BECKMAN RESEARCHERS MAP INTRICACIES OF LUNG CANCER IN ONE OF THEIR OWN

By Krista Conger

It was around 3 p.m. on a Thursday last October when James Spudich and Suzanne Pfeffer poked their heads into the office of Mark Krasnow, on the fourth floor of the Beckman Center for Molecular and Genetic Medicine, interrupting a meeting he was having with a colleague.

“Can we talk to you for a minute?” Pfeffer said.

Impromptu conversations were nothing new for the three. The longtime colleagues and friends have a lot to talk about. Spudich, PhD, whose research focuses on understanding the molecular forces that drive muscle contractions, earned a doctoral degree from Stanford in 1968 under Arthur Kornberg, MD, a founding member of the Department of Biochemistry. Krasnow, MD, PhD, who studies lung development and disease, completed his postdoctoral work under former department chair David Hogness, PhD. Krasnow, Spudich and Pfeffer, PhD, are all professors of biochemistry and have chaired the department. (Pfeffer, who joined the department in 1986, is completing her second stint as chair.)

But this time, the topic was more personal. Spudich, 76, had come to share some unexpected news: He had lung cancer. “I was wondering if you were looking for any human tissue samples for your research,” he said.

Shocked and saddened, Krasnow asked when Spudich would undergo surgery to have the tumor removed.

“I told him it was scheduled for 8 a.m. the next morning,” Spudich recalled. “I remember Mark went kind of pale.”

Spudich had no way of knowing it, but the meeting he’d interrupted between Krasnow and assistant professor of pediatrics Christin Kuo, MD, had been called to discuss how Krasnow could broaden his research, which he had been conducting mainly in mice and small primates called mouse lemurs, to include human cancers. But the logistical and legal hoops that would need to be cleared prior to any kind of human-based research were daunting, and Krasnow knew it would likely take months to obtain the necessary approvals.

Kuo, a specialist in pulmonary medicine, had been working with Stephen Quake, PhD, professor of bioengineering and of applied physics and co-president of the Chan Zuckerberg Biohub, and postdoctoral scholar Spyros Darmanis, PhD, a group leader at the Biohub, to study the development and function of lung neuroendocrine cells using single-cell RNA sequencing. As a pulmonary fellow, Kuo had worked with Krasnow to conduct her initial studies in mice. But in 2016 she’d established her own laboratory in the Department of Pediatrics and had begun to develop the methods and key reagents to efficiently isolate human lung cells. Not only did she have all the necessary protocols and approvals in place to begin her study, she had also already established a collaboration with Joseph Shrager, MD, professor and chief of thoracic surgery, who was slated to perform Spudich’s procedure the next morning.

Within moments, the researchers had initiated a remarkable series of events that would, over the next 16 hours, lead to the beginning of what will likely be the world’s largest study of healthy and diseased human lung tissue. Dubbed the “lungome,” the effort has brought together researchers and clinicians from throughout the Beckman Center, Stanford Bio-X and

the Biohub to perform a kind of unprecedented molecular poking and prodding of Spudich's lung tissue —analyzing gene expression profiles, signaling pathways, cellular architecture and immune responses —to identify for the first time exactly what goes wrong when lung cells become cancerous.

'A remarkable story'

"This is a massive attack on a human disease with the most modern of research tools on one tumor from one patient," said Krasnow, who estimates the project will cost $1 million or more. "In five or 10 years, I believe that the knowledge we will learn from this study will guide the standard of care for all lung adenocarcinoma patients. It's a remarkable story."

The effort isn't intended specifically to help Spudich, whose prognosis is good because his cancer was caught early and removed quickly. Rather, it promises to offer an intimate glimpse into the earliest steps of cancer development in human lung tissue and will likely lead to better diagnosis and treatment of lung adenocarcinomas.

In the hours leading up to the surgery, the researchers struggled to balance their excitement about the scientific opportunity with their concern for a colleague. To say James Spudich is well-known in the Stanford Medicine community would be an understatement.

