadopoulos and a former student at the Hopkins Center, Isaac Kinde, the method (SafeSeq-S) harnesses massively parallel sequencing to fish out between one and five genuinely mutated DNA sequences among 10,000 normal ones.

The team first assessed whether the system could detect ovarian and uterine tumor DNA in fluid from a Papanicolaou (Pap) smear, a routine cytology test for cervical cancer developed in the 1950s by another Greek immigrant. "We thought we don't need to add burden on patients," says Papadopoulos. "If women are willing to get a Pap smear, why not extend that test to cover some other tumor types, especially ovarian cancer,

Photo by Flynn Larsen

PapSEEK was nearly 99% specific for cancer, which is essential to avoiding false positives. It also picked up 81% of endometrial cancers and 33% of ovarian cancers.

where right now we have no approved screening test?"

The researchers reported in two papers in *Science Translational Medicine* in 2013 and 2014 that their methods could detect all uterine tumors and 41% of ovarian tumors in Pap smears, as well as tumor DNA shed into the blood by a variety of common cancers. A biotech named PapGene was established in Baltimore to develop liquid biopsies based on the technology. In 2019, after receiving a large infusion of additional venture funding, the company emerged with the name Thrive Earlier Detection.

Toward early detection

With the feasibility of the tests established, Papadopoulos and his colleagues began improving the method's sensitivity and accuracy with support in part from the cancer prevention initiative launched by Ludwig and the Conrad N. Hilton Foundation. The test they developed for endometrial

and ovarian cancers, PapSEEK, looks for aneuploidy—abnormal numbers of chromosomes typical to malignant cells—and mutations in 18 genes. It was evaluated in 658 cancer patients and 1,002 healthy women.

PapSEEK, they reported in *Science Translational Medicine* in 2018, was nearly 99% specific for cancer, which is essential to avoiding false positives. It also picked up 81% of endometrial cancers and 33% of ovarian cancers. When fluid was collected with a brush that extends deeper into the cervical canal, PapSEEK detected tumor DNA in 93% of endometrial cancer patients and 45% of those with ovarian cancer. Sensitivity was further boosted to 63% for ovarian cancer when both blood and pap fluid were tested.

To further develop the blood-based test for a more general cancer screening, Papadopoulos and his colleagues had to figure out ways to dramatically improve both its sensitivity and specificity—the latter being critical to avoiding potentially traumatizing false positives. To improve sensitivity, the researchers added to their DNA screen a panel of proteins that are known to be elevated in various cancers. The proteins would also help locate the source of the mutated DNA circulating in the blood, which picks up DNA from everywhere.

The test they came up with, CancerSEEK, looked for eight proteins along with 16 mutated gene sequences and was evaluated in a multi-institutional study on hundreds of controls and patients with nonmetastatic cancers of the ovary, lung, liver, pancreas, stomach, esophagus, colorectum and breast. Papadopoulos and his colleagues reported in *Science* in 2018 that the specificity of the test exceeded 99%, while its median sensitivity was 70%—ranging from almost all ovarian cancers (98%) down to a third of breast cancers. "The sensitivity of the test is not where we want it to be, but we are working on ways to increase it," Papadopoulos says. They are, for example, adding DNA probes for aneuploidy and other chromosomal aberrations associated with malignancies.

Photo by Monty Rakusen

The team has also devised a liquid biopsy for urothelial and bladder cancers based on the analysis of urine samples. They reported in *eLife* in 2018 that the test, UroSEEK, detected 75% of urothelial cancers and—when combined with cytology, an existing method of surveillance—95% of bladder cancers. All three of the tests are being developed further by Thrive, which Papadopoulos says is in a better position to put them through clinical trials. He and his colleagues, meanwhile, continue to innovate. That is, after all, something they do rather well.

THE TUMOR MAPPERS

The unique cooperative research model of the Ludwig Center at Harvard is being productively harnessed by the Tumor Atlas Project, an ambitious effort to create high-dimensional maps of any and all tumors.

