LUDWIG
CANCER
RESEARCH

# 2018 RESEARCH HIGHLIGHTS

LIFE-CHANGING SCIENCE

# WELCOME

Ludwig Cancer Research has many gifted scientists who have many interesting findings to share. Every year we pick out a few fascinating discoveries reported by Ludwig researchers in the previous year and tell the stories of how they came about. Not the short version, of the discovery itself, but the long one, of the journey of scientific inquiry that led to each finding and the lives, careers and fascinations of the scientists who led the effort. We highlight in this report a cross-section of Ludwig's life-changing science that illustrates how we're pursuing our mission to advance cancer research and care.

One theme that leaps out in this report is the importance of teachers—the sort who turn science into poetry and transform students into independent investigators. These teachers go the extra mile to engage and excite their students with the power of the scientific method and its ability to illuminate the mysteries of nature. They are mentors who stick out their necks for young scientists, take a chance on them and help them fulfill their scientific aspirations. Many of the scientists we profile express an immense gratitude for their teachers, and in turn become mentors and take genuine pride in the young scientists they themselves have trained.

The profiles also bear out the global scope of our effort to conquer cancer. In establishing the Ludwig Institute for Cancer Research, Daniel K. Ludwig argued that "the rare vision and ability needed in the battle against cancer are not limited by frontiers, and the scientists who possess these gifts must be sought wherever they are to be found." You will notice that many of the researchers profiled here are immigrants and world travelers. Others made their contributions while remaining in their native countries. Together, these researchers represent an endeavor that transcends country, creed or color to harness talent from all corners of the world to a common and humane cause.

Ludwig is proud to be a leader of this cause, and we hope you enjoy this small sampling of our contributions.

Happy reading!

Sincerely,

Ed and Chi

**Edward A. McDermott Jr.**
President and Chief
Executive Officer

**Chi Van Dang**
Scientific Director

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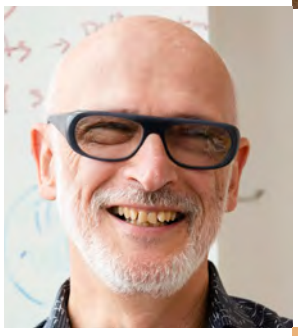
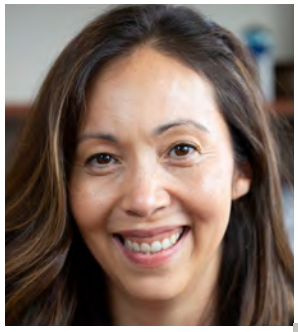










SANJIV SAM

# GAMBHIR

STANFORD

LUDWIG

Photo by Steve Fisch

# THE QUINTESSENTIAL IMAGER

His mission to detect disease early and visualize cells and molecular processes hidden deep within living bodies is transforming cancer diagnosis and therapy.

Sanjiv Sam Gambhir, all of 19 and with a bachelor's degree in hand, had no doubt he wanted to be a physicist.

Gambhir planned to train his keenly analytical intellect on nuclear fusion, hoping to enroll in the PhD program at Princeton University in the fall of 1983. But his economist father, an alumnus of the London School of Economics, had other ideas. One evening, the younger Gambhir found a bunch of physicists in his family's modest living room in Tempe, Arizona, several of them unemployed and all of them invited to the Gambhir home to dissuade Sanjiv from making what they felt was a potential mistake.

The gambit worked. A compromise was reached. Gambhir applied to MD/PhD programs around the country, ultimately enrolling in a 10-year program at the University of California, Los Angeles (UCLA). But he didn't entirely abandon his first love. Unable to do the PhD portion of the program in physics, he obtained one in applied mathematics instead. "This is where I developed a real interest in using mathematics to solve biological problems and physics to develop new instruments to visualize what's going on within the body," says Gambhir, who is today a Virginia and D.K. Ludwig Professor of Cancer Research at Stanford and, not coincidentally, chairman of the Department of Radiology at the Stanford University School of Medicine.

That fascination has endured, and it has in large measure inspired Gambhir's extraordinarily prolific research career. Though best known as a pioneer of molecular imaging, Gambhir has broadened the ambit of his highly interdisciplinary studies to include minimally invasive diagnostics, nanotechnology, early cancer detection, and the development of novel instruments for biomedical imaging. Over the past three decades, he has published more than 625 papers, filed more than 40 patents—pending and granted—and helped spin off three biotech startups to commercialize his inventions.

Photo by Stewart Marcano

In December 2016, Gambhir and his team published a paper in the *Proceedings of the National Academy of Sciences (PNAS)* on an inexpensive nanotechnology-based blood test to monitor lung tumor evolution. He and his colleagues also reported in a 2017 paper in *Science Translational Medicine* a method for the live imaging and monitoring of therapeutic immune cells in humans and demonstrated its use in brain cancer patients. Finally, in a recent *Cancer Research* paper, Gambhir's team reported the development and evaluation of a technology that permits the visualization of all activated T cells in the body, which will likely be of great use in optimizing a wide variety of cancer immunotherapies.

## Finding a place

Sanjiv Gambhir's family immigrated to the US from India when he was just seven years old. He had health issues and did better in relatively dry and hot climates, so his family—including his father; his mother, a former teacher; and his sister, who is today a radiologist in San Francisco—settled in Tempe. His father, who had worked for an oil company in India, eventually found employment with the Bureau of Indian Affairs, but the adjustment wasn't exactly easy. "We were pretty affluent in India, but in Arizona we really started from scratch," Gambhir recalls. "We lived in a small apartment, and had no car for several years."

Though Gambhir had a gift for math and physics, he struggled in high school. "I almost dropped out because I was never able to learn the way most people learned and had a very difficult time with a limited attention span," he says. A physicist, Michael Wells, who had left Motorola to teach, and a biology teacher, Kathy Aspey, who took an unusually quantitative approach to the subject, saved him from that fate. "If they hadn't been my teachers, I don't know what would have happened to me."

Gambhir majored in physics when he enrolled at Arizona State University at the hopelessly awkward age of 15, joining a physics department that he says was as pedagogically exceptional as it was small. "Those undergrad days made me appreciate even more strongly how much physics could do," says Gambhir.

When he began at UCLA in the Medical Scientist Training Program, Gambhir was fortuitously placed in the program headed by Michael Phelps, one of the co-inventors of positron emission tomography (PET)—an imaging technology based on the detection of radiolabeled chemical tracers that are taken up by cells. It is today a common tool of clinical oncology. At the time, however, PET scanners were bulky, expensive machines used only in academic labs. Phelps and his team were working to bring them to the clinic.

## Seeing the unseen

After Gambhir completed his MD/PhD and did his medical internship in medicine and residency in nuclear medicine at UCLA, the Molecular and Medical Pharmacology Department hired him as an assistant professor in 1994 and soon thereafter appointed him director of its new Crump

"All of these technologies sought to visualize biology inside a subject … and to use that visualization to understand molecular behavior."

Institute for Biological Imaging. "My initial research started with the goal of studying biology without perturbing the animal or human," he explains. "How do you not have to remove tissue from a mouse or do a biopsy of a human? How do you see the unseen and detect disease at a molecular level? The ultimate goal was to really bring together the fields of cell and molecular biology with that of biomedical imaging."

Gambhir's UCLA lab sought to capture disease and other biological processes early and within living things. "It required building many kinds of new tools: imaging agents, new approaches to imaging, new ways to quantify data," says Gambhir. Researchers were already using green fluorescent proteins (GFPs), which glowed to report molecular events, like the expression of a gene or the interaction of a pair of proteins. But GFP fluorescence could not capture molecular events deep within the living human body, and that's what Gambhir wanted to see.

Starting in 1997, under the mentorship of Harvey Herschman, a cell biologist, Gambhir's lab began engineering a viral gene for that purpose. The proteins encoded by those genes would be expressed within targeted cells, where they'd trap radiolabeled tracers injected into the blood. The expression of the reporter gene could even be linked to that of another gene of interest, allowing clinicians and researchers to monitor its expression. The tracer's signals would be converted into an image by a PET scan.

Gambhir demonstrated in 2000 that this strategy generated quantifiable images of reporter gene expression in targeted internal tissues of living animals. In 2003, he was recruited by Stanford University to start the Molecular Imaging Program at Stanford and head up a Division of Nuclear Medicine at the university hospital, where he is today also chairman of the Department of Radiology. By 2005, he and his colleagues had demonstrated the viability and utility of his leading PET reporter in humans.

"The first place we applied these PET reporter genes was in human gene therapy," says Gambhir. "People were delivering viruses to treat liver cancer. We came in with a virus that also carried our reporter gene. So now we could tell not only whether the virus had gone to a particular place but if the gene it carried was being expressed."

Gambhir was simultaneously developing PET reporters to visualize everything from heart muscle cells after cardiac cell transplantation to T cells infused into mice to attack tumors. His team also began exploring bioluminescent and fluorescent technologies to similarly image organs and tissues sans radiation in living bodies, and novel detection technologies like photoacoustic imaging (in which light pulsed into a living subject generates sound as a readout of molecular information).

"All of these technologies sought to visualize biology inside a subject, including a human, and to use that visualization to understand molecular behavior—and then use that to solve the problem of early disease detection

and improved disease management," says Gambhir.

His research interests also began moving beyond imaging to the exploration of minimally invasive DNA and protein diagnostics and nanotechnology for disease detection. In 2008, he launched the Canary Center at Stanford for Cancer Early Detection, funded by Don Listwin, a former Cisco executive who had lost his mother to ovarian cancer initially misdiagnosed as an infection. Gambhir also established a National Cancer Institute-funded cancer nanotechnology center at Stanford, where after 14 years he is a principal investigator.

