PHOTO: DREW GURIAN

The littlest patient

Cutting-edge mouse models fuel hope for understanding and treating cancer

By **Jennifer Couzin-Frankel**, *in New York City*

The worst day of Kenneth Olive's career began unremarkably. He woke up in his two-bedroom apartment in Harlem and tag-teamed breakfast for his 1-year-old son as he and his wife raced to get ready for work. At the 116th Street subway stop nearby, Olive hopped on a C train uptown to 168th Street. His lab is about a block away, in the cancer center at the heart of Columbia University's medical complex. There, the young assistant professor was banking his career on a mouse—one he hoped would pinpoint new drugs for pancreatic cancer, among the deadliest diagnoses in oncology.

Sitting on the train, Olive opened an e-mail message he had downloaded earlier but hadn't yet read. It was from an executive at a biotech company with which he was working closely. What he read sucked the air right out of him. The company, Infinity Pharmaceuticals Inc., had been running a clinical trial in 122 people with advanced pancreatic cancer; about half were getting a new drug thanks to impressive results in Olive's genetically engineered mice. Days earlier, data monitors had noticed a striking disparity between patients who were on the experimental treatment and those who were not—but because the trial was blinded, they did not know which group was which. Now, the blinds had been lifted. To everyone's horror, the patients dying more quickly were on the new drug. It was the first big test for Olive's mice—and the animals had, by all appearances, failed spectacularly.

Kenneth Olive holds one of his mice engineered to develop pancreatic cancer. He tests drugs on the animals in hopes of identifying the best ones for cancer patients.

"I remember being numb," Olive says. It was January 2012, 2 years since he had joined Columbia's faculty. He was 35 years old. "We had built our entire laboratory based on this mouse," he says. Intended to mimic human cancer with unusual precision, the animals were even being monitored and treated in a "mouse hospital" custom-built just for them. Rushing into work, Olive called a lab meeting for 10:30. After his group gathered, he broke the news. "It was a very quiet and heavy room. I told them that we were allowed to be mopey for 3 hours, and then we were going to have a meeting that afternoon to brainstorm" what went wrong.

Olive is trying to shift a dismal statistic that plagues his field: About 90% of cancer drugs that enter clinical trials based on upbeat mouse data fail. For some tumors, the need for new therapies is especially acute. In pancreatic cancer, the 5-year survival rate is about 6%. "These patients," Olive says, "don't have any options."

> ***"I told them that we were allowed to be mopey for 3 hours, and then we were going to have a meeting that afternoon to brainstorm."***
>
> **Kenneth Olive,** Columbia University

To change that, he and a growing number of other cancer researchers are trying to build a better mouse. Their approaches are a radical break from the past: taking human cancer cells grown on plastic over many years and injecting them into an animal. Dozens if not hundreds of drugs have subdued cancer in these mice. A handful have done the same for people.

Olive believes he can do better with a genetically engineered mouse. At conception, he endows the animal with the same gene mutations that show up in most human pancreatic cancer cells; the mutations become active during early development, in cells destined to form the pancreas. Just like certain people, these animals spontaneously develop pancreatic tumors.

Changing the paradigm requires not just a superior rodent. It also takes rethinking how and how thoroughly the animals are studied. To wring as much information as possible from his mice, Olive schedules every one of them for ultrasounds twice a week to check for tumors and monitor existing disease. The "treatment room" includes boxes of surgical gloves, sterile drapes, a surgical microscope, and a venting hood to protect researchers from inhaling the anesthetic they use on the animals.

But that day in 2012 underscored just how difficult it is to mirror human cancer in a wriggling 30-gram ball of fur, where tumor size is measured in millimeters and lifespan in days. Every couple of weeks Olive hears from a scientist or company eager to test a favorite therapy. "Can you just throw this into a few mice for me?" they ask. That isn't quite how it works, Olive says. The risks and the challenges sometimes feel overwhelming. But the payoff, he believes, will make it all worthwhile—if his mice pan out.

OLIVE STEPPED INTO THE WORLD of pancreatic cancer by chance. In 2005, fresh out of graduate school, he was recruited to join the lab of David Tuveson, an oncologist and cancer biologist then at the University of Pennsylvania. Tuveson had just published a paper describing an uncommonly accurate mouse model for pancreatic cancer.

To create it, Tuveson mutated two of the mouse's genes. One, *Kras*, shows up in about 95% of human pancreatic tumor samples. The second, *p53*, appears in 70%. The model isn't an exact replica of humans. Unlike people, the animals have these mutations throughout their pancreas, not just in their tumor cells, and throughout their life rather than just when cancer makes its appearance.