Many of the more than 20 people involved in this last-minute research effort had known Spudich for years. Quake had received a bachelor's degree in physics at Stanford in 1991 and had also done postdoctoral work under professor of physics Steven Chu, PhD. At the time, researchers in the Chu and Spudich labs were collaborating to build a single-molecule laser trap that

Mark Krasnow, M.D., Ph.D.,
Professor of Biochemistry and Howard Hughes Medical Institute Investigator.

"This is a massive attack on a human disease with the most modern of research tools on one tumor from one patient," said Krasnow. "I believe that the knowledge we will learn from this study will guide the standard of care for all lung adenocarcinoma patients. It's a remarkable story."

Spudich would use to study the force generated by the interaction of actin filaments with myosin molecules responsible for muscle contraction. (Chu was awarded the Nobel Prize in physics in 1997 for related work.) Together, Chu and Spudich launched Stanford Bio-X in 1997 to promote interdisciplinary collaborations between researchers in Stanford's schools of Medicine, of Engineering and of Humanities and Sciences.

Nearly everyone speaks of Spudich with admiration, not just for his prodigious scientific accomplishments — he received the 2012 Albert Lasker Basic Medical Research Award for his research into the molecular motors that drive muscle contraction — but also for his warm and kind personality.

"Everyone knows and loves Jim," Quake said. "So it wasn't surprising that our whole community mobilized immediately to make this happen."

"This was an amazing scientific opportunity that came much earlier than expected," said Krasnow, who is also a Howard Hughes Medical Institute investigator. "But, of course, there was also the realization that we were talking about our beloved colleague, mentor and friend. When that person is right in front of you, and with you, it gives this effort an urgency and a poignancy that brings science and medicine together in such a beautiful and powerful way."

Spudich, who refers to the effort as Project Lung, is far less sentimental. In fact, some might say he's unabashedly enthusiastic. "There's no place else in the universe where the biology, the biophysics and the technology — everything that we can muster to throw at these tissues — exists that will allow us to really understand the lung in unprecedented molecular detail," Spudich said. "What is going to emerge is an understanding about lung biology at a level of depth no one has previously imagined. And it's kind of special that this is my lung."

Common form of lung cancer

Lung adenocarcinoma is the most common type of lung cancer. It usually occurs in current or former smokers, but it is also the main type of lung cancer in nonsmokers. It's frequently diagnosed at a late stage, after patients report symptoms of coughing or other vague symptoms like weight loss or unexplained pain.

Spudich was lucky that his cancer was discovered early. "I was at my annual physical on Sept. 20, 2017, when my doctor asked how I was. I said I felt great, but my wife, Anna, said, 'No, he's not alright,'" Spudich recalled. "We looked at her, and she continued, 'Jim's more tired than he usually is, and he has a slight cough, and we just came back from India.' I felt both of these symptoms were unremarkable, as I'm getting older, but heeding her concern, my doctor ordered a chest X-ray."

The X-ray, taken nine days later, showed a slight cloudiness in the upper lobe of Spudich's left lung. Subsequent CT and PET scans suggested the presence of a possible adenocarcinoma, about 2 centimeters long, which was confirmed by a biopsy. Surgery to remove the lobe, containing both normal and cancerous tissue, was scheduled for the morning of Oct. 27.

"So, totally unexpectedly, I went in one month from feeling normal and fit to having to deal with lung cancer," Spudich said. "I knew it was time to talk to Mark, who is the world's expert on lung development and who was carrying out some of the most cutting-edge research on the origins of lung adenocarcinomas in laboratory mice."