## Nanosifting

In collaboration with the laboratory of Shan Wang, a Stanford professor of electrical engineering and materials science who is a colleague at the nanotechnology center, Gambhir and his team developed extraordinarily sensitive magnetic nanotechnologies for the detection of cancer and other disease biomarkers. (These are already in commercial development.) In December 2016, they and Viswam Nair, a pulmonologist at the medical school who was mentored by Gambhir, led a publication in *PNAS* describing a method of capturing and analyzing rare circulating tumor cells (CTCs) that cancers shed into the blood.

Such "liquid biopsies" could significantly improve the management of cancer therapy, allowing physicians to routinely monitor their patients' tumors. "By analyzing CTCs we can track how a tumor is evolving and determine whether someone is about to fail treatment," says Gambhir. "We can then switch patients early to another therapy that might be more effective."

The researchers took blood from lung cancer patients and labeled it with antibodies specific to CTCs, which in turn were tagged with magnetic nanoparticles specific to antibodies. They then used a device developed in Wang's lab known as the MagSifter to pick out the CTCs and drop them individually into minute wells, where they were analyzed for the presence of a few cancer-driving genes. If the approach passes muster in larger clinical studies, the test will likely be just as useful in the treatment of a variety of other cancers.

## On the T cell beat

By 2003, Gambhir was already preparing to test his PET reporters on the human immune system. A team at City of Hope, in Duarte, California, was planning to infuse engineered immune cells—chimeric antigen-receptor T cells (CAR-T)—into patients to treat the brain cancer glioblastoma multiforme (GBM). Gambhir could introduce the PET reporter into the T cells as part of the engineering. The reporter would give the clinical researchers an immediate and invaluable handle on how the therapeutic T cells were doing inside patients.

"It took a decade to move it into humans because of all the regulatory challenges," says Gambhir. "No one had ever put genes for PET imaging into cells and, in those days, immunotherapy had not caught on either. And these were a very complex set of patients who were very sick because they had recurrent GBM." Ultimately, the PET reporter took more than a decade—and, Gambhir estimates, some 50 papers worth of work in all—to reach its destination inside patients.

Along the way, Gambhir's own son, Milan, would be diagnosed with GBM. Despite the best efforts of Gambhir and his colleagues around the world, including treatment with several experimental immunotherapies, Milan died from his illness at the age of 16, some 21 months after his diagnosis. "This disease is very deadly, and very few people survive it," says Gambhir.

Through this difficult period, Gambhir's work with City of Hope proceeded steadily forward and, in 2017, the team published its results in

*Science Translational Medicine*. Those results showed that PET reporters could be used to track where engineered T cells went as they hunted down tumors and to determine whether they arrived, in what number, and if they were still alive. And that was not all. "We could see T cells going to other sites in the brain, and we realized that there were hidden tumors in those places that were unknown to us," says Gambhir. "What a surprising result that was!"

Gambhir's reporter gene strategies are now being used in clinical trials to track not just CAR-T cells but other immunotherapies that involve extracting, manipulating and reinfusing immune cells. They're also being used in other applications such as stem cell therapy to track, for instance, therapeutic stem cells after they've been injected into the heart. Like the GFP technology of the 1990s, the PET reporter gene has become a general molecular tracking tool but, in this case, for small and large living subjects.

## A general reporter

Building on that work, Gambhir and his team reported in *Cancer Research* in 2017 the development of a novel PET radiotracer that, when injected into the bloodstream, preferentially accumulates in activated T cells. In their study, the researchers demonstrated the safety and distribution of one of those agents in humans. They also showed in a mouse model how it could be used to quickly detect graft versus host disease, a potentially lethal condition in which T cells transplanted into a patient attack the recipient's tissues.

"We showed we can monitor what the immune system is doing without first having to put a reporter gene into T cells," says Gambhir. "Often you don't have the luxury of having the T cells outside the body and then reinfusing them after they've been genetically modified." The ability to skip that step has significant implications for cancer therapy. Checkpoint blockade and many experimental immunotherapies activate killer T cells while they're inside the body. Being able to monitor how patients receiving such therapies are responding would significantly improve the management of cancers, allowing physicians to adjust the therapy as needed.

> "Right now, in most of medicine, including cancer immunotherapy, we're shooting blind."

"Right now, in most of medicine, including cancer immunotherapy, we're shooting blind," says Gambhir. "If I give you an immunotherapy and it doesn't work—like in my own son—we don't know why it didn't work. Is it because the T cells never made it to the tumor, or did they make it and then get exhausted? Or is there some other reason the cancer spread?

"This technology also lets us look at toxicity. If the cells are making it to their targets and revving up, but we also see them in activated in the bone marrow and other non-target sites, we can potentially predict you're in for a toxic crisis."

Gambhir's PET tracers and reporters, spun out to a start-up named CellSight, are already being evaluated in clinical trials for cancers of the lungs, bladder, head and neck, as well as urothelial cancers. Yet tracking immune cells is only one part (albeit an important part) of the promise of Gambhir's work.

"What it comes down to is that once you have the tools and technologies to track molecular processes in living patients you fundamentally change how disease diagnosis and management is handled," he says. That change, it would appear, is coming fast.

JOHANNA **JOYCE**

LUDWIG **LAUSANNE**

Photo by Gilles Weber

# THE
# TUMOR ECOLOGIST

Her ongoing investigation of how noncancerous cells in the microenvironment of tumors contribute to malignant growth, drug resistance and metastasis is also revealing how such relationships might be disrupted to treat cancer.

Johanna Joyce was puzzled. The cause of her befuddlement: positive results from an experiment in a mouse model of glioma, an aggressive brain cancer.

The year was 2012, and it was already known that macrophages—immune cells that ordinarily gobble up cancer cells and infectious agents—often turn traitor, multiply within tumors and drive cancer progression. Joyce, who was at Memorial Sloan Kettering Cancer Center (MSK) at the time, wanted to see what would happen if such turncoat macrophages in gliomas were targeted with an inhibitor of the CSF-1 receptor (CSF-1R), whose activity is normally essential to their survival.

"The results were striking," says Joyce, who joined the Ludwig Branch in Lausanne, Switzerland, in 2016, where she is a Member. "Even after treatment of just one week, we saw a pretty dramatic regression of the tumors." But what puzzled her was that the tumors were still teeming with macrophages. As Joyce and her team reported in *Nature Medicine* in 2013, the glioma cells were producing factors that helped macrophages survive the therapy. But the loss of the receptors' signal, rather than killing the tumor-associated macrophages (TAMs), had "reeducated" them, altering their gene expression programs to convert them back into cancer cell gourmands.

That study, with its scientific and therapeutic implications, put the Joyce laboratory on the map of tumor biology. In the years since, she and her lab have continued to expose the intricate interplay between cancer cells and a motley crew of noncancerous cells in the tumor microenvironment. In 2017, she and her team at Ludwig Lausanne reported in *Oncogene* how macrophages help gliomas resist targeted drug therapy and how such resistance might be overcome with CSF-1R inhibition. Another Joyce lab publication in *Cell Reports* described a similar macrophage role in chemotherapy resistance. Finally, a

"It was teaching as teaching should be done. They taught us how to think about science through stories of how the discoveries happened. That made it so fascinating that you just wanted to learn more and more."

*Nature Cell Biology* publication elucidated how obesity, through its effects on another type of immune cell, the neutrophil, drives the spread of breast tumors to the lungs.

## Student days

When Joyce was 14 years old, her parents moved her and her four younger siblings from London to a farm they bought outside Dublin. Joyce's omnivorous appetite for science intensified under the influence of the teachers at her new school—particularly, she recalls, an enthusiastic chemistry instructor named Mr. Bennett. After finishing school, she enrolled in an honors program in the natural sciences at Trinity College, in Dublin, where she ultimately focused on genetics. "I thought the inherent logic of it was quite beautiful," she says.

The professors of genetics at Trinity, she says, were the best teachers she ever had. "They instilled in us an absolute love for genetics of all types," she says. "It was teaching as teaching should be done. They taught us how to think about science through stories of how the discoveries happened. That made it so fascinating that you just wanted to learn more and more, and enjoyed going to the classes so much. It's something I try, to the extent I can, to bring into how I teach my own students."

Joyce's honors thesis at Trinity, on genomic imprinting—the regulation of a subset of genes depending on which parent they're inherited from—led directly to doctoral research in clinical genetics at Cambridge University, in the laboratory of Paul Schofield. There she explored how the faulty regulation of imprinted genes causes Beckwith-Wiedemann syndrome, which predisposes children to cancer.

## Into the microenvironment

An urge to go beyond cancer genetics and plunge deeper into the multicellular complexity of cancer took Joyce to Douglas Hanahan's laboratory, then at the University of California, San Francisco, where she started her postdoctoral studies in 1999. Collaborating with the chemical biologist Matthew Bogyo, Joyce explored how cathepsins—a family of protein-snipping molecular scissors—participate in multiple aspects of pancreatic cancer progression.

Their studies also revealed that immune cells are notably avid expressers of cathepsins. "That early result," says Joyce, "ultimately led me to focus on the roles of TAMs in cancer initiation, progression, invasion and response to therapy, and so it set the stage for the whole program that I developed in my own lab in New York and that continues here in Lausanne."

Photo by Eric Déroze

After joining MSK in 2004, Joyce expanded her studies to investigate TAMs in breast cancer and, later, in gliomas, ultimately leading to the *Nature Medicine* paper on CSF-1R inhibition and TAM reprogramming.

"That was a different way of thinking about targeting the tumor microenvironment," says Joyce. "You don't necessarily want to deplete these and other immune cells in cancers because they have critical housekeeping functions. But by re-educating them so that they can again execute those functions we could potentially get better therapeutic outcomes."