Yet the mice captivated Tuveson and Olive. The disease's choreography closely matched its dance in people. It metastasized to the same sites—the liver, the lymph nodes, the lungs. The animals developed many of the same complications as people, including fluid collections in the abdomen and a muscle-wasting syndrome. When Tuveson slipped a slide from a mouse's tumor into a stack of slides from people, a pathologist "couldn't tell the difference," Olive says. But would a mouse whose cancer generally

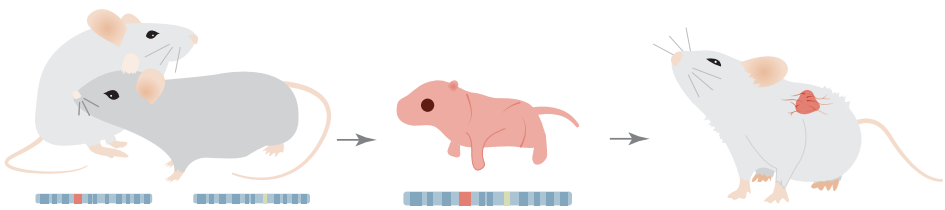
Three model mice

Traditional mouse xenografts have been used for years as cancer models, but many researchers say they can do better. They are experimenting with mice genetically engineered to develop cancer and ones that carry a patient's specific tumor.

Traditional mouse xenograft

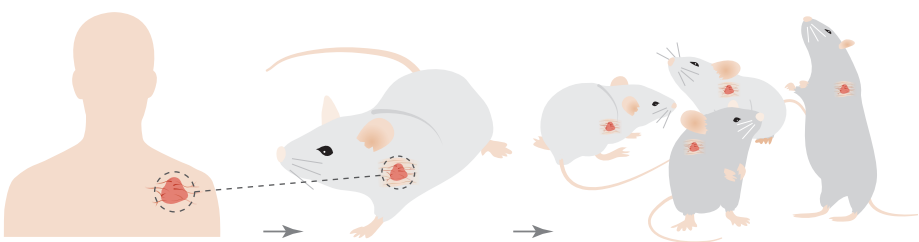
Cells from a human tumor are grown in the lab and maintained for many years. They are then injected into a mouse or in some cases inserted surgically into certain organs.

Genetically engineered mice



In one version of this model, two types of mice are engineered: one with the mutated genes that are inactive, and one with an "activator." When the animals are bred, the offspring carry active mutations that lead to a specific cancer.

PDX mice



Tumor and surrounding tissue from a patient are implanted into a mouse. After they engraft, tumor samples from that mouse are removed and implanted into others, creating a cohort with the patient's tumor.

resembled a human's and behaved like it also respond similarly to therapy? "You have to go into it without any assumptions," says James Doroshow, who directs the division for cancer treatment and diagnosis at the National Cancer Institute (NCI) in Bethesda, Maryland. NCI is working on both genetically engineered models and another called patient-derived xenograft (PDX) models, in which tumor and some healthy tissue are removed from a patient and engrafted into a mouse (see p. 28).

Olive, just beginning his postdoc, was eager to plunge ahead with the new model. "I said, 'Great, I'm here, I want to put drugs' " in these mice. " 'Let's go.' "

Hang on, Tuveson shot back. How do you know which animals have cancer?

Olive paused. As he now explains, the mice "don't let you know that they're sick until they're very sick—and then you only have a few days" until they die. Olive wasn't much interested in imaging, but he didn't have a choice. He began running around the university, "begging and borrowing" time on different machines—some built for mice, some for people—to visualize what, if anything, was growing deep inside the animals' bellies.

In a stroke of luck, he happened upon an ultrasound machine designed for rodents in the cardiology department. He asked if he could bring one of his mice along and discovered the machine was just right. It was small, easy to use, and, by the standards of such equipment, relatively cheap—about "$350,000 instead of $3.5 million," says Olive wryly. Tuveson, a young faculty member, pooled money from his startup package and grants to buy one for the lab.

Now, Olive was ready to put the mice through their paces. The obvious starting point was the chemotherapy drug gemcitabine, which until the mid-2000s was the only therapy approved to treat pancreatic cancer. Like so many before it, it had performed impressively in early mouse trials, but only about 5% to 10% of patients benefit from it.