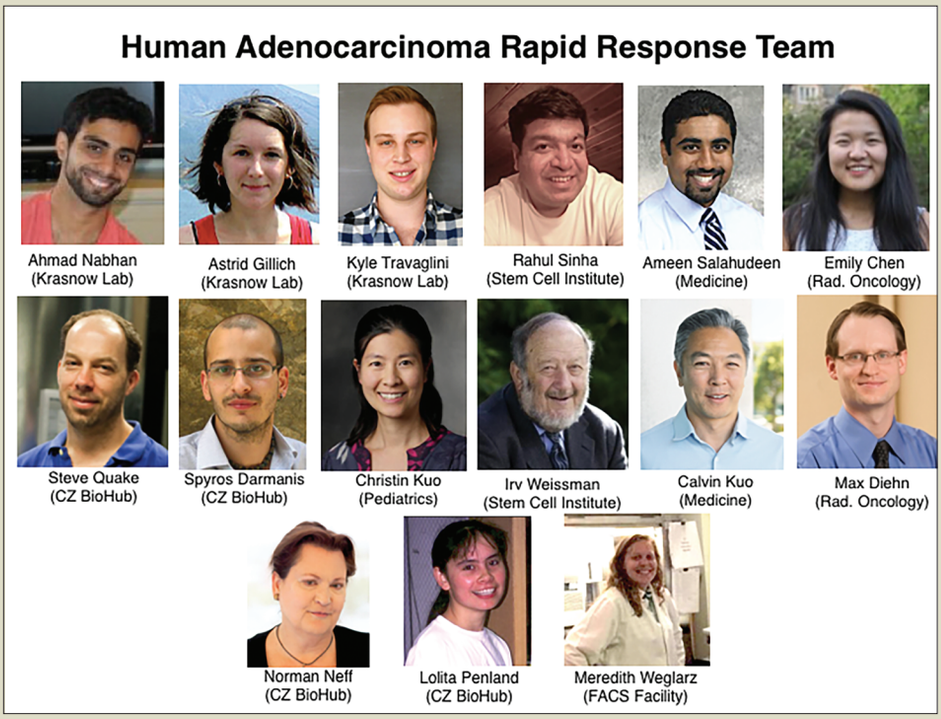
At the time, Krasnow was part of a large collaboration organized by Quake and Tony Wyss-Coray, PhD, professor of neurology, to understand the diversity of cell types across many tissues as part of the Chan Zuckerberg Biohub's Cell Atlas project. Using technologies developed by Quake and others, researchers are performing comprehensive profiling of the transcriptomes of single cells, enabling them to analyze cell type and state with unprecedented sensitivity and precision in both health and disease.

"Steve, Tony and their teams were working on a mouse cell atlas," Krasnow said. "We were in charge of the lung aspect. To do so, we had been assembling what we termed 'rapid response teams' to quickly collect and analyze the mouse tissue. So we knew how to do something like this, and how to do it well. But we hadn't done it before in humans, and nowhere nearly as quickly as we had to act with Jim's tumor. Normally we had a lead time of days or weeks; now we had hours."

'A unique opportunity'

In addition to ensuring all aspects of the protocol were followed, including explaining to the patient, Spudich, exactly what would happen, Krasnow faced another hurdle: how to get funding for the surprise project.