## Resolving resistance

But would the effect last? Or would gliomas, among the wiliest of malignancies, develop resistance?

In a 2016 paper published in *Science* after Joyce joined Ludwig Lausanne, she and her colleagues addressed those questions. They found that after prolonged treatment, about half of the gliomas in mice became resistant to the CSF-1R inhibitor and every tumor that recurred did so in the context of a scar. "We identified a prominent and quite complex resistance mechanism involving many different cell types within these treated lesions that ultimately led to the reemergence of glioma cell proliferation and invasion," says Joyce.

Prolonged treatment with the anti-CSF-1R drug in the context of recurrent disease prompted macrophages to adopt a wound healing response. That includes secreting growth factors such as insulin-like growth factor-1 (IGF-1), which they do in response to another factor (interleukin-4) produced by infiltrating T cells of the immune system. IGF-1, for its part, activates a signaling pathway in the cancer cells that drives their growth—a pathway mediated by a protein named PI-3 kinase (PI3K). The CSF-1R resistance, the researchers showed, could be overcome with drugs that block the receptor for IGF-1 or PI3K activity. Combining either with

Photo by Gilles Weber

CSF-1R blockade extended survival in a mouse model.

## Restorative interventions

Like many other cancers, gliomas are driven in large part by the unbridled activity of a diverse and ubiquitous clan of signaling enzymes known as tyrosine kinases. But drugs that inhibit various kinases have had little or no effect on gliomas. Joyce and her team noticed, however, that tyrosine kinase inhibitors were nonetheless very effective in killing glioma cells in culture. "Whenever you see something like that, it grabs your attention," says Joyce.

It suggested, for one thing, that the observed drug resistance might stem from the tumor microenvironment. In 2017, Joyce and her team reported in *Oncogene* that inhibiting CSF-1R with a drug could restore sensitivity to other tyrosine kinase inhibitors in mouse models. In this case, they showed, the reprogramming of TAMs by CSF-1R inhibition was directly involved in making gliomas susceptible to the cancer cell-targeted inhibitors.

"We used the knowledge we had of macrophages and of CSF-1R signaling and inhibition to overcome this microenvironment-mediated resistance to therapy—something we and others are finding is extremely important to the efficacy of multiple therapies in many different cancers," says Joyce.

Indeed, she and her group had already shown in 2011 that treating breast cancer with Taxol tends to boost TAM numbers, which drives resistance to chemotherapy. In 2017, her laboratory demonstrated in a *Cell Reports* paper that macrophages also secrete factors that interfere directly with Taxol's effects on cancer cells—which is to force an extended arrest during cell division that prompts their suicide. TAMs, Joyce and her colleagues found, shortened the duration of the mitotic arrest induced by Taxol. They also showed that inhibiting a signaling pathway involved

in this interference, mediated by a protein named MEK, could restore sensitivity to Taxol.

### Prep work

While at MSK, Joyce's team had held joint meetings with the laboratory of Andrew Dannenberg, a colleague at Weill Cornell Medical College in New York. Dannenberg and his team were interested in the link between obesity and different cancers, including breast cancer; Joyce and her team were particularly intrigued by the effects of obesity on systemic inflammation and potential connections to metastasis.

With a shared expertise in TAMs, the researchers looked at the effects of obesity on these cells first. But they quickly noticed that neutrophils—another type of immune cell—were more intimately linked to inflammation in the obese. "We found that in the normal lung, outside of the context of cancer, there was already a profound accumulation of neutrophils," says Joyce. This was evident in obese mice as well as in blood samples from obese women.

In 2017, Joyce and her colleagues reported in *Nature Cell Biology* that neutrophils accumulate in the lungs of obese mice and that the effect is exacerbated in the presence of a breast tumor. Neutrophils, it appeared, prepare a niche for colonizing breast cancer cells—which would explain why obese women with breast cancer have an increased risk of developing lung metastases and a typically worse prognosis.

The increased metastasis is dependent on the immune factors interleukin-5 and GM-CSF, and blocking those factors pharmacologically inhibited the effect in mice. Intriguingly, and potentially important from a public health perspective, they found neutrophil migration and the enhanced metastasis could also be reversed by weight loss—at least in mice.

Such microenvironmental discoveries will be incorporated into the ambitious cancer immunotherapy program now underway at Ludwig Lausanne. Joyce is already working with neuro-oncologists and surgeons at the Lausanne University Hospital (CHUV) to that end. "We have established some fantastic collaborations with CHUV to perform immune cell landscaping in every brain malignancy that is operated on in the hospital," she says, "and in parallel we are preparing to use our preclinical models to try to develop novel immune therapies within the brain."

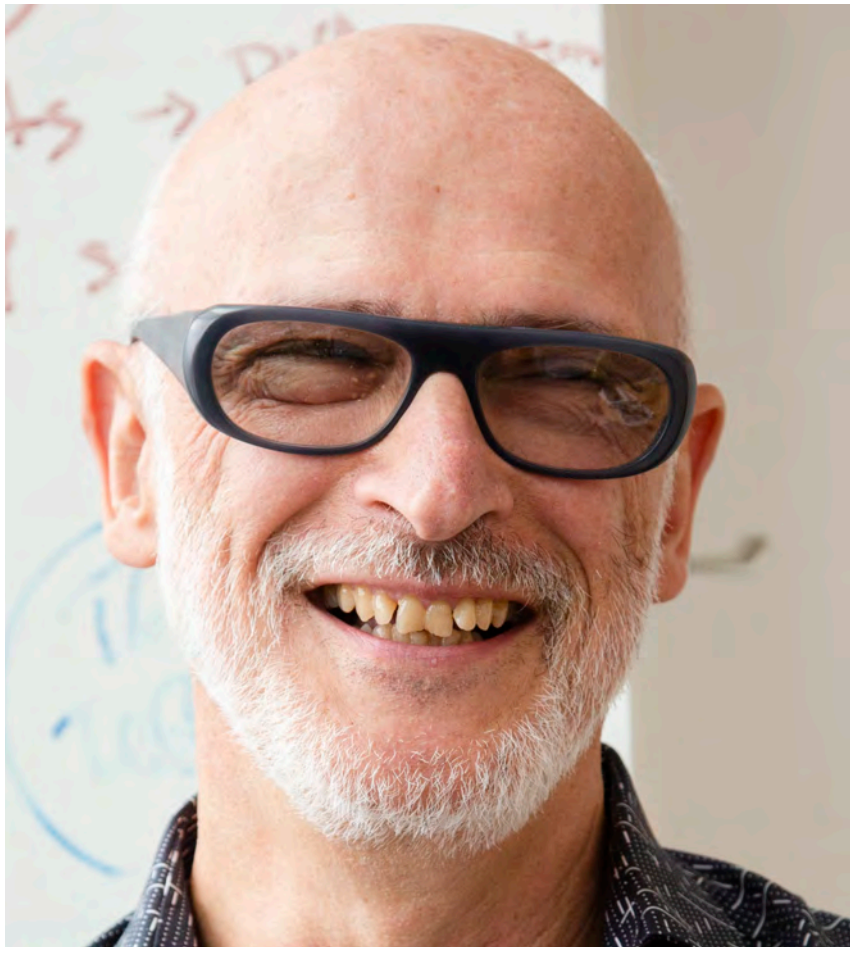
"We have established some fantastic collaborations with CHUV to perform immune cell landscaping in every brain malignancy that is operated on in the hospital."

Meanwhile, Joyce's breakthroughs have generated intriguing opportunities—including in a team that won a £20 million Cancer Research UK Grand Challenge award in 2017. Led by Greg Hannon of Cambridge, the international team will construct an immersive, 3D version of breast tumors that can be studied through virtual reality. "This Grand Challenge project is completely unbiased in terms of the cells we're looking at," says Joyce. "We want to explore all of it, to tackle that complexity head on."

Joyce, in other words, plans to keep doing what she's been doing all along.

ALEXANDER

# RUDENSKY



LUDWIG

MSK

Photo by Flynn Larsen

# THE
# TREG MASTER

His decades-long study of the regulatory T cell continues to yield surprises, exposing new ways in which the suppressive immune cells function and how they inhibit and fuel malignancy. His discoveries illuminate powerful new approaches to cancer prevention and therapy.

The year was 1989, the Soviet Union was on the verge of collapse and Alexander Rudensky was in the kitchen of his Moscow apartment, dialing the legendary immunologist Charles Janeway.

Worried that a possible backlash to Mikhail Gorbachev's *perestroika* might plunge Russia into brutal totalitarianism once again, Rudensky and his wife were hoping to spend a couple of years abroad with their children until things settled down. In that time Rudensky planned to gain valuable experience working in a Western laboratory. With that in mind, he had shot off a letter to Janeway—whose publications he greatly admired—asking if the immunologist would consider hiring him as a postdoc. He fully expected to be ignored. But Janeway, who took some pride in the international flavor of his lab, did respond. And now Rudensky was in for another surprise. "The first thing he said was, 'When do you want to come?'" recalls Rudensky.

Janeway's intuitive brilliance, it appears, extended to spotting scientific talent. The Russian immunologist who walked into his lab three months later would go on to help lay the foundations of an invaluable subfield of immunology dedicated to T regulatory cells—a lineage of the immune system's T cells essential to suppressing immune responses and preventing deadly autoimmunity. Over the years, Rudensky and his colleagues have methodically unraveled the biology of these cells, showing how the lineage is formed and maintained, how the cells function, and how their ability to dampen inflammation can contribute to human disease, including both the containment and progression of cancers.