Other researchers developing cutting-edge mouse models have taken a similar tack: testing whether their models mimic known human drug responses. At the University of Turin in Italy, molecular oncologist Livio Trusolino gathered colon cancer tissue from patients whose disease had spread to their liver and engrafted this tissue into animals without a functioning immune system to create PDX mice. Then, he gave the animals an antibody that's often used in patients. The proportion of mice whose tumors shrank or stopped growing "were pretty much the same as the ones in the clinic," he says. In Boston, researchers tested a genetically engineered mouse model for lung cancer in parallel with an AstraZeneca clinical trial of a targeted therapy. The mice, which had the same genetically driven disease subset as the patients, received the same treatment. "The moment that was striking for me was when the human data were announced, and our data matched exactly," says thoracic oncologist Kwok-Kin Wong at the Dana-Farber Cancer Institute, who led the work.

Olive hoped for a match, too. Most scientists want to see their drug cure mice. He was praying his would fail.

It did. Tumors shrank in just two of 17 animals. Flush with success, Olive proceeded to his next drug: Infinity's.

"THESE ARE OUR OUTPATIENTS," Stephen Sastra says with a sweep of his hand. "These are our inpatients. And this is our maternity ward." Sastra is a research scientist originally from Melbourne, Australia, who has spent 15 years operating on small animals. The mouse hospital, with its racks of cages and musty smell, is his domain.

Clad in protective booties, gloves, a yellow gown, and a hair covering, Sastra keeps a close eye on more than 750 animals. Some (the "outpatients") are healthy but, thanks to their mutations, at high risk of cancer; others are sick; and others are nursing their newborns, the next wave of study enrollees. Real estate in New York is at a premium even for mice, so the Olive lab owns another 207 animals in a facility in Massachusetts.

Some mice with cancer have a biopsy at diagnosis, so that the tumor can later be compared with its treated version—in humans and mice, cancer cells often change after treatment. That can help the researchers decipher why a drug succeeds,

ILLUSTRATION: C. SMITH/*SCIENCE*

or fails. After gemcitabine flopped in their mice, as they'd hoped it would, Olive and Tuveson found that the stroma—the healthy cells surrounding the tumor—shield the cancer from chemotherapy. In a 2009 paper in *Science*, they speculated that this might help explain why so many drugs don't help pancreatic cancer patients.

Other scientists are using revamped mouse models to explore the diversity of cancer in people. "Personalized medicine and targeted therapies in fact stand on exceptions," Trusolino says. He is particularly interested in patients who are resistant to drugs, especially those whose resistance can't be explained by known genetic mechanisms. When he gave his PDX mice an antibody commonly used in metastatic colon cancer, a subset didn't respond, just as happens in people. Trusolino found that about 20% of these nonresponders had alterations in a gene called *HER2*, which is a drug target in breast cancer. He experimented with giving them two breast cancer drugs. The tumors receded. A clinical trial, called HERACLES, is now enrolling colon cancer patients with the same alterations to test the same drugs. Results aren't out yet, but Trusolino says tumors are shrinking or stable in many patients. "It is the first time in my life that I see results from my lab translated into a clinical benefit," he says.

Knowledge like this can inspire streamlined clinical trials. It can also clarify how to use drugs already on the market. "Sometimes we're catching up," says William Sellers, vice president and global head of oncology at the Novartis Institutes for BioMedical Research in Cambridge, Massachusetts. "We are going back and doing this to get more data on drugs we have."

Most believe that these new mice adhere far more closely to human biology than did their predecessors. But they are not without pitfalls. In PDX mice, the healthy human tissue, or stroma, that's transplanted is replaced over time by a mouse version, and it's not clear if that alters drug responses. The animals lack a functioning immune system—otherwise, the transplanted human tumor won't engraft. But that limits which drugs can be tested, ruling out immunotherapies, and it may also affect how closely the mice reflect human biology. In the genetically engineered mouse, the tumors may lack the genetic diversity seen in people, and the animals often have lesions scattered throughout their organ. "I think we've got to stop looking at models as faithfully recapitulating disease," says Carol Bult, scientific director of the PDX program at the Jackson Laboratory in Bar Harbor, Maine. "You pick a model to address the question you're asking."

What's more, the animals aren't for everyone. Each of Olive's mice comes with a $1380 price tag from breeding until death. His mouse hospital costs many hundreds of thousands of dollars a year to run. There is also a high cost in time: for PDX mice to engraft the tissue, and for genetically engineered mice to develop cancer. Olive's animals live 5 1/2 months on average, which, he says, "is a long time sitting on a shelf."