When Peter Sorger set out to develop a method for mapping the different cells in tumors, he didn't expect actual mapmakers would be involved. So when members of Harvard's Department of Architecture approached him one day following a presentation, Sorger was surprised. "They said, 'That's really cool. Let's work together,'" recalls Sorger, an investigator at the Ludwig Center at Harvard and professor of systems pharmacology at Harvard Medical School. "Unknown to me, Harvard was the place where the initial GIS"—geographic information system—"was developed back in the 1950s."

The Harvard cartographers' expertise would prove useful for organizing and visualizing the flood of tumor data that the Ludwig Tumor Atlas Project (TAP), led by Sorger, was generating. Launched in January 2019 with funding from Ludwig Cancer Research, TAP aims to develop a multi-dimensional "map" that captures the locations and identities of not just cancer cells but also the noncancerous immune and supporting cells that contribute to tumor evolution, progression and response to therapies. It is also a sort of technological avatar of an idea central to the structure of the Ludwig Center at Harvard: to bring together diverse biomedical disciplines and their associated technologies to tackle the most intractable problems of cancer research and care.

It takes a village

It's no coincidence that TAP originated at Ludwig Harvard, which has a special focus on drug resistance in cancer. "The Tumor Atlas Project fits into every single project we have," Ludwig Harvard Co-director Joan Brugge observes. "The technology makes it feasible to follow many different proteins in real human tumors, which is key to an understanding of the state of individual cells in tumor tissue prior to and after drug treatment."

TAP, Sorger expects, will not only help transform our understanding of cancer biology but drive innovations in diagnostic pathology as well. The first phase of the project will map tumor cells, unraveling their


PETER
SORGER
LUDWIG
HARVARD
Photo by Flynn Larsen

"The technology makes it feasible to follow many different proteins in real human tumors, which is key to an understanding of the state of individual cells in tumor tissue prior to and after drug treatment."

interactions with supporting noncancerous cells and immune cells, and pin down the cell signaling pathways involved in driving tumor growth and drug resistance. The second, Sorger says, will deploy machine vision, artificial intelligence and multi-dimensional visualization to combine data from many specimens, facilitate expert annotation by human pathologists and develop algorithms for predicting the responses of individual patients to specific therapies.

The technology benefits from a unique "cooperative research model" that Brugge and Co-director George Demetri have implemented at Ludwig Harvard. That model seeks to bring together researchers from multiple disciplines at the outset of every inquiry. The framework is vital to TAP, which relies on contributions from not just oncologists and pathologists but also software developers, computational biologists and, of course, geographic information systems specialists.

"The foundational technology that underlies modern digital maps is conceptually applicable to our Atlas," Sorger says. "On our website, you can zoom in and out on millions of tumor cells from different diseases. The technology behind that is the same one used in Google Earth."

Community building

Ludwig Harvard's model was forged in the earliest days of its establishment, when Brugge and Demetri were appointed its co-directors and had to decide how to distribute the annual interest of the $90 million endowment from Ludwig Cancer Research. "George and I were in sync from the very beginning," says Brugge, whose own thinking was influenced by her experience co-founding a biotech company. "I saw how well it can work when you have multiple people with different expertise coming together to help solve a problem."

With the new funding, the co-directors saw an opportunity to build a truly multidisciplinary model for cancer research. "What we wanted to do was to bring the other people who are really interested in a given problem from multiple areas of science, and then together develop the strategy to attack the problem, so that from the very beginning, we would be functioning as a unit."

In practice, this means that every research team that is part of Ludwig Harvard receives about $150,000 in seed funding annually to pursue its research. This has helped forge a community, says Demetri, who is also the associate director for clinical sciences at the Dana-Farber/Harvard Cancer Center.

"Our pitch to faculty was, 'If you join our community, we will have the ability to come up with new ideas, intersect in different ways, and provide seed money to get great multi-institutional, multi-investigator grants going forward,'" Demetri says. "Did we get

Ludwig Harvard Co-directors George Demetri and Joan Brugge.