In 2017, Rudensky and his team at the Ludwig Center at Memorial Sloan Kettering Cancer Center (MSK) added another dimension to T regulatory cell (Treg) biology, reporting in the *Journal of Experimental Medicine* how a

functionally distinct role of Tregs in tissues drives the progression of lung tumors in mice. In another paper, published in *Nature*, Rudensky and his team reported results from their analysis of a distinct subtype of Tregs. Their findings illustrate a significant complexity in Treg biology that is essential to fine-tuning the cells' containment of inflammation.

## Getting qualified

Rudensky was raised in a relatively scholarly atmosphere in an apartment in the heart of Moscow. His mother had earned a degree in law at the height of the Doctor's Plot—Stalin's last anti-Semitic campaign—but was effectively barred from its practice. She re-enrolled in university and went on to teach Russian language and literature. His father, a former gyroscope engineer with the Soviet missile and space programs, was a bureaucrat in the Academy of Sciences who edited books on spaceflight and rocket science on the side. The Rudensky household was thus host to a stream of physicists and engineers, who would drop by the apartment to work on their manuscripts.

In school, Rudensky fell in love with chemistry, concocting explosives and other chemical mischief at home. Soon he was taking night classes in chemistry at Moscow State University, fascinated by organic synthesis and, later, biochemistry—which became his major at the Second Moscow State Medical School. While working toward a master's degree in the subject, he took a summer job in an immunology lab and was soon working nights and weekends with an immunochemistry group at the Academy of Medical Sciences in Moscow.

Rudensky wrote his master's thesis on his work there mapping a bacterial protein's interactions with antibodies and the lab director, Alexander Kulberg, asked him to stay on as a graduate student. The academy, however, rejected him. "I was told there were some 'administrative issues,'" says Rudensky. "It was in part—maybe significantly—because I am Jewish."

That, oddly enough, turned out to be a stroke of luck: A colleague at the lab introduced Rudensky to his brother, Vitalij Yurin, at the Institute for Genetics of Microorganisms, and Rudensky joined the lab in 1979. "I was really fortunate that I joined the institute," says Rudensky. "Vitalij Yurin was a leader in molecular immunology in the Soviet Union."

In Yurin's lab, Rudensky focused on how antigens are processed for recognition by T cells, a step known as antigen presentation that is critical to the elicitation of T cell responses. It was inspiring but challenging work. "We would use beakers and candles to create the concentrations of carbon dioxide we needed to culture our cells," Rudensky recalls. "It may seem somewhat heroic. Our publications took time, but they were well received, and on par with work being done in more advanced laboratories in Europe and elsewhere."

To get his doctorate in 1986 from the Immunology PhD Council at the Gabrichevsky Institute of Epidemiology and Microbiology, Rudensky used an alternative path available to researchers. It involved compiling his research, defending his thesis before a group of scientists and passing a few exams—not just on biomedical subjects but foreign languages and Marxist philosophy as well. Doctorate in hand, he stayed on as a senior researcher in Yurin's group for another four years, racking up publications in the *European Journal of Immunology* before joining Janeway's lab at Yale University.

## Treg mining

Rudensky continued working on antigen presentation at Yale, focusing on the recognition of self-antigens by T cells and publishing papers and reviews in *Nature* and other leading journals. On the strength of this work, and with Janeway's active support, Rudensky was recruited in 1992 to

Photo by Flynn Larsen

be an assistant professor at the University of Washington, in Seattle, where he continued that research.

In 1995, a Japanese researcher named Shimon Sakaguchi, after a decade of persistent investigation, published a landmark study describing a class of cells that were essential to suppressing autoimmune reactions. "Sakaguchi had worked on this problem even though a number of people in immunology did not regard it with much respect," says Rudensky. "Don Mason's group in the UK also contributed immensely. This early work culminated in the discovery of the cell-surface marker CD25 as a defining feature of a subset of T cells enriched for suppressor activity."

Rudensky started a program to explore the biology of these cells, which would eventually come to be known as regulatory T cells. He and a postdoc, Marc Gavin, quickly found that the cell's function was not entirely defined by the expression of CD25, which is a receptor for the immune factor interleukin-2. Rudensky, Gavin and graduate student Jason Fontenot then started looking for a more categorical genetic determinant of Treg identity and reported in a landmark publication in *Nature Immunology* in 2003 that the transcription factor FoxP3 fit that bill. This discovery made the precise identification of these cells easier, fueling an explosion of research into Tregs.

Rudensky's lab has since been a mine for pretty much everything Treg. He and his colleagues established that FoxP3 is not only required for the establishment of the Treg lineage during development but also essential to their function throughout life. They demonstrated that FoxP3 loss in mice causes severe autoimmunity, and established that human diseases linked to a deficiency of the transcription factor are also associated with a paucity of Tregs. They discovered the signals that regulate the activation of FoxP3 and detailed the many mechanisms by which Tregs suppress immune responses. In this bonanza of discovery, Rudensky's lab also generated numerous mouse models that are used around the world today by researchers studying everything from cancer biology to autoimmune disease.

## When good Tregs go bad

It was only after moving to New York in

"The important message of this study is that most effector and regulatory T cells in the tumor can have effects beyond the ones people expect."

2008—he was appointed director of Ludwig MSK four years later—that Rudensky began experimentally probing Tregs in cancer. "I think it was because of the environment here that we became interested in their role in tumors," he says. "We were particularly interested in looking at the role of T cells that would not be amenable to checkpoint blockade." That made sense: Both MSK and the Ludwig Center had played outsize roles in the development of checkpoint blockade and other immunotherapies.

With the arrival of postdoctoral fellow Paula Bos, the group began developing mouse models to examine Tregs in tumors. They found that the depletion of Tregs in mice significantly delayed the progression of breast tumors. But this, they reported in the *Journal of Experimental Medicine* in 2013, wasn't due to their suppression of killer T cells, which attack cancer cells and are often suppressed by Tregs. Rather, the anti-tumor effects of Treg depletion appeared to be dependent on helper T cells, which orchestrate inflammatory immune responses, and the production of an immune signaling factor called interferon gamma. Further, the effects could be magnified by subsequent radiotherapy, which reduced tumor burden and extended the lives of the mice.

Rudensky's team also examined some 100 breast tumors and blood samples from patients, looking for markers to distinguish Tregs that infiltrate tumors from others of their ilk. The effort, spearheaded by MSK surgeon and postdoctoral fellow George Plitas and reported in a paper published in 2016 in *Immunity*, found several—most notably a cell-surface receptor involved in immune cell migration named CCR8. "This has led to efforts in our lab to generate therapeutic antibodies for the more selective depletion of regulatory T cells in human tumors," says Rudensky.

His studies have also shown that Tregs have a complex and long-term influence on cancer initiation and progression. He and his colleagues reported in *Nature Immunology* in 2016, for example, that Tregs play a dual role in gastrointestinal cancers that are fueled by inflammation—initially inhibiting their progression but then fueling it after the tumors turn malignant. The lab has also shown that gut bacteria produce a metabolite, butyrate, from certain dietary fibers that boosts the generation of Tregs. This suggests that diets rich in those fibers might suppress the inflammation associated with many GI cancers. Both these findings are of relevance to Rudensky's participation in a program for cancer prevention launched by Ludwig and the Conrad N. Hilton Foundation.

## Hazards of healing

Rudensky's ongoing characterization of the multifaceted Treg has yielded other surprising discoveries about its role in cancer. In 2015, he and his colleagues reported in *Cell* that Tregs residing in the lungs appear to play an important part in the repair of tissue damaged by viral infection. This function, they demonstrated, is mediated by a protein named amphiregulin and is unrelated to the Treg's immunosuppressive duties.

"We thought that such functions of regulatory T cells, and perhaps other T cells, might be

found not only in tissue injury but also in situations where tissue function is altered," says Rudensky. "Cancer was one such example."

In 2017, Rudensky and his colleagues reported in the *Journal of Experimental Medicine* that this is indeed the case. Amphiregulin production by Tregs and other types of T cells that flood into tumors, they found, contributes significantly to the progression of lung cancer in mice. Neither the loss of amphiregulin across T cell types nor its selective depletion in Tregs has any effect on their immune functions. But its loss does significantly retard the growth of lung tumors transplanted into mice. Amphiregulin produced by T cells, they found, most likely acts on other normal cells present within the tumors' microenvironment—including noncancerous epithelial cells and other immune cells, like macrophages and neutrophils—to promote tumor growth.

"The important message of this study is that most effector and regulatory T cells in the tumor can have effects beyond the ones people expect," says Rudensky.

## A flavorful symmetry

In another 2017 publication, Rudensky and his team took on a lingering puzzle of Treg biology.

"We and others have observed that regulatory T cells, which express FoxP3, can also paradoxically express transcription factors associated with pro-inflammatory, effector immune responses," says Rudensky. One such factor, T-bet, is known to enhance the activity of helper T cells, which orchestrate the T cell attack. Whether this expression is transient or lasting and essential to Treg function was an open question.

In their *Nature* paper, Rudensky and his colleagues reported that T-bet expression supports a late-stage specialization of Tregs. Eliminating T-bet-expressing Treg cells, they

Photo by Flynn Larsen

showed, resulted in severe autoimmunity in mice that was driven by a T-bet-expressing subtype of helper T cells (TH-1) and the killer T cells they activate.

When Treg cells that do not express T-bet were selectively depleted, the T-bet expressing Treg cells that remained specifically inhibited TH-1 cells and killer T cells—but not another subtype of helper T cells that stimulates antibody responses. The T-bet expressing Tregs were also found in the company of T-bet-expressing target cells. That is, they appear to be specialists and to become so by expressing T-bet in the latest stages of their development.