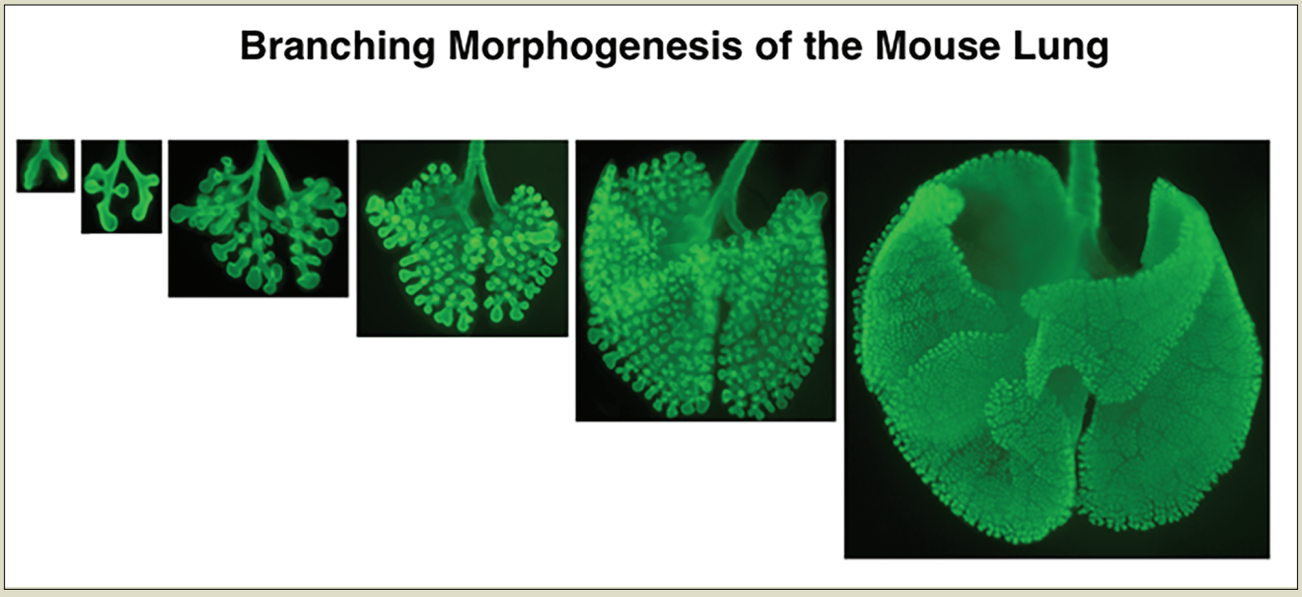
"I immediately contacted Steve and described how this was an amazing opportunity to extend the CZ Biohub studies in mice into human lungs and lung cancer," Krasnow said. "We could analyze normal and diseased human tissue, and compare it to what we had learned



in mice and mouse lemurs. And then I told him who the patient was. I don't think it took Steve even a second to say, 'Go for it.'"

That afternoon, members of the rapid response team, including Kuo and several students and postdoctoral scholars in Krasnow's lab, went to work to "humanize" their mouse studies. "Christin worked on the clinical side," Krasnow said, "getting forms and making connections with surgeons, clinicians and pathologists. Meanwhile, the students and postdocs in my lab were figuring out what was needed to be done differently with human tissue."

For one thing, the sheer amount of tissue that would be removed from Spudich would be much larger than a tiny mouse lung. Furthermore, the researchers couldn't use mouse-specific antibodies to separate the human tissue into specific cell types. And although some types of cells are relatively abundant and easy to analyze, others require meticulous care to isolate. "We're not just interested in the tumor cells themselves," Krasnow said. "We also want to understand the roles played by the stromal cells that surround the tumor, the immune cells that have infiltrated the tumor and even the endothelial cells that form the vessels that deliver blood to the growing mass. Bulk analysis, in which several cell types are combined, obscures much of the most interesting information. We wanted to sample all major cell types in the tissue in and around the tumor, and also in healthy neighboring lung."



"There's no place else in the universe where the biology, the biophysics and the technology – everything that we can muster to throw at these tissues – exists that will allow us to really understand the lung in unprecedented molecular detail," Spudich said. "And it's kind of special that this is my lung."

'Moving quickly'

Krasnow's team pulled out all the stops to find the appropriate materials for the research.

"All of these people scurried about until late in the night to scrounge reagents and antibodies from other labs and the CZ Biohub, either here on campus or nearby," Krasnow said. He also alerted Lisa Nichols, PhD, who directs the Beckman Fluorescence Activated Cell Sorting (FACS) Facility, to prepare for an influx of samples. "We had to line up time on the cell-sorting machines and find FACS operators who could be on-call after the surgery," Krasnow said. "And all of this had to be put in place by 8 a.m. the next morning."

In addition to facilitating the efforts of the rapid response team, Krasnow marshaled other lung adenocarcinoma researchers at Stanford.

