

Immune cells invade a rodent islet (cell nuclei in blue) in a diabetes-prone mouse. In type 1 diabetes, the islet's insulin-producing β cells (green) come under attack by immune cells (CD4+ T cells in red) when certain aspects of the immune system's "brakes" are lacking. Vignali's lab has discovered several pathways that contribute to this process.

COURTESY MARIA BETTINI, VIGNALI LAB

THE POWER OF NEGATIVE THINKING

MEET DARIO VIGNALI

BY ELAINE VITONE

Two immunologists walk into a bar.

The scientists—a PhD from St. Jude named Dario Vignali and an MD/PhD Yale prof named Mark Shlomchik—catch up over beers, as they often do at these scientific conferences. And then Vignali confides: "I'm considering moving to a major adult cancer center."

“Well,” says Shlomchik (*speaking of major cancer centers!*), “I’m moving to the University of Pittsburgh as chair of immunology. Maybe you could think about Pitt.”

Joking aside, landing a recruit like Vignali—an eminent scientist who’d been courted many times before—took a lot more than beer. Vignali is now vice chair and professor of immunology at the University of Pittsburgh,

a common language with transplant biologists, molecular oncologists, vaccinologists, and most every other immunology expert you can shake a stick at; and launched his independent research career with basic science papers on autoimmune disease. (In fact, their beer summit was at an autoimmunity meeting.)

And *then* he tackled cancer.

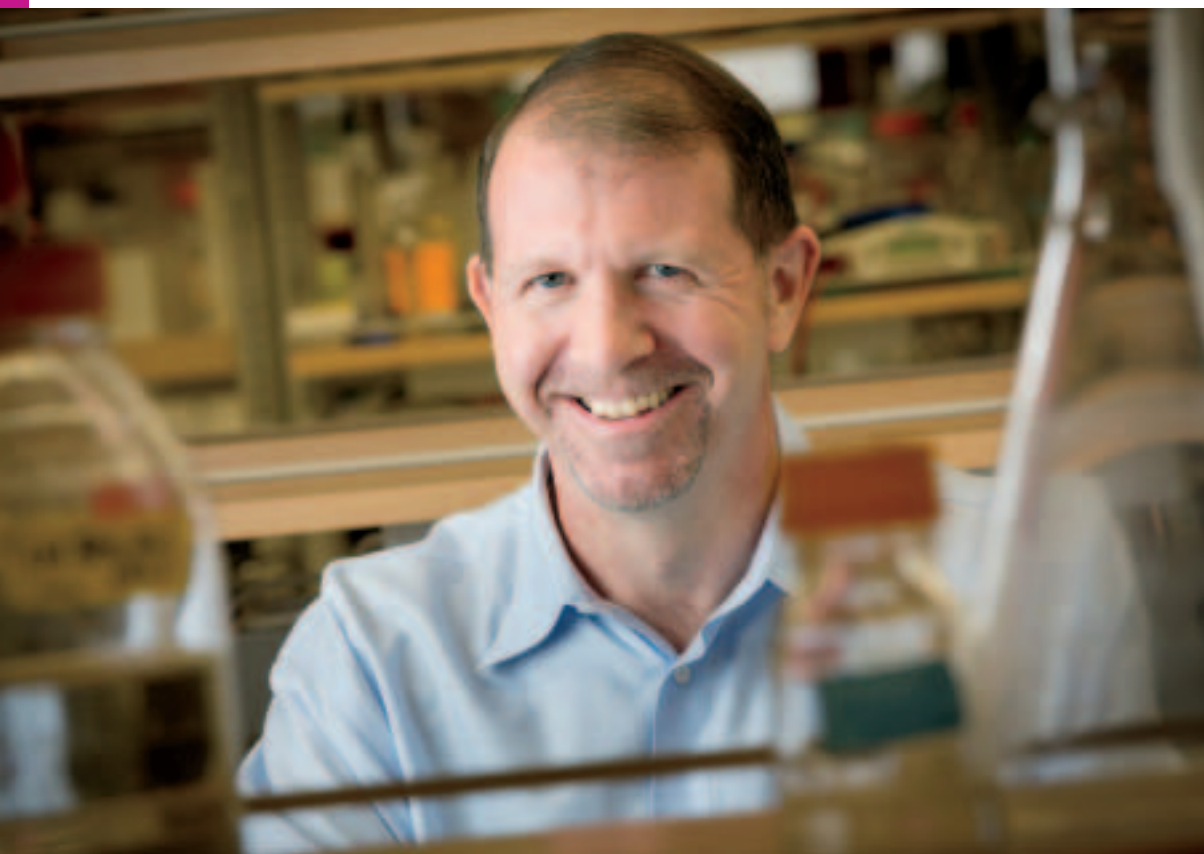
Vignali arrived at Pitt this summer as it was launching Act II of a massive effort to raise the profile of immunology on campus. (Act I was put on by Olivera Finn, PhD Distinguished Professor of Immunology and Surgery, who founded Pitt’s department in 2002.) Using Pitt/UPMC’s cancer immunology program as a model (which was started by Finn in 1991), Pitt plans to strengthen immunology not only in its traditional realms—autoimmune disease, cancer, infectious disease/vaccines, and transplant medicine—but also in its less obvious ones, such as inflammatory diseases of the lung and the microbiome.