"To generalize this finding would be to say that there are different flavors of Tregs that each specifically controls different types of inflammatory responses," says Rudensky. In this way, he and his colleagues propose, Tregs divide their labor, specializing in silencing distinct aspects of the immune response without compromising others.

"We are looking currently at whether T-bet-expressing regulatory T cells can be found in cancer, and then we can see what they do there," says Rudensky.

Few people are in a better position to find out—or to put the answer to good use.

BENOÎT

# VAN DEN EYNDE

LUDWIG

BRUSSELS

Photo by Flynn Larsen

# THE TUMOR DEFENSE DISMANTLER

He began his career helping to lay the scientific groundwork for modern immunotherapy. Now he's unraveling the myriad ways tumors thwart immune attack—and showing how to undo those defenses.

In science, as in many other things, it's the surprises that tend to stick with you—and sometimes in more ways than one.

Benoît Van den Eynde got a big one nearly three decades ago, while working with Thierry Boon, the founding director of the Brussels Branch of the Ludwig Institute for Cancer Research. Boon had previously shown in a series of milestone studies in the late 70s and early 80s that the mammalian immune system can detect and clear cancer, a possibility most scientists doubted at the time. By the mid-80s, his team was racing to find in mice and humans the first example of a naturally occurring cancer antigen, a molecular flag that marks diseased cells for targeting by T cells of the immune system. Van den Eynde was working on the mice.

Based on their previous studies on tumors with chemically induced mutations, the researchers expected the antigen would be a randomly mutated version of a normal gene—a neoantigen—which would appear foreign to T cells, provoking attack. "To our surprise, the antigen turned out to be identical to the normal gene," recalls Ludwig Member Van den Eynde. "We called it P1A and realized quite quickly that the gene is expressed in the tumor but mostly silent in normal tissues." Reported in 1991, it was the first of what would come to be called the "MAGE-type" or "cancer testis" antigens, which are expressed in human cancers as well and would become central to several immunotherapy strategies.

P1A, for its part, stuck around as a useful tool. Roughly a decade and a half later, Van den Eynde used it to construct a mouse model for an inducible cancer that provides a venue for a more realistic assessment of immunotherapies. In 2017,

"People are trying to confirm those findings but, if correct, spliced peptides will have to be taken into account in vaccine design and across immunology."

he and his colleagues reported in *Nature Communications* how they used that model to elucidate a novel mechanism of immune resistance in tumors. In another study published in *Cancer Immunology Research* in 2017, Van den Eynde and his team probed a separate mechanism of malignant immunosuppression and showed that it might be overcome with the use of an anti-inflammatory drug already on the market.

## Becoming a scientist

When Benoît Van den Eynde was in high school near Brussels, his grandparents bought him a subscription to a science magazine. The gift opened his mind to scientific discovery. "I thought, 'This is a cool job to do,'" he recalls.

The thought stuck with him and, at 18, in his second year of medical school at Université catholique de Louvain in Brussels, he asked a biochemist if he could join his laboratory as a student researcher. After graduating with honors with his medical degree, Van den Eynde qualified for a five-year program in internal medicine. But, still feeling the tug of science, he exercised an option to claim a year of credit in his clinical training for two spent on research and joined Boon's newly opened Ludwig Branch in 1985.

Based on its studies of mice, Boon's team was by the mid-'80s creating what amounted to personalized cancer vaccines for a small group of melanoma patients. The vaccines worked quite well, even curing a German patient's widely metastasized cancer—a landmark, if rarely repeated, event in the history of cancer immunotherapy. Van den Eynde, for his part, joined an effort to identify the melanoma antigens and asked his medical school administrators for another two years to continue his research. Once again, his request was granted.

In 1989, Van den Eynde published a paper in the *International Journal of Cancer* showing that the German patient's T cells appeared to target at least six naturally occurring antigens on her melanoma cells. Thrilled, Van den Eynde dropped his medical studies and, leading a small group by 1994, set about discovering antigens in melanoma and other human cancers. He received his PhD in 1995.

Over the next few years, Boon's team raced to translate its discoveries—particularly the MAGE cancer antigens—into cancer vaccines for more general use. Van den Eynde's research, however, would take him down a scientific path more fundamental in nature yet just as relevant to cancer immunotherapy.

## Incisive science

Sick cells alert the immune system to their condition by chopping up abnormal proteins associated with their pathology and presenting the fragments, or peptides, to T cells. The chopping is done by an enzymatic machine known as the proteasome, the presenting by a family of proteins called MHC (HLA in humans and H-2 in mice). In 2000, Van den Eynde's group published a paper in *Immunity* describing a cancer antigen derived from a protein that was expressed in all cell types; the antigen seemed normal in every

Photo by Flynn Larsen

way, yet it elicited a T cell attack only on cancer cells, not healthy ones.

"There was a paradox there," says Van den Eynde, "and it was in trying to understand that paradox that I became interested in antigen processing."

Van den Eynde's subsequent exploration of the anomaly—which continues today—was rich with discovery. He and his colleagues reported in 2004 in *Science* an entirely novel type of antigen processing, in which peptides are spliced and then shuffled so that their amino acid sequence no longer resembles any part of the original protein. A recent independent study suggested as many as a third of the peptides presented to T cells could be of that variety. "People are trying to confirm those findings but, if correct, spliced peptides will have to be taken into account in vaccine design and across immunology," says Van den Eynde.

His team also discovered that cancer cells tend to deploy a standard proteasome, while normal antigen-presenting cells express what is known today as the immunoproteasome—which is built from a different mix of enzymatic subunits that generate distinctly different peptides for presentation. "If you want to trigger an immune response that is meaningful in cancer patients," explains Van den Eynde, "it would be better to trigger T cells activated by peptides produced by the standard proteasome."

## La resistance

While exploring cancer antigens, Van den Eynde also became increasingly interested in the mechanisms by which tumors evade immune attack. In 1998, he came across a

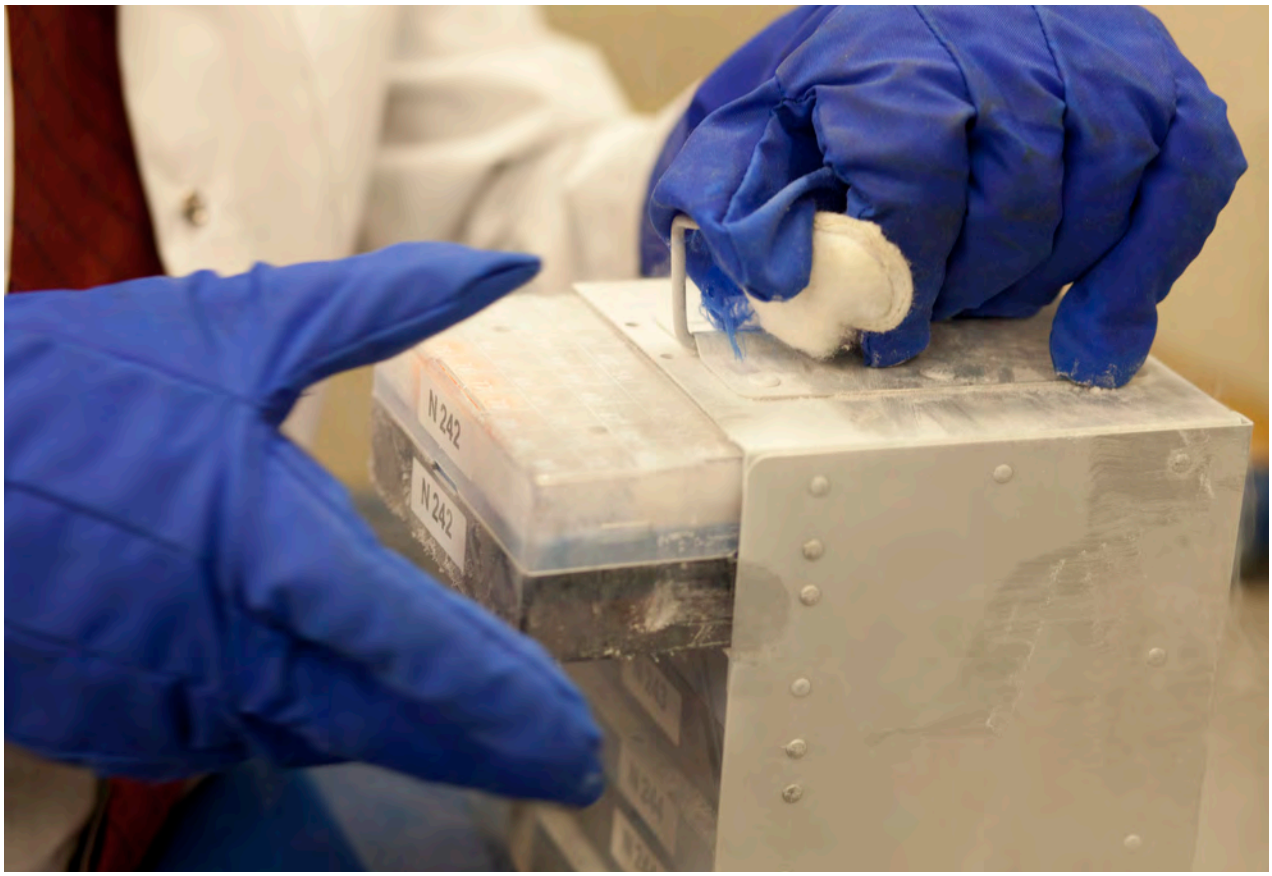



Photo by Flynn Larsen

paper showing that cells in the mammalian placenta help prevent T cell attack of the embryo by harnessing an enzyme known as indoleamine 2,3-deoxygenase-1 (IDO-1), which deprives killer T cells of a vital nutrient—the amino acid tryptophan. Van den Eynde and his colleagues reported in *Nature Medicine* in 2003 that tumors do the same. This sparked an industrywide race to develop IDO inhibitors as cancer therapies. Van den Eynde himself launched, with Ludwig's support, a spinoff named iTeos—a story covered in the 2014 Ludwig Research Highlights report.