"I was on the East Coast at a meeting that afternoon when I received a phone call from Mark," Maximilian Diehn, MD, PhD, associate professor of radiation oncology, recalled. "Would I be interested in collecting plasma for study?"

Diehn, who earned his doctorate in biophysics in the Department of Biochemistry at Stanford, is studying whether the presence and levels of circulating tumor DNA, or ctDNA, which is shed into the bloodstream by tumor cells, can be used to diagnose or to predict the recurrence of the disease after initial treatment. He and Ash Alizadeh, MD, PhD, associate professor of oncology, have shown that if ctDNA is detected after radiation and surgery, that patient is at high risk for recurrence.

"Jim's cancer type and stage basically fits perfectly into one of our ongoing clinical studies," Diehn said. "By collecting blood before and after surgery, and then intermittently during and after subsequent chemotherapy, we can look for the presence of ctDNA and possibly predict the chance of recurrence."

Diehn and Alizadeh have also helped to develop an assay in Stanford's Molecular Pathology Laboratory to identify the presence of genetic changes in a tumor that can be targeted by existing drugs or treatments.

Krasnow still had a few more experts he wanted to involve.

"Immunotherapy is a very exciting field of research right now, and we wanted to reach out to experts of the immune and blood system to systematically analyze immune cells infiltrating Jim's tumor," Krasnow said. Irving Weissman, MD, director of Stanford's Institute for Stem Cell Biology and Regenerative Medicine and of its Ludwig Center for Cancer Stem Cell Research and Medicine, fit the bill perfectly. He and Spudich are old friends.

Rahul Sinha, PhD, a former postdoctoral scholar in Weissman's lab and an instructor at the institute, came on board to collect the necessary blood and tissue samples and initiate the analysis of tumor-infiltrating immune cells.

Finally, Krasnow called professor of medicine Calvin Kuo, MD, PhD, and hematology and oncology fellow Ameen Salahudeen, MD, PHD. The two had been working to establish new ways to culture normal and cancerous human lung tissue from surgical biopsies to study human-specific biology. Together with Tushar Desai, MD, assistant professor of medicine, they had

"We're not just interested in the tumor cells themselves," Krasnow said. "We also want to understand the roles played by the stromal cells that surround the tumor, the immune cells that have infiltrated the tumor and even the endothelial cells that form the vessels that deliver blood to the growing mass."

recently identified the presumed cell-of-origin for lung adenocarcinoma in humans.

"Again, we were just so fortunate to have world experts, or in many cases the world expert, right next door," Krasnow said.

"Jim's cancer is going to be one of the most heavily analyzed human cancers ever," Diehn said.

'A very busy patient'

By the next morning, Spudich himself was "a very busy patient," signing multiple consent forms and being briefed on what his participation in each study entailed.

"Jim's attitude was amazing," Krasnow said. "In everything he does, he has a wonderful blend of vision and hope. This was no different." Meanwhile, the rapid response team was gowned and waiting, somewhat nervously, outside an operating room at Stanford Hospital.

"These are PhD students and postdocs," Krasnow said. "I don't think any of them had ever been in an operating suite before, or even seen or touched fresh human tissue. But there they were, after being up much of the night, as Shrager removed the tissue through the tiniest of incisions."

Kuo was in the operating room to discuss with Shrager and the pathologists how to get the best samples of normal and diseased tissue for the planned analysis. "These sections were precisely annotated and hand-delivered to the lab immediately, to ensure the freshest tissues," Kuo said.

"From that point on, except for the size of the samples, it was pretty much like handling mouse tissue," Krasnow said. "Except this time it was someone they knew."

Under the care of Shrager and his team and Heather Wakelee, MD, professor of oncology and a specialist in lung cancer, Spudich recovered quickly from the surgery. "I was up and walking the halls that evening," he recalled. "I really can't say enough about Drs. Shrager and Wakelee and all the oncology care team at Stanford. They are phenomenal, and I am so thankful."