“[Pitt] is going to be an exciting place,” says Vignali. “In the next five years, we’ll probably have one of the largest expansions of immunology in the country.”

In the early ’90s, cancer immunology research was still somewhat phenomenological and descriptive, Vignali says. “A tumor grows. You look inside. There are a bunch of [immune] cells. But why is the tumor not being cleared? ... I felt that, until we really understand the immune system, we’re not going to be able to manipulate the immune response to cancer.”

To pay the bills through his graduate studies at the London School of Hygiene & Tropical Medicine, Vignali worked as a technician in a lab that studied the immune response to a parasitic worm and the deadly infectious disease it carries, schistosomiasis. It turned out to be a productive day job. “Infectious disease models are terrific for studying the immune system,” he says.

If you’re an immune cell, “know thyself” is a fundamental imperative. For every passerby you come upon, recognizing “self”—healthy bodily cells—versus *personae non-gratae* like viruses, bacteria, and cancer, is crucial. So, for each of his two postdoctoral fellowships, Vignali trained with a scientist who was expert at the mechanisms of



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Vignali joined Pitt’s faculty in 2014.

as well as coleader of the cancer immunology program and codirector of the Tumor Microenvironment Center at the University of Pittsburgh Cancer Institute (UPCI).

Many of Vignali’s papers in recent years were at the forefront of a sea change in oncology known as cancer immunotherapy, which *Science* named breakthrough of the year in 2013. But Shlomchik is quick to point out that Vignali is not “just” a cancer researcher.

Vignali got his PhD in infectious disease immunology; did postdoctoral training in fundamental molecular processes, giving him

In his new scientific home, Vignali has two labs: one in the Immunology Department, where he studies certain pathways of what’s known as immunoregulation, and another at the Hillman Cancer Center, where he’s applying his benchside insights to new cancer therapies.

One reason Vignali chose Pitt is that, at a time of dwindling federal research dollars, many immunology departments are shrinking—but thanks to the investment of the School of Medicine, as well as UPMC, Pitt immunology is growing.

this self-knowing: Günter Hämmerling at the German Cancer Research Center, who uncovered some of the basic mechanisms used by the immune system to recognize “self” and distinguish “self” from “foreign,” and Jack Strominger at Harvard, who isolated and solved the structure of some of the molecules known as MHC (major histocompatibility complex). (Peter Doherty, who recruited Vignali to St. Jude in 1993, won a Nobel prize for showing that it’s MHC molecules, in fact, that define that process of self-knowing.)

Vignali’s Harvard mentor remembers

ance your blood sugar. In your immune system, there’s stimulation—to fight off pathogens—and the yang to its yin is regulation, ever keeping your immune response in check. Without efficient immunoregulation, we’ve long known, a body can attack itself: It damages its own pancreas in type 1 diabetes; its central nervous system in multiple sclerosis; and a host of organ systems in lupus.

On the flip side, scientists have long suspected that when the immune system is subdued, it leaves the door open for cancer. In animal models as in humans, we’ve seen tumors spark and spread like a gas fire when

a molecule called LAG-3; its potential is directly informed by Vignali’s work.