Unfortunately, the 2018 failure in Phase III trials of an IDO-1 inhibitor prompted developers to pull back from the therapeutic class. But Van den Eynde remains optimistic that IDO inhibition still holds promise. A better selection of tumors for IDO inhibition, he believes, could improve efficacy in trials. It might, for example, work better in tumors that continuously express IDO and lack killer T cells almost entirely, rather than those in which IDO expression is induced by stimuli such as immunotherapy.

Tumors of the former category were, in fact, a focus of the study Van den Eynde and his colleagues published in *Cancer Immunology Research* in 2017. Van den Eynde and his colleagues suspected steady IDO expression might account for the immunologic chill of such "cold tumors" and set about probing why it occurs. Their study revealed that the steady expression of IDO depends on COX-2—an enzyme involved in inflammation—and its primary product, a long fat molecule named prostaglandin E2 (PGE2).

PGE2, they showed, is produced by those tumors and activates a signaling cascade within cells that triggers IDO1 expression. Van den Eynde and his team showed in an immunologically reconstituted mouse model of human ovarian cancer that blocking COX2

with a drug named celecoxib effectively shut down the constitutive expression of IDO-1 and led to tumor rejection.

"Celecoxib is already on the market, so you don't need to do a drug development program before you test it in patients," says Van den Eynde. Indeed, he is already in discussions with oncologists at the University Hospital Saint-Luc in Brussels about running a small clinical trial combining celecoxib and checkpoint blockade as a cancer therapy.

Photo by Flynn Larsen

"I thought, 'This is a cool job to do.'"

## Countering countersurveillance

Around the time Van den Eynde began mulling IDO in the late 90s, he was also thinking about how to develop a tumor model that might more faithfully recapitulate immune suppression in tumors. Mouse models available in the 90s were made by injecting cancer cells into mice to seed fast-growing tumors. But as Boon's team had formally shown, tumors in patients evolve gradually against a patrolling immune system. The transplanted models don't quite recapitulate that process.

In 1998, Van den Eynde began working with colleagues in Marseille, France, to construct a model that would. By the middle of the last decade, he and his colleagues had engineered a mouse in which melanoma could be induced with the administration of a breast cancer drug and whose tumors expressed P1A. Next, the researchers engineered a nearly identical mouse to make T cells targeting P1A. "This was a cool tool because we could now isolate large numbers of T cells that recognize the P1A antigen and inject them into a mouse with an induced tumor that expresses that antigen," says Van den Eynde.

As they reported in *Nature Communications* in 2017, the induced tumors were resistant to a battery of immunotherapies, including anti-P1A vaccines and even adoptive T cell therapy (ACT) that involved injecting 10 million P1A-targeting T cells into the mice. "Honestly, I was expecting that in this case the T cells would be able to reject the tumor," says Van den Eynde. "But they had no effect at all."

When the same P1A-expressing cancer cells were transplanted into mice, however, they were cleared by ACT. Comparing the noncancerous cells present in both types of tumors revealed that one type of cell, the polymorphonuclear myeloid-derived suppressor cell (PMN-MDSC), was present exclusively in the induced tumors. These cells, it seemed, were engaging a previously unknown system of immune suppression to thwart the T cell attack.

Van den Eynde and his team showed that the PMN-MDSCs express high levels of a surface protein known as Fas-ligand, which induces T cell suicide when it binds its receptor on the T cells. Blocking this interaction restored the ability of the T cells to kill the induced tumors.

"We didn't have a full rejection of the tumor, but we did get a reduction in T cell suicide and better control of the tumor," Van den Eynde explains. This is, in his view, a good sign, as it suggests the tumors are engaging other methods of immune suppression as well, all waiting to be discovered and undone. "I think this mouse model will give us many more important findings on tumor immunosuppression," he says.

In Van den Eynde's hands, it probably will.

MARCIA

# HAIGIS

HARVARD

LUDWIG

Photo by Flynn Larsen

# THE MITOCHONDRIAL NETWORKER

She has shown how biochemical discord in the powerhouse of the cell can shape the aberrant metabolism of cancer cells. Disrupting the relevant metabolic circuitry could help treat a variety of malignancies.

Some good deeds, it appears, do go unpunished. Consider Ludwig Harvard's Marcia Haigis. As a freshman at the University of New Hampshire in the early 90s, the young Haigis got certified as an emergency medical technician, working the night shift to rack up the volunteer hours required to retain membership in the ambulance corps. It was after one of those shifts, before 7 a.m., as she took a shortcut to her dormitory through the biology research building, that Haigis discovered her calling. Fascinated by the posters in the hall, she wandered through the only office door open at the time and asked the professor in there, biochemist Rick Cote, if he had a few minutes to talk. A few hours later, she emerged, bleary-eyed but inspired—and with a summer job offer in hand. "That," she recalls, "is how I got hooked on research."

Probing the structural intricacies of an enzyme in Cote's lab that summer awakened in Haigis a fascination with fundamental protein chemistry—not only with its intrinsic beauty but also its potential for answering larger questions about human health and disease. Today, her laboratory at Harvard Medical School explores the biochemical maze of the mitochondrion, the bean-like organelle best known as the cell's power station. Over the past dozen years, she and her colleagues have methodically exposed how the interplay of enzymatic networks within the mitochondrion transmits signals that modulate the cell's metabolism at large, exerting a systemic influence on everything from obesity to immunity to aging and cancer.

In 2017, Haigis and her colleagues published a paper in *Science* revealing that a waste product of metabolism lethally toxic to ordinary tissues—ammonia—looks like good grub to breast tumors. Tracing the fate of the

Photo by Flynn Larsen

toxin in cancer cells, her team revealed how the cells recycle the toxin to fuel unfettered growth. She and her team also showed that targeting that process could open a new approach to treating breast cancer.

## Finding a calling

Haigis was born in Las Vegas and then moved with her family to South Korea as an infant, where her father, an officer in the US Air Force, had been stationed when he met her mother. After the family returned to the US, they hopscotched between states from Nebraska to Alabama, and ultimately New Hampshire, where they settled in Portsmouth. Haigis and her two younger siblings spent most of their childhood there.

After majoring in biochemistry in college, Haigis joined the graduate program at the University of Wisconsin–Madison. Her doctoral research under the guidance of the chemical biologist Ronald Raines explored how the molecular geometry of an enzyme that slices up RNA molecules contributes to the enzyme's function. "It was a lab where you learned the fundamentals about protein folding," says Haigis. "With this background, I was eager to work in a field where enzymology and biochemistry had center stage but the driving questions would be directly related to biology."

Haigis found a perfect fit in the study of sirtuins—a family of enzymes that chemically

modify other enzymes in distinct ways to alter their activities. One member of the family, named SIRT1 in mammals, had come about as close as any enzyme gets to pop culture celebrity. Giving yeast, fruit flies and roundworms an extra copy of their respective versions of the SIRT1 gene significantly extended their lives. Other sirtuins, however—mammals have seven in all—languished in obscurity.

Joining Leonard Guarente's lab at the Massachusetts Institute of Technology, Haigis turned her attention to the neglected sirtuins that reside in the mitochondrion (SIRT3, 4 and 5). Her work provided among the first bits of evidence that the mitochondrial sirtuins play a significant role in controlling metabolic processes outside the organelle, a finding that upended prevailing dogma. SIRT4, she showed, represses an enzyme essential to amino acid metabolism called glutamate dehydrogenase. This has the effect of suppressing insulin secretion by pancreatic islet cells in mice.

## Touring cancer metabolism

In 2006, Haigis joined Harvard Medical School, focusing her laboratory on how mitochondrial processes, initially involving sirtuins, participate in aging and cellular adaptations to stress. A graduate student in her lab, Lydia Finley, noticed that the loss of SIRT3 activity ultimately boosted the expression of genes essential to glycolysis. This is a metabolic pathway active in the cytoplasm through which the sugar glucose is broken down to generate energy. It also furnishes molecular building blocks essential to cell proliferation.

While healthy cells only resort to glycolysis when there's a shortage of oxygen, cancer cells have long been known to keep it going even when oxygen is abundant—a phenomenon known as the Warburg effect, a hallmark of cancer metabolism. Haigis, Finley and their colleagues found that the loss of the SIRT3 gene induced gene expression patterns and metabolic activity that mirrored the Warburg effect.

> "I was eager to work in a field where enzymology and biochemistry had center stage but the driving questions would be directly related to biology."

Examining a variety of tumor cells for their SIRT3 status, the researchers discovered that the SIRT3 gene had been deleted in most. Their study, published in *Cancer Cell* in 2011, revealed how the SIRT3 enzyme counters the metabolic reprogramming that drives cancer cell proliferation and survival. Other researchers subsequently reported that mice lacking the SIRT3 gene spontaneously develop breast tumors.

"Our entry into cancer research was the observation that these mitochondrial sirtuins have profound effects on cellular metabolism," says Haigis. "A lot of the metabolic pathways they regulate are central to tumor cell growth."

With that in mind, Haigis and her colleagues began exploring when the mitochondrial sirtuin genes are switched on in cells. They noticed that damage to DNA, which can cause mutations that drive cancer, activated SIRT4. It did so, they reported in a 2013 *Cancer Cell* paper, through its suppression

"If we identify and understand new metabolic vulnerabilities that are unique to each cell type, we may be able to tailor a metabolic cocktail or precisely target those pathways."

of glutamate dehydrogenase and the metabolism of an amino acid named glutamine. That in turn had the effect of arresting cell division.