Because his cancer was caught early and removed fully, it's presumed to be cured. However, the current standard of care recommends several rounds of chemotherapy to reduce the chance of any possible recurrence. For patients with metastatic disease, the prognosis is less positive.

"In metastatic disease, chemotherapy is used to prolong life rather than cure the patient," Wakelee said. However, new approaches include an immunotherapy approach, known as checkpoint blockade, and personalized treatment based on the genetic sequence of the tumor to more precisely target cancer cells.

"Previously, we would treat all metastatic lung adenocarcinomas the same, while now we look very closely at the cancer's molecular underpinnings," Wakelee said. "Now we wouldn't think of starting treatment without understanding the genetic changes present in each patient's cancer."

Wakelee is the principal investigator for a clinical trial testing the effect of checkpoint inhibitors on the cure rates for people with early stage lung adenocarcinoma. Not surprisingly, Spudich has enrolled in the trial. After completing post-surgery chemotherapy, half the participants will receive the active drug and half will be part of a control group.

'A full participant in the project'

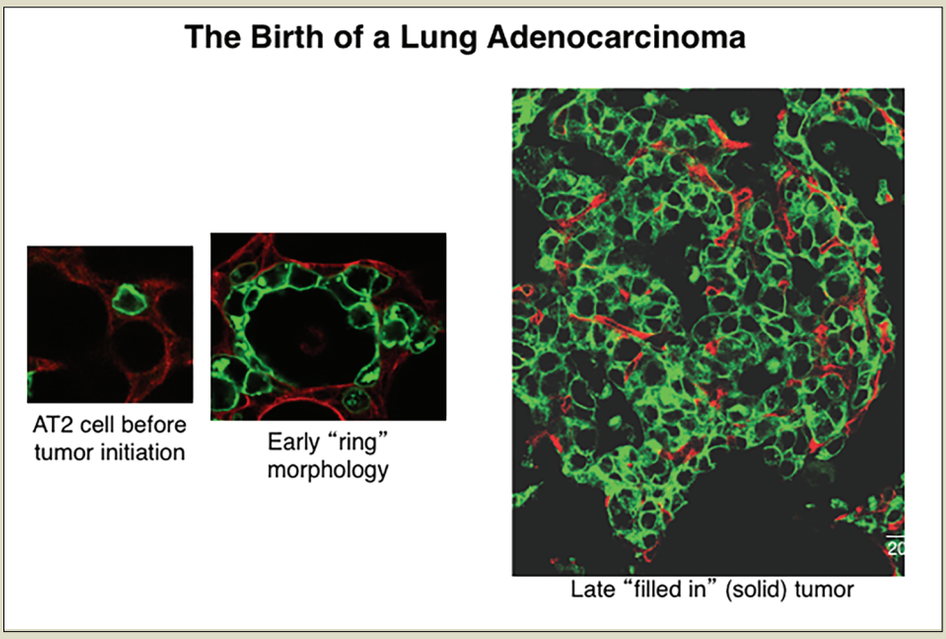
"It's so interesting to be the patient — I'm receiving chemo — but also to be a researcher deeply involved in the decision-making and data-analysis process. For example, I attend regular meetings of the team to decide what to do with the tissue samples," Spudich said.

"Jim's not just a patient, he's also a scientist," Krasnow said. "He started reading all our papers in the field. He wants to know everything. He quickly became not just a huge motivation for us all, both scientifically and personally, but a full participant in this project."

Raj Rohatgi, PhD, associate professor of biochemistry and of medicine and a close friend of Spudich, facilitated Spudich's involvement in the scientific aspects of the work. "Raj is an expert in lung cancer and a close colleague of Heather Wakelee's," Spudich said. "He is extremely knowledgeable about the molecular and cell biology of the disease and he's been invaluable to me. Mark, Raj and I confer almost daily about the latest developments in Project Lung."

In the months since the surgery, the scientists have amassed unprecedented amounts of data as a result of Spudich's impromptu visit to Krasnow's lab.