It wasn’t an easy road. For many years prior, studies along these lines were considered dangerous academic territory. See, everyone had a sense that immunoregulation was important. Everyone suspected some kind of specialized “brakes” must be at work—regulatory T cells (Tregs), or suppressor T cells, as they were called back then, explains Lotze. “But the immunogenetics, in a series of unfortunate studies, failed to confirm their existence.” Tregs remained the Bigfoot of immunology.

“Suppression became a dirty word,” recalls Vignali. “It was just labeled phenomenology, and many questioned whether it really existed.”

him as efficient, precise, and extremely hard-working. “He’s a self-made scientist,” says Strominger, adding that Vignali is a technological innovator, as well. In 2006, Vignali’s lab first detailed, in *Nature Methods*, a new way to develop mouse models for studying T-cell biology. Vignali’s process allows scientists to express various immune proteins, like T-cell receptors (which are used by T cells to identify MHC molecules), and employs a retrovirus as a gene vector. The old way of creating mouse models could take several years. These retrogenic mice, as Vignali dubbed them, can take as little as six weeks to develop, from start to finish.

Strominger is one of a host of scientists now using retrogenic mice to study a range of diseases. “He improved the technology enormously,” Strominger says.

In the early ’90s, when Vignali was just starting out, the big buzzword in molecular immunology was costimulation. A long list of cell-surface molecules known to play some part in fine-tuning and revving up the immune response—in applying the gas, if you will—was growing.

But very little was known about the other side, the brakes.

Medicine is full of binaries like this: triceps versus biceps to control your arm; insulin versus its opposite, glucagon, to bal-

the immune system is compromised. So for decades, cancer researchers have attempted to compensate for these imbalances with immune-boosting cancer vaccines—and Pitt teams, notably that of Finn (immunology’s founding chair emerita), have been testing these experimental techniques.

As Finn wrote last summer in *Cancer Immunology Research*, cancer vaccines have largely proven unsuccessful at treating cancer. However, these studies have opened new possibilities in cancer prevention research and pointed the way to an entirely new approach to cancer treatment. This is because these past two decades of cancer vaccine research have helped to uncover new insights about how cancer develops in the first place.

Namely, by slamming a foot on the brakes. Cancer cells actually boost immunoregulation.

In the past five years, the FDA has approved three cancer drugs that take aim at this mechanism—that stop the foot from hitting those brakes. “It’s the power of negative thinking,” says Michael Lotze, MD professor of surgery, immunology, and bioengineering and assistant vice chancellor, health sciences. These drugs, he says, are the most promising things to happen to cancer research in decades. Another drug in this class that’s currently being evaluated in clinical trials targets

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But that made it all the more appealing for Vignali.

When he started his St. Jude lab, he began using the LAG-3 molecule as a study control for his experiments, just because it happened to be structurally similar to the molecule he was studying called CD4. But he noticed that LAG-3 happened to have “very interesting inhibitory properties,” he says. He’s been studying them ever since.

Vignali found that when LAG-3 is removed from a mouse with a genetic predisposition to type 1 diabetes, the disease goes into hyperdrive. LAG-3 seemed to be a crucial part of the immune system’s brakes. He would go on to publish extensively on this and other pathways implicated in diabetes and other autoimmune diseases, and eventually in cancer.

Some 20 years ago, immunoregulation became not so dirty anymore when Tregs’ existence was finally confirmed. A flurry of interest surrounded Tregs; Vignali, at first, opted not to follow the pack. “Then, sort of ironically, we got dragged into it,” he says with a laugh.

As it turned out, T cells—the immune

system's infantry—are turned off by LAG-3. A scientist who played a key role in this discovery, Johns Hopkins's Drew Pardoll, didn't know much about LAG-3 (very few people outside of Vignali's lab crew did) when the molecule's intriguing abilities first caught his attention. As it happened, Pardoll and Vignali had met just a year prior when Pardoll was touring St. Jude labs. So in 2002, Pardoll gave Vignali a call. "And [Vignali] said, *Yeah, absolutely. Happy to send you our antibodies, which are always very useful tools to study a molecule,*" recalls Pardoll. More than a decade later, they're scientific collaborators, copatent holders, and close friends. Pardoll is even a scientific godfather of sorts to Vignali's second-eldest son, who's working in Pardoll's lab at Hopkins.