"SIRT4 seems to dampen mitochondrial metabolism and help cells deal with stress," says Haigis. "It induces a metabolic pause, or what we call a metabolic checkpoint, and gives cells time to repair the damage." Illustrating the importance of that checkpoint, Haigis' team showed that mice engineered to lack the SIRT4 gene developed spontaneous lung tumors within 15 months.

In another study, Haigis' lab took a closer look at what was once a poorly understood enzyme named PHD3, a close relative of a pair of enzymes through which SIRT3 suppresses the Warburg effect. The group's findings, reported in *Molecular Cell* in 2016, revealed that PHD3 silences an enzyme involved in the breakdown of fats inside the mitochondrion for energy, an option normal cells only take when they're stressed out by low nutrient supplies.

Haigis and her colleagues also found that expression of the PHD3 gene is severely suppressed in a subset of cancers that include acute myeloid leukemia. "Certain tumors do not rely on the Warburg effect and are not glycolytic, but they do have an addiction to fat oxidation, or burning fat, and they need it to survive," says Haigis. "We speculated you can target those tumors with inhibitors of fat oxidation."

## A toxic treat

Given how often the amino acids glutamate and glutamine pop up in cancer metabolism, Haigis' lab wanted to know what happened to a toxic byproduct of their breakdown: ammonia.

Ordinarily, the body quickly clears ammonia and sends it to the liver, where it is processed and excreted as urine. But

Photo by Flynn Larsen

cancer cells metabolize nutrients furiously as they grow, so ammonia tends to accumulate in tumors. Graduate student Jessica Spinelli observed that breast cancer cells even seem to thrive when it is added to their cultures. This suggested the cells were using it for something. What exactly was less clear.

To find out, Haigis, Spinelli and their colleagues tried first to figure out whether the ammonia was going down certain metabolic pathways that make molecules rich in nitrogen, like the constituents of DNA. After several months of negative results, they decided to scan all the nitrogenous metabolites in the cell at once—more than 200 in all— before finally calling it quits. Adapting an obscure chemical reaction concocted by the 18th century chemist Pierre Berthelot and a procedure for the quantitative analysis of ammonia metabolism—both reported in a 2017 *Scientific Reports* paper—the team stuck an isotopic label on ammonia and fed it to the cancer cells.

As Haigis and her colleagues reported in *Science* in 2017, ammonia was being used by breast cancer cells to generate amino acids, most often glutamate and amino acids generated downstream from glutamate. What's more, the breast cancer cells don't just thrive on the ammonia, they're almost addicted to it: Blocking glutamate dehydrogenase activity retarded breast tumor growth in mice.

The team is now looking at whether ammonia has the same effect in other types of tumors, especially those of the liver, where it is abundant. They are also examining how the high levels of ammonia affect other cells in the environment of the tumor.

"If we identify and understand new metabolic vulnerabilities that are unique to each cell type, we may be able to tailor a metabolic cocktail or precisely target those pathways," says Haigis. The task, she admits, will not be easy, since tumors vary so much. "Identifying the metabolic fingerprint of a tumor before starting a therapy is a major challenge in cancer biology and treatment."

On the plus side, Haigis is on the case.

FRANK

# FURNARI

LUDWIG

SAN DIEGO

Photo by Stewart Marcano

# THE BRAIN TUMOR DECIPHERER

His sustained exploration of the signaling networks, communications and genetic idiosyncrasies of brain cancer cells is yielding valuable clues to new therapies.

Inspiration loves a change of scenery. This may be why it visited Frank Furnari once, roughly a decade ago, during an afternoon stroll at the gardens in San Diego's Balboa Park.

Working out of Web Cavenee's lab at Ludwig San Diego, Furnari had been picking apart how the mutant receptor EGFRvIII drives the brain cancer glioblastoma multiforme (GBM). Though a more potent engine of cell proliferation than its unmutated "wild-type" counterpart, EGFRvIII is typically found only on a minority of cells in any given GBM tumor. This is puzzling because rapidly growing EGFRvIII cells should take over the whole tumor. Eyeing the lush vegetation in the garden, Furnari found himself pondering ecological interactions. "I'm looking at these trees and thinking, 'The tree is helping the orchid grow, and there's no damage to the tree, even though the orchid is thriving, embedded in the tree,'" he recalls. "Then I got to thinking how if you mix crops, you get a much higher yield in your harvest. I wondered, 'Could something like this be happening in the tumors?' "

He was onto something. Furnari and his colleagues reported in *Genes & Development* in 2010 that signals from EGFRvIII prompt cells to secrete a factor named interleukin-6 (IL-6), which fuels the proliferation of both cell types, keeping their proportions within the tumor steady. But do cells expressing EGFRvIII also protect their wild-type cousins from therapy—much as plant diversity shields crops from weeds and pests?

In 2017, Furnari and his colleagues reported in *Genes & Development* that the answer, once again, is yes and that the process works by driving the expression of a protein that prevents the suicide of GBM cells deprived of the receptor's signals. The team's findings have opened a novel strategy for treating

"I got to thinking how if you mix crops, you get a much higher yield in your harvest. I wondered, 'Could something like this be happening in the tumors?' "

GBM, a currently incurable cancer that typically causes death within 14 months of diagnosis. In another study published in 2017, Furnari and his colleagues showed how GBM cells lacking the tumor suppressor PTEN can, paradoxically, be killed by disrupting the activity of a second tumor suppressor named DAXX. This effect, known as synthetic lethality, illustrates a novel and actionable approach to devising new drugs for GBM therapy.

## A work ethic

Furnari grew up in Queens, New York, in an apartment above his father's butcher shop. "He would go down to the docks in the wee hours and bring back these huge sides of beef," Furnari recalls. "It was a hard life."

It got much harder, and for the whole family, when Furnari was eight years old, after his father was so disabled by a heart attack that he could no longer work. Furnari's mother, until then a homemaker, found a fulltime job as a bookkeeper to keep the family afloat. "The dynamics of our household completely changed," says Furnari. "Mom became the breadwinner. From our house in Queens, she would take buses and trains into Manhattan every day, come back in the evening and then do all the things that women did at home at the time."

The parental work ethic rubbed off on Furnari, who ran a newspaper route starting at 12 and held various jobs throughout high school. At Hofstra University, he majored in biology, minored in biochemistry and worked six to seven hours a night as a technician in a toxicology lab. After obtaining his bachelor's degree in 1985, he worked for two years as a technician in the laboratory of John Mendelsohn, who was then the chairman of the Department of Medicine at Memorial Sloan Kettering Cancer Center, in New York.

It was here that Furnari first encountered the EGF receptor while working on an antibody that targeted the protein as a potential cancer therapy. Furnari developed a fascination with cancer research that led him to begin graduate school at the University of North Carolina at Chapel Hill, where Joseph Pagano—an expert on DNA tumor viruses—was his adviser.

Furnari worked on the Epstein-Barr virus—which causes mononucleosis and is linked to nasopharyngeal carcinoma and Burkitt's lymphoma—studying how its cancer-promoting genes resemble human oncogenes. "That was the beginning of doing what I wanted to do," he says. "I wanted to study human diseases. In particular, cancer."

As his graduate work wound to a close in 1993, Furnari became interested in tumor suppressor genes, then a red-hot field. A faculty member got him in touch with Cavenee, who was opening a new Ludwig Branch at the University of California, San Diego (UCSD). Cavenee had started a GBM research program in his lab, which Furnari


Photo by Stewart Marcano

joined, focusing on the EGF receptor and, later, PTEN. His research was the first to demonstrate that restoring PTEN function in GBM tumors in which the gene was mutated suppressed their growth.

## Tapping the crosstalk

Over the next 15 years, Furnari worked as section head in Cavenee's lab, becoming a tenured professor in the Department of Pathology at UCSD in 2011 and a Member of the Ludwig Institute in 2016. His research over those years continued to explore EGF receptor signaling and PTEN function and dysfunction in glioblastoma.

After the GBM genome was sequenced in 2008, it was clear that extreme genetic diversity is something of a hallmark of the tumors. This diversity is reflected in the counterintuitive distribution of EGFRvIII-expressing cells in GBM tumors that had so puzzled Furnari and led to his moment at the botanical garden.

In the 2017 *Genes & Development* paper Furnari and his colleagues reported that the IL-6 secreted in response to EGFRvIII signaling results in the activation of a nuclear factor. This factor, in concert with a protein named BRD4, boosts the expression of survivin, which saves cancer cells from death by EGF receptor inhibition. Silencing survivin expression with an experimental inhibitor of BRD4 restored sensitivity to EGF receptor inhibitors in both EGFRvIII and wild-type cells and extended the survival of mice bearing GBM tumors.

"Perhaps we can leverage the GBM tumor's heterogeneity for therapy if we can understand how interactions between genetically diverse tumor cells lead to the use of common signaling pathways that are important to survival," says Furnari. "That's the next phaseof this project."

## An induced vulnerability

The study published in *Nature Communications* in 2017 stemmed from the observation that about 40% of GBM tumors sport deletions of

Photo by Stewart Marcano

PTEN, which would make them resistant to EGF receptor inhibitors. This is because PTEN inactivates a pathway involved in the EGF receptor's signaling cascade—the PI3 kinase pathway—that would, in its absence, be constantly active.

Furnari and postdoc Jorge Benitez wondered whether cells with the PTEN deletion might be susceptible to synthetic lethality. "Are there signaling pathways or other growth-promoting mechanisms in cells that are only essential when PTEN is deleted?" Furnari recalls thinking. "Something that creates a vulnerability in PTEN-deleted cells?"

