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 his medical internship 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 Institute for Biological

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

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 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.