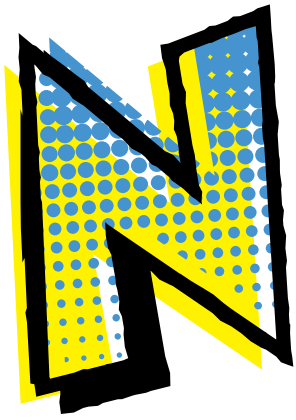
MY MIGHTY MOUSE

Personal drug regimens based on xenograft mice harboring a single patient's tumor still need to prove their true utility in medicine.

BY MEGAN SCUDELLARI



No two cancers are alike. But tumors, across the myriad permutations of the disease, share one characteristic: unpredictability. That unpredictability includes how the tumor will react to treatment. Because of the toxicity of chemotherapy, no patient wants to find out by trial and error how his or her particular tumor will respond to a given drug. So doctors have long sought ways to identify which therapies will be most beneficial—before actually treating the patient.

In the early 1980s, researchers commonly tested drug efficacy against patient tumor cells in a petri dish, but the method often failed to predict treatment success. At the University of Freiburg in Germany, oncologist Heinz-Herbert Fiebig had a different idea. Fiebig had been implanting pieces of human tumors into mice with compromised immune systems, which do not reject foreign tissue. He surmised that human tumor cells grown in a living, breathing mouse, instead of a dish, would be more likely to predict the drug response of an actual human tumor.

Over eight years, Fiebig tested his idea by transplanting fragments of more than 400 human tumors, each about the size of the head of a pushpin, just below the skin of immunocompromised mice. Using 80 of the successful tumor xenografts—from the Greek "xenos" meaning "foreign"—he compared how the mouse's tumor responded to a drug or drug combination with the treatment response of the corresponding human patient. Of 21 patients who responded to a particular drug or drug combination, their xenograft mice correctly predicted the outcome 90 percent of the time. Similarly, of 59 patients who did not respond to treatment, the mice correctly reflected such tumor resistance in 96 percent of cases.[1]

"We found the system was very predictable and very useful for screening drugs," says Fiebig. But the idea of building mouse models for individual patients to guide treatment was impractical, he found. The xenografts often grew too slowly to provide timely results; more than half of the 80 patients needed treatment before their mice yielded actionable data. Sometimes the grafts did not grow at all; Fiebig obtained successful engraftments only 50 percent of the time. Simply making and maintaining the mice was also prohibitively expensive.

Some see mouse avatars as the epitome of personalized medicine. Others argue that they are far from ready.

In a 1988 paper summarizing his findings, Fiebig concluded that xenograft mice were wonderful models for broadly testing new drugs against human tumors, but they "cannot be used as a clinical routine method" for predicting patient treatment.[1] The idea of using xenograft mice as personal avatars for cancer patients was discarded. But not forgotten.

More than two decades later, avatars are making a comeback, driven largely by New Jersey–based Champions Oncology. The company will develop a personalized mouse model for any cancer patient who can afford the service, which is not covered by insurance.

"The idea of really trying to match the perfect drug to the perfect patient is something we're all really trying to invest more effort into," says Keren Paz, chief scientific officer of Champions, which has ongoing collaborations with numerous universities and hospitals.

These days, mice grafted with human tumors, known as patient-derived xenograft (PDX) mice, are common in cancer research laboratories. In academia, researchers often create the mice as live mammalian models of whatever type of cancer they are studying. Additionally, organizations such as Freiburg-based Oncotest, a company founded and directed by Fiebig, and the Jackson Laboratory in Bar Harbor, Maine, provide access to a wide range of PDX mice made from donated tumor tissue. After growing the donated tissue in mice, they cryopreserve some of it for future use, and offer drug-testing services to researchers and pharmaceutical companies. Oncotest, for example, provides drug-testing services to 16 of the 20 largest pharmaceutical companies, using a library of more than 350 PDX mouse models.