In exploring that possibility, they discovered a three-way interaction between PTEN, a protein involved in packaging DNA known as histone 3.3 (H3.3), and DAXX, a so-called chaperone protein, which helps guide the attachment of H3.3 to DNA.

H3.3, however, is no ordinary packager of DNA. It also appears to play a role in suppressing the expression of cancer genes. Knocking out DAXX in cells lacking PTEN, they discovered, silenced the same cancer genes suppressed by PTEN. "It was as if we'd restored PTEN to these cells," says Furnari. "In the absence of DAXX, histone 3.3 was able to repress the activation of these oncogenes, and the effect was only seen in cells that lacked PTEN. It was a great example of synthetic lethality."

The researchers reported that PTEN works against cancer in part by boosting the deposition of DAXX and H3.3 onto chromatin—the general term for DNA and its protein packaging. They proposed that in the absence of PTEN, DAXX and chromatin compete for H3.3, freeing up cancer genes for expression.

But when both PTEN and DAXX are deleted, H3.3 is once again free to bind to the chromatin. In support of that model, they found that if either PTEN or DAXX was

Photo by Stewart Marcano

eliminated, tumors continued to grow in a mouse model of GBM. But when both were deleted, that growth slowed considerably.

Furnari and his team are now collaborating with Geoff Wahl at La Jolla's Salk Institute to find small molecules and protein fragments that disrupt DAXX's interaction with H3.3. Such molecules could be useful for the treatment of GBM tumors that have PTEN mutations.

Today Furnari is increasingly turning his attention to developing better animal models for GBM using novel genome editing techniques. So far, he says, his team's models have faithfully recapitulated the mutations and biology of various subtypes of GBM. "We're very excited by this program because we think we can make just about any tumor type given the right combination of mutations that we dial in using genome editing."

Those models will no doubt be put to excellent use.

"We're very excited by this program because we think we can make just about any tumor type given the right combination of mutations that we dial in using genome editing"

PING-CHIH

**HO**

LUDWIG

LAUSANNE

Photo by Eric Déroze

# THE IMMUNOMETABOLOMIC DISRUPTOR

He eavesdrops on the metabolic chatter between cancer cells and immune cells. Manipulating this malignant crosstalk could significantly boost the efficacy of immunotherapy.

Growing up in Kaohsiung, a southern port city in Taiwan, Ping-Chih Ho was lucky to have the kind of parents who cultivate curious minds. "They gave me a lot of freedom to learn everything I wanted to learn," he recalls. As it turned out, a mix of freedom and curiosity would characterize the best moments of Ho's future career as well.

They would, most notably, propel him to the front of a fledgling field of growing importance to cancer research known as immunometabolomics, which explores how the molecular byproducts of metabolism mediate a conversation between the immune system and the tissues it patrols. That chatter often proves fateful in tumors, which manipulate their metabolic environment to thwart immune attack.

In 2017, Ho's laboratory reported in *Nature Immunology* that relative levels of two run-of-the-mill metabolites involved in the breakdown of the amino acid glutamine and the tricarboxylic acid cycle, a metabolic pathway, can have profound effects on the function of immune cells known as macrophages. His team showed how this balance can determine whether macrophages assume a state in which they can gobble up cancer cells and instigate an anti-tumor immune response, or an alternative one that can suppress such responses and support cancer progression. The findings suggest that the classical enzymatic networks that generate those metabolites might be pharmacologically tweaked to boost the effects of cancer immunotherapy.

## Lucky break

Soon after getting a master's degree in biochemistry from National Taiwan University in 2006, Ho met a visiting University of Minnesota researcher, Li-Na Wei, and convinced her to hire him as a technician in her lab. "That was a very big transition," says

"Since tumor cells and T cells show similar metabolic activity in the same environment, my gut feeling was that they would be communicating with each other through metabolic crosstalk and that this might be one of the reasons immune cells fail against tumor cells."

Ho. "I expected I'd only be there a year." But Li had other ideas. She invited Ho to join her team as a PhD candidate, which he did in 2008.

Li and her team were studying the dysfunctions of fat cells that contribute to insulin resistance in Type II diabetes, and one of their interests was a transcription co-suppressor—a regulator of gene expression—known as RIP140 that can contribute to metabolic diseases. Some of their experiments had shown that macrophages express RIP140 at relatively high levels; other researchers reported that the factor boosts their inflammatory effects. Li suggested Ho make RIP140 activity in macrophages and fat cells the subject of his doctoral research. Learning about metabolic disorders and probing macrophage immunology, Ho traced the links between signaling networks that drive lipid transport and metabolism and those that induce inflammation.

"This was how I started getting interested in immunology," he says.

## Freedom and curiosity

So interested, indeed, that he decided to become an immunologist. Despite a strong record of publications in journals like *Cell Metabolism* and *Nature Immunology*, Ho had some trouble landing a postdoctoral position in immunology. "Many people believed back then that studying signaling cascades of macrophages is not real immunology and so I appeared to lack the required expertise in cellular immunology," he says. Fortunately, the Yale University immunologist Susan Kaech, who is today director of Nomis Center for Immunobiology and Microbial Pathogenesis at the Salk Institute, was not one of them. Ho joined her group as a postdoc in 2012.

Kaech had long explored how chronic viral infections induce a paralyzing exhaustion in the immune system's T cells, which are charged with clearing such infections. She was now interested in probing a similar phenomenon observed in tumors. Less clear was which angle to take. "We had a number of

Photo by Eric Déroze

discussions and she gave me a lot of freedom to determine what I should work on," says Ho.

It was already becoming clear at the time that activated T cells, among the fastest growing cells in the body, have metabolic profiles that resemble those of cancer cells. "Since tumor cells and T cells show similar metabolic activity in the same environment," says Ho, "my gut feeling was that they would be communicating with each other through metabolic crosstalk and that this might be one of the reasons immune cells fail against tumor cells."

It was an original idea, and it appealed to Kaech. "We were very lucky," says Ho, "because it looks like we were right."

The crosstalk they detected arose from a fundamental process known as glycolysis, by which cells break down glucose to generate energy. Normal cells only switch on glycolysis when they're starved of oxygen. Cancer cells, on the other hand, keep it on regardless—a phenomenon known as the Warburg effect—because it generates not just energy but also raw materials essential to cell proliferation. Ho, Kaech and their colleagues discovered that the cancer cell's induction of the Warburg effect coincides with the exhaustion of killer T cells and helper T cells (which orchestrate immune responses). Their study, reported in *Cell* in 2015, detailed why this is the case.

It turns out that a metabolite generated by glycolysis—phosphoenolpyruvate—is a critical switch for T cell activation. After the immune cell's surface sensor, the T cell receptor (TCR), has been engaged by a cancer antigen, the glycolytic metabolite induces a flood of calcium into the cell. That influx is critical to the T cell attack. Trouble is, cancer cells tend to consume most of the glucose in their microenvironment.

"Without glucose," says Ho, "the TCR still gets stimulated, but there's only a transient calcium influx. That is not sufficient to induce


Photo by Eric Déroze

a T cell response, but it *is* enough to induce T cell exhaustion."

Most notably, Ho and his team showed that by engineering T cells to produce phosphoenolpyruvate by breaking down alternative nutrients instead of glucose, they could ameliorate the T cell exhaustion. Injecting those T cells into mice with melanoma shrank tumors and extended the survival of the mice. "This was proof of concept that we can rewire a metabolic pathway in T cells to get them to do their job," says Ho.

## Conflict resolution

A month after that paper appeared in *Cell*, Ho arrived in Ludwig Lausanne, starting a research program in his new laboratory that is integral to the Branch-wide effort to develop personalized cell-based cancer immunotherapies (detailed in the *2017 Research Highlights* report). Ho still primarily focuses on T cells, with the aim of engineering their metabolism to further boost their activity for such therapies. But he has not forgotten the humble macrophage.

With good reason. Most macrophages in tumors are of the M2 variety that suppresses immune responses, rather than the M1 type that eats cancer cells. "We wanted to understand how tumor cells use their metabolic activity to coopt macrophages," he says. "Understanding that mechanism might allow us to reprogram those macrophages to improve immunotherapy."

Soon after opening his lab, Ho was discussing with a student and a postdoc a pair of papers that had come up with conflicting answers on the matter. One concluded that the amino acid glutamine promotes the formation of M1 macrophages, the other that it promotes M2s. This was of special interest to the team because many tumors are highly dependent on glutamine and drugs are currently being developed to block an enzyme, glutaminase, that is involved in its metabolism.

The *Nature Immunology* paper published by Ho in 2017 resolved the dispute and showed that both papers were right, in a way. It wasn't so much glutamine itself that determined the fate of the macrophage as the balance between two molecules in the chain of biochemical reactions that process the amino acid.

"The balance between these two metabolites in the cell determines whether the macrophage becomes an M1 type or an M2," says Ho. If a macrophage is fed glutamine and is prone to making succinate from the amino acid, it becomes an M1 cell in attack mode. If, on the other hand, it is set to make α-ketoglutarate, it turns into an M2. The paper also traced the distinct signaling pathways and patterns of genomic activation that contribute to each of these fates, explaining how and why the ratio of these metabolites in macrophages drives such starkly divergent fates.

"If we can artificially change this balance by providing cell-permeable metabolites or targeting a particular metabolic pathway," says Ho, "we might be able to guide macrophages in the direction we want." Such a capability could be invaluable to a variety of immunotherapies, since it is becoming increasingly clear that many of them are compromised by M2-like macrophages and other immunosuppressive cells in tumors.

Ho and his team are now working toward that goal.

"We wanted to understand how tumor cells use their metabolic activity to coopt macrophages. Understanding that mechanism might allow us to reprogram those macrophages to improve immunotherapy."

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