Photo by Flynn Larsen

pushback? You bet we did. But in the end it worked. Remember, this was right around the time when team science was starting to catch fire. People were realizing that the translation from basic science to patients is too complicated for any one person, and we need to figure out how to work together."

Brugge and Demetri also implemented a weekly Monday meeting to which anyone associated with Ludwig Harvard research—from principal investigators to postdocs, graduate students, and clinicians—is invited. The "Ludwig Monday meetings," as they've come to be known, are a chance for researchers from different disciplines to come together to learn what their colleagues are working on and determine how their projects might intersect.

Jennifer Guerriero, who has been attending the weekly gathering since her postdoc days, says the meeting had a strong influence on her as a young scientist. "I remember sitting there in awe and watching and listening to all of these senior and junior people just talking about science together," recalls Guerriero, who now directs the Breast Immunology Laboratory in the Women's Cancer Program at the Dana-Farber Cancer Institute. She too has become a key part of the TAP and tCyCIF team, with a special focus on the roles of macrophages in chemotherapy and immunotherapy.

The effect Guerriero described was by design. "In some ways, it felt like part of what we did was introduce people to other people," says Demetri. "It was like a junior high school dance, where the basic scientists were on that side of the room and the clinical scientists were on the other side, and the two groups were too shy or unable to talk to each other."

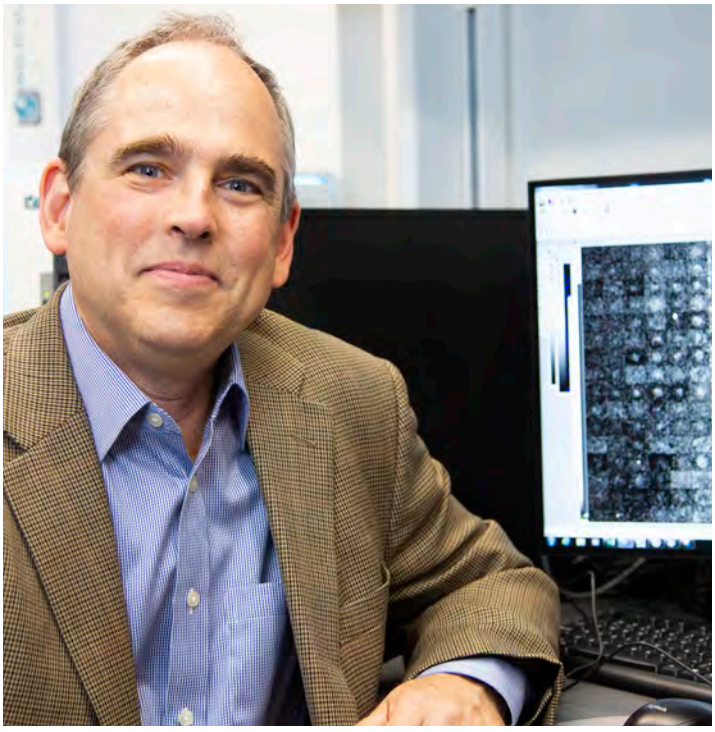


Photo by Flynn Larsen

E pluribus unum

At the heart of TAP is a method dubbed tissue-based cyclic immunofluorescence, or tCyCIF, which is being developed at Ludwig Harvard under Sorger's direction. tCyCIF allows researchers to obtain images containing multiple layers of protein information about tumors—including their cancer cells and their associated immune and other noncancerous cells—at subcellular resolution. It combines the output of multiple existing instruments and reagents into a workflow that can scan a tissue sample dozens of times without damaging its constituent cells. Each scan looks for three to five different protein markers. When compiled, the information generates a composite image of a tumor constructed from 40 to 60 "channels" of information.

Sorger likens the process to getting to know a stranger by asking many simple yes and no questions. "But instead of asking just one stranger, imagine asking these questions to a stadium full of people simultaneously. tCyCIF is very much like that," he explains.

