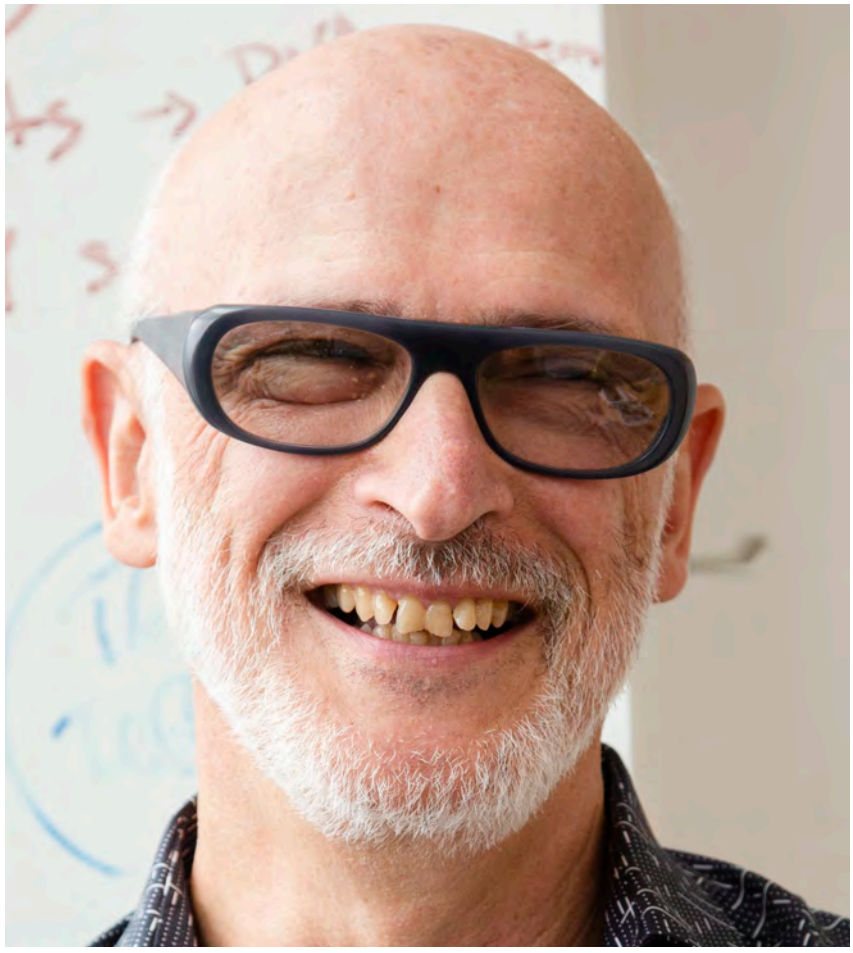
ALEXANDER

# 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

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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.