"Major results are starting to pour in," Krasnow said. "We've already learned that there are cell types in humans that are either not present or not detectable in mice, which is very interesting. We've also identified a potential driver mutation for Jim's tumor."



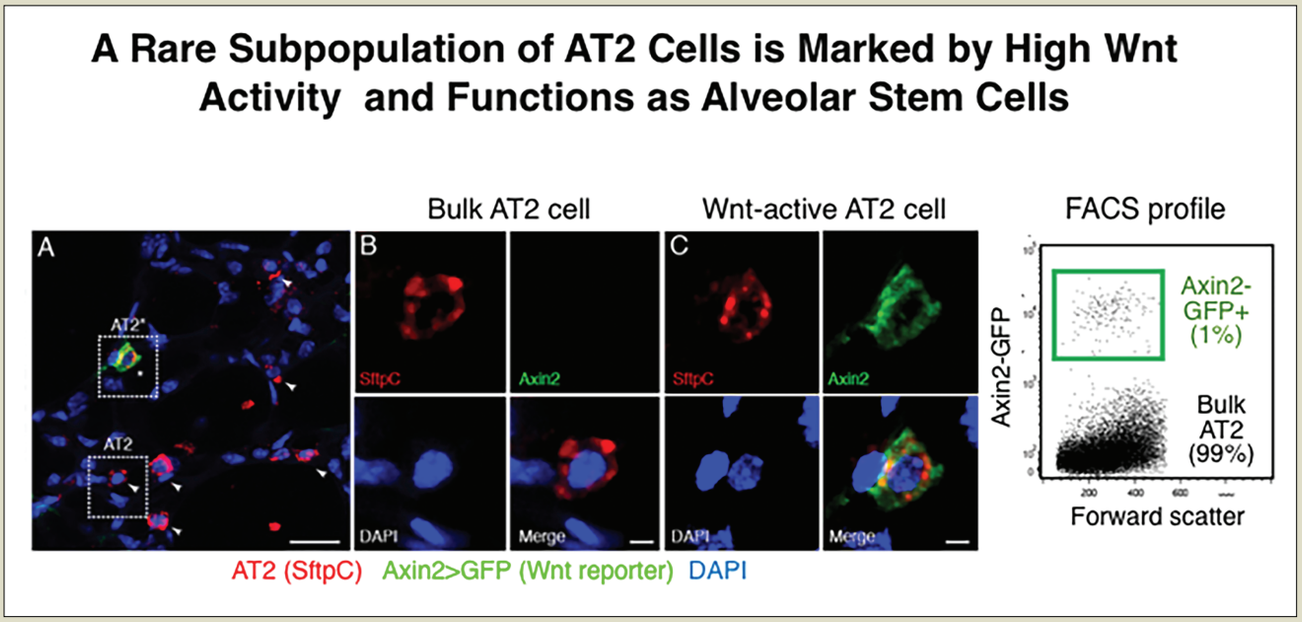
"This is a foundational study," Krasnow said. "We've marshaled numerous experts and techniques in an effort to truly understand for the first time the full cellular and molecular complexity of a single tumor in a way that will push us to a whole new level of potential therapies for this disease."

Diehn and his colleagues have identified a genetic change in Spudich's cancer that, although relatively rare in lung adenocarcinoma, is common in melanoma. Drugs exist that target cancer cells with that mutation when the cancer is metastatic, but they are not yet approved for use in early stage cancers.

Krasnow and his colleagues are working to identify further subpopulations of cells in the lung. "We want to get to know the tumor and what is driving it," he said. "Are there subpopulations that might be responsible for maintaining and expanding the cancer?"

Previous work in Krasnow's lab has found that a small fraction of cells called alveolar type 2 cells also have stem cell capabilities. To maintain their stem cell identity, the cells require the presence of neighboring cells called fibroblasts that secrete a signaling molecule called Wnt. "These alveolar type 2 cells are basically stem cells with a day job," Krasnow said, "and they have a private niche of just one adjacent fibroblast." If these stem cells are inappropriately activated, it's possible they could begin dividing uncontrollably and give rise to an adenocarcinoma, the researchers believe.