The result is a remarkably rich and nuanced picture of tumors. "Before, we had a very unidimensional view of individual cells," says Sandro Santagata, an investigator at the Ludwig Center at Harvard, co-leader of the TAP and an associate professor of pathology at Brigham and Women's Hospital. "Now we can not only spot an immune cell, but determine specifically which type it is and define the functional state that it's in, and then compare it to a slightly different immune cell that occupies a different space. Not only do you get to really probe deeply the identity and the properties of individual cells, now you also get to see how they interface with each another."

tCyCIF at work

tCyCIF is designed to use the kinds of standard biopsy samples that hospitals and researchers have been collecting from patients for nearly a century (it also works with mouse models of cancer). "Our goal was to hack directly into the standardized clinical workflow," says Sorger, who is also a principal investigator in the U.S. National Cancer Institute's Human Tumor Atlas Network, for which he is mapping premalignant tissues associated with certain skin and blood cancers. "We wanted to develop a method that allows us to get deep molecular insights from a sample that is collected from virtually every single cancer patient." This continuity means that tumor samples collected in completed clinical trials can still be analyzed, as can accumulated samples of rare cancers collected over the decades.

Sorger envisions tCyCIF as a complement rather than a competitor to other cell-screening technologies. For example, single cell RNA sequencing can provide a wealth of information on the gene expression profile of individual cells. "You get detailed information on individual cells, but you get no information about their locale," Sorger says. By contrast, while tCyCIF tracks only a few dozen proteins, it can interrogate many square centimeters of tissue—hundreds of thousands of individual cells—and determine the precise

morphologies of cells and their spatial relationships to each other. Combining the results of RNA sequencing and other large scale, or "omics", assays with maps generated by tCyCIF is a central goal of TAP.

To help people expand on TAP using research data from their own labs, Ludwig Harvard is placing details about tCyCIF and TAP in the public domain. "The patient data is anonymized, but all of the data and any insights we glean from it will be publicly accessible," Sorger says. "We want to make the data and the code freely available to the Ludwig community to demystify and democratize high dimensional histology."

Currently, TAP consists of dozens of images of six types of tumors—including triple-negative breast cancer, ovarian cancer, and acute myeloid leukemia—but Sorger envisions the project growing to encompass a greater variety of cancers as other centers and hospitals contribute samples. A key step will be combining image data from many different patient specimens into general-purpose maps. It is not yet clear how this will be accomplished, and Sorger and Santagata look forward to investigators from many Ludwig Centers becoming involved.

A higher order experiment

The idea for TAP was partly inspired by Ludwig Harvard's cooperative research model, and Demetri thinks the project could be a vehicle for disseminating the model to other centers. "I see it as a social experiment," Demetri says. "Can we use it as a testing ground to see how we can all work better together? Everybody wants this kind of information, and there are whole companies being formed to do this, but if we can do it academically and openly distribute the tools and the data to people, we will engender trust and enable people to ask better questions and get answers faster."

All tissue samples for TAP have so far come from U.S. hospitals, but members of the Ludwig Harvard team have travelled to Ludwig Lausanne to initiate a collaboration on ovarian organoid cultures. As another step toward more open collaboration, the Ludwig Center at Harvard and the Lausanne Branch are experimenting with a workflow in which tumor samples scanned with tCyCIF at one center are analyzed with software at the other. Work on Barrett's esophagus with Ludwig Oxford is being planned as well, and Ludwig Harvard is working closely with Ludwig MIT to apply t-CyCIF to mouse models of cancer.

"We envision interactions with additional Ludwig Centers and Branches, either through direct collaboration or the transfer of technologies and methods," Sorger says. "We're comfortable with either approach. Within a few years we hope that this grows beyond a technical collaboration into a Ludwig-wide effort to efficiently ask and answer questions about shared data on drug resistance and the prospects for improving therapeutic responses."

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