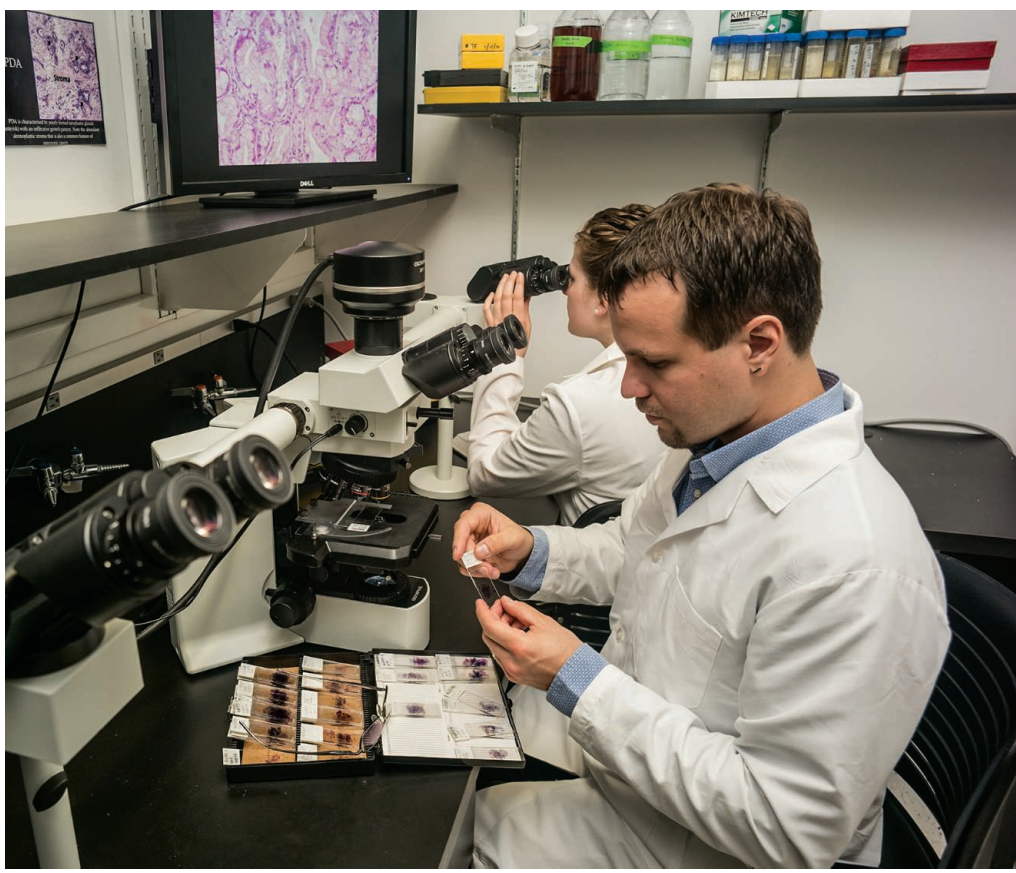
FAILURE PROPELS OLIVE FORWARD. After the Infinity news broke, his dejected group generated 21 hypotheses, then whittled them down with the help of patient data shared by the company. Ultimately, one theory rose to the top. The animals were treated for a few weeks. The patients were treated for months. What if there was an acute response to the drug that was positive, Olive wondered, and a chronic one that was not? They began a new experiment, enrolling mice with precancerous lesions and treating them longer.

"One hundred percent were dead by 4 months," Olive says. "It was a dramatic difference" from the study that had swayed Infinity, in which the mice getting the drug lived twice as long after the study began as the others. The animals' tumors, he also discovered, were poorly differentiated, a type that grows and spreads especially quickly.

The therapy had been designed to disrupt the stroma in the hope that doing so would make it easier to treat with chemotherapy. In the short term, that worked. In the long run, it destabilized the cancer and changed its pathology.

Olive could breathe again. His mice, and his career, were safe for now. But the episode exposed how easily everyone had misread the original experiment. Although the animals had lived an extra 2 weeks, any tumor regression had been fleeting. The experience "changed my outlook on what constitutes a response" in a mouse, Olive says. He now wants to see impressive tumor shrinkage, not just slower growth.

At lunch one Wednesday in early September, Olive ran into an oncologist colleague at a sandwich shop across the street. The two chatted amiably. That doctor, Olive later shared, is caring right now for a 28-year-old with pancreatic cancer. It's "unheard of" for the disease to strike someone so young, he says. These are the stories that keep him focused on saving patients, one mouse at a time. ■



Probing cancer biology, Kenneth Olive and graduate student Jaime Eberle examine pancreatic tumor samples from their genetically engineered mice.

PHOTO: DREW GURIAN