The team found that when LAG-3 is blocked or deleted, T cells divide like

detailed how IL-35 could turn T cells into highly potent immune-response quashers.

Most recently, Vignali's team discovered a Treg-surface pathway with promise as a target for cancer. Preliminary findings (published in *Nature*) suggest that the pathway appears to be important to Tregs' astounding durability in what's called the tumor microenvironment. What's the tumor microenvironment, you ask? Good question.

Cancer doesn't happen in a vacuum. In its natural habitat—a living, breathing host—tumor cells are interspersed with connective tissue, blood vessels, and a multitude of immune cells of various stripes, some of which are defending the body and others that have been co-opted to contribute to its demise. Cancer-host crosstalk is dynamic, evolving, and complex.

Most often, though, scientists tend to

"We said, *Gee, what would it take for you to move here?*" recalls Ferris. "And he said, *One thing I've always wanted to do is really focus on the tumor microenvironment.*"

Well, Ferris replied (*speaking off*), as it happened, he'd recently gotten the green light—and some greenbacks—to finally pursue that very same goal.

As codirectors of Pitt's Tumor Microenvironment Center, Ferris and Vignali lead a multidisciplinary effort to essentially meet tumors where they are—which is all over the place, in a constantly shifting biome in terms of genomics, metabolism, oxygen, and inflammation. UPCI teams are studying cancers in mice—and comparing that activity to what's happening in Hillman Cancer Center patients a few dozen yards away. They're enlisting Pitt experts from all walks and recruiting a few

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crazy. And when the researchers administered Vignali's LAG-3 antibodies to a mouse model of immune regulation in the lungs, the antibodies blocked immune-regulation-causing disease.

Even better, mice that lack LAG-3 do not suffer crushing autoimmune disease, as one might fear. As it turns out, LAG-3 is specific only to inflammatory sites—like a tumor.

And if you administer LAG-3 antibodies along with antibodies to another known immunoregulatory receptor, PD-1, you get even more bang for your buck. These encouraging results, published in *Cancer Research* in 2012, are the bases of clinical trials now under way.

While hunting for ways to target Tregs, in 2007 Vignali discovered that an important product of these cells, called IL-35, is one of the few cytokines that regulate rather than excite the immune response. His team published its initial findings in *Nature* in 2007. In a *Nature Immunology* paper, Vignali et al.

focus on one particular aspect of a cancer cell and how it responds when, say, a given pathway is removed. Vignali is interested in complementing this approach with a bigger-picture perspective: What's the impact on the body when a pathway becomes unstable? How do bodily cells then, in turn, affect the cancer?

Studying this big, biomolecular picture, the whole physiological enchilada formally known as the tumor microenvironment, is like "looking at the community rather than the individual," says Vignali. "That's what we've felt was lacking. That's what we wanted to do."