"We really want to know what goes on in the very earliest stages of cancer development," Krasnow said. "Are there any signs that it initiates in these alveolar type 2 cells? Intriguingly, it turns out that Jim's tumor DNA does have an oncogenic mutation in one of these activating pathways. And now we have the opportunity to study them at single-cell resolution and compare them much more carefully to normal lung cells."



Potential approach for cure

One concept Krasnow and his collaborators are pondering is the idea that it might be possible to cure, rather than treat, lung cancers with a two-pronged approach: blocking the pathway that stimulates the cells' growth while also removing the Wnt signal that is necessary to confer the cells' stem cell properties.

"This is a foundational study," Krasnow said. "We've marshaled numerous experts and techniques in an effort to truly understand for the first time the full cellular and molecular complexity of a single tumor in a way that will push us to a whole new level of potential therapies for this disease."

"It's so amazing what you can do in 2018," Spudich said. "A lot of these techniques were invented right here at Stanford, many in the Biochemistry Department in which I spent so much of my career. If someone asks me exactly what we're going to learn from this study, I would answer the same way I answered when people asked me what would come out of Bio-X when it was first proposed. 'If I could tell you that, then we have failed.' But it's very exciting to imagine that I have a chance to help unravel what causes these cancers in the first place and the molecular details that might lead to new treatments."

Photo credits:

Page 7. Photo by Justin Lewis.

Page 9. Image by Mark Krasnow. Krasnow Lab.

Page 10. The growth and branching of the mouse bronchial tree is shown in these stereoscope images of a series of lungs from different embryonic stages immunostained to visualize the airway epithelium. Image by Ross Metzger. From Metzger RJ, Klein OD, Martin GR, Krasnow MA, (2008). The Branching Programme of Mouse Lung Development. Nature. 453(7196):745–50.

Page 13. Confocal image of a mouse lung AT2 cell (Green) within a healthy alveolus. Image by Tushar Desai, see Nature… Desai, T., Brownfield, D. and Krasnow, M.A. (2014) Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507, 190-194.

Confocal image of a typical lesion at an early stage of adenocarcinoma development. Note expansion and spread of Kras-activated AT2 cells circumferentially around the alveolus, which precedes appearance of the adenoma nodule. Image by Tushar Desai, see Nature… Desai, T., Brownfield, D. and Krasnow, M.A. (2014) Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507, 190-194.

Confocal image of mouse lung adenoma driven by expression of oncogenic Kras in AT2 cells. Green marks AT2 cells within tumor and red indicates other cell types within the tumor mass. Image by Tushar Desai, see Nature... Desai, T., Brownfield, D. and Krasnow,

M.A. (2014) Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507, 190-194.

Page 14. Confocal image of mouse lung stained for the AT2 marker (SpC), green fluorescent protein (Axin2 lineage tag), and DAPI (nuclei) with boxed areas shown in close up showing Axin2-negative (middle) and Axin2+ AT2 (right) cells. Image by Ahmad Nabhan, see Science... Nabhan,, N., Brownfield, D.G.., Harbury, P.B., Krasnow, M.A.*, and Desai, T.J.* (2018) Single cell Wnt signaling niches maintain stemness of alveolar type 2 cells. Science, in press. (*, corresponding authors) PMID: 29420258, doi: 10.1126/science.aam6603.

Flow cytometry plot shows that ~1% of mouse lung AT2 cells are Axin2+ stem cells (indicated by green box). Image by Ahmad Nabhan, see Science... Nabhan,, N., Brownfield, D.G.., Harbury, P.B., Krasnow, M.A.*, and Desai, T.J.* (2018) Single cell Wnt signaling niches maintain stemness of alveolar type 2 cells. Science, in press. (*, corresponding authors) PMID: 29420258, doi: 10.1126/science.aam6603.