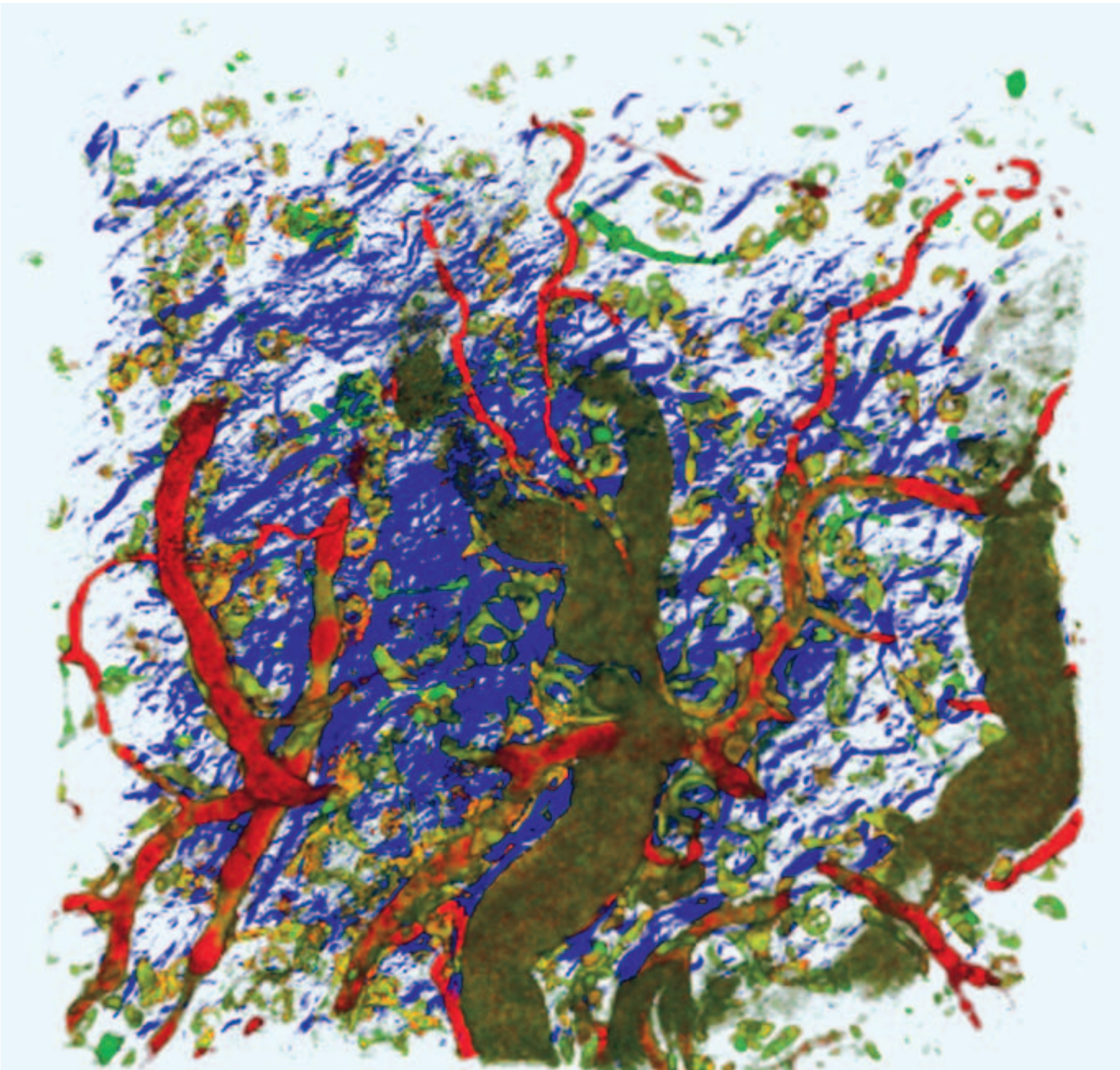
As Vignali was scoping out Pitt, he got to talking and e-mailing with Shlomchik and with Pitt's Robert Ferris—an MD/PhD and UPMC Professor of Advanced Oncologic Head and Neck Surgery and chief of the Division of Head and Neck Surgery within the Department of Otolaryngology (among other titles).

new ones, too. (The first recruit, assistant professor of immunology Greg Delgoffe, a PhD who did his postdoc with Vignali at St. Jude, arrived last summer.)

"We need to move past the animal models and move [the study of the disease] into humans as quickly as possible," says Ferris.

An immunologist, an oncologist, and two pharmaceutical company researchers walk into a bar . . . and, casually and unceremoniously over beers, the pharma researchers show Vignali and Pardoll a small, empty vial encased in a solid block of plastic for posterity. The label indicates the vial was designed to hold LAG-3 antibodies—the first ever produced for human use. The company would also explore how the antibody's efficacy might be boosted by combining it with other drugs.

Today, a promising class of therapeutics is being tested in patients. And that's no laughing matter. ■



Rendering of immune cells infiltrating a rodent melanoma. During tumor growth, regulatory T cells (green) invade the tumor (blue) and use a number of mechanisms to prevent the host's immune system from attacking the tumor (vasculature in red). Vignali's lab has discovered several pathways that contribute to this process.