But while most PDX mice are used as general models of human cancer in the laboratory, others seek to use them as Fiebig originally hoped—as avatars to guide customized patient care. Indeed, some see mouse avatars as the epitome of personalized medicine and point to the handful of success stories in which such mice have revealed new treatment options for patients who'd failed to respond to traditional therapies. Others, however, argue that PDX mice are far from ready to be used as a routine part of clinical care.

"No one has really shown that [using PDX mice to evaluate drug responses] actually changes and improves outcome," says Judy Boughey, an oncologist and researcher at the Mayo Clinic in Minnesota, who is using PDX mice in a breast cancer study. "[The technique] requires

further evaluation and validation before it's ready for everyone to just go and have their tumor xenografted." Indeed, PDX mice have yet to undergo a controlled, prospective clinical trial to compare avatar-based predictions with physician recommendations, she notes.

"This platform has tremendous potential to advance precision oncology, where we can use a patient's tumor DNA to really tailor therapy, but we have to be really rigorous about the science of developing the platform," agrees Carol Bult, director of the PDX/Cancer Avatar Program at the Jackson Laboratory.

for each patient in the trial. "If you made a prediction from a human *and* it works in a mouse avatar, now the chances of it working back in a human are much, much higher," says Califano.

The goal, for now, is to prove that the drug predictions from the computer model successfully treat their corresponding mouse tumors. So far, they do: the team identifies a drug or combination therapy that works in the mice almost 100 percent of the time, says Califano. The next step will be to use the computer model results and PDX mice in a clinical trial to guide patient care by sharing the results with physicians.

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>
> —Andrea Califano, Columbia University

Corralling cancer

Fueled in part by interest generated by Champions, more and more scientists now use PDX mice to study the potential cancer drug responses of individual patients. Their hope is that these personalized cancer models might allow doctors to more effectively treat cancer based on the specific molecular makeup of a patient's tumor, achieving the ideal of precision medicine, as touted by President Obama in this year's State of the Union address. (See "The Challenges of Precision" on page 31.)

At Columbia University in New York City, systems biologist Andrea Califano and pediatric oncologist Andrew Kung are using patient-specific PDX mice to assess breast, neuroendocrine, and brain cancers. First, the team sequences DNA and RNA from tumor biopsies and inputs that information into a computer model that identifies a handful of dysregulated proteins necessary for survival of the tumor cell, what Califano calls the "master regulators." The computer then predicts three drugs and three drug combinations likely to target those master regulators. Instead of testing the drugs in cultured cells as he might have done in the past, Califano tests them in PDX mice, developed individually

Researchers at the Mayo Clinic are taking a similar approach, using PDX mice to match genetic characteristics of an individual's tumor to specific drugs. From 2012 to 2014, the Breast Cancer Genome-Guided Therapy (BEAUTY) study enrolled 140 women with high-risk breast cancer. Each of the patients underwent tumor biopsies before, during, and after a 20-week regimen of chemotherapy. A team led by Matthew Goetz and Boughey used the biopsies for sequencing and to create PDX mice for each participant. The researchers are now linking the genetic information with the human drug responses and exploring drug options in the chemotherapy-resistant PDX mice. Once again, the next step will be to see if the results beneficially impact patient care, Goetz says.

Before that happens, however, there are still a handful of challenges that must be overcome. As Fiebig found in his early work, tumor samples do not always successfully graft onto the mice. Boughey and her colleagues achieved only about a 40 percent graft rate, she notes. Not surprisingly, the more aggressive a tumor, the more likely it is to engraft—probably because those cancer cells are particularly adept at growing and spreading. Mayo's Prostate Cancer Medically Optimized Genome-Enhanced Therapy (PROMOTE) study, inspired by BEAUTY, has had even more difficulty getting grafts of prostate cancer to take hold in mice. Since the study began in June 2013, only 6 of 80 total tumor grafts, or about 7 percent, have been successful.

"There is some talk of going into 3-D cell models rather than xenografts, because it is so difficult and challenging to grow [the transplanted tumors]," says Manish Kohli, the Mayo Clinic oncologist leading the PROMOTE study. "We're probably going to have to make a call by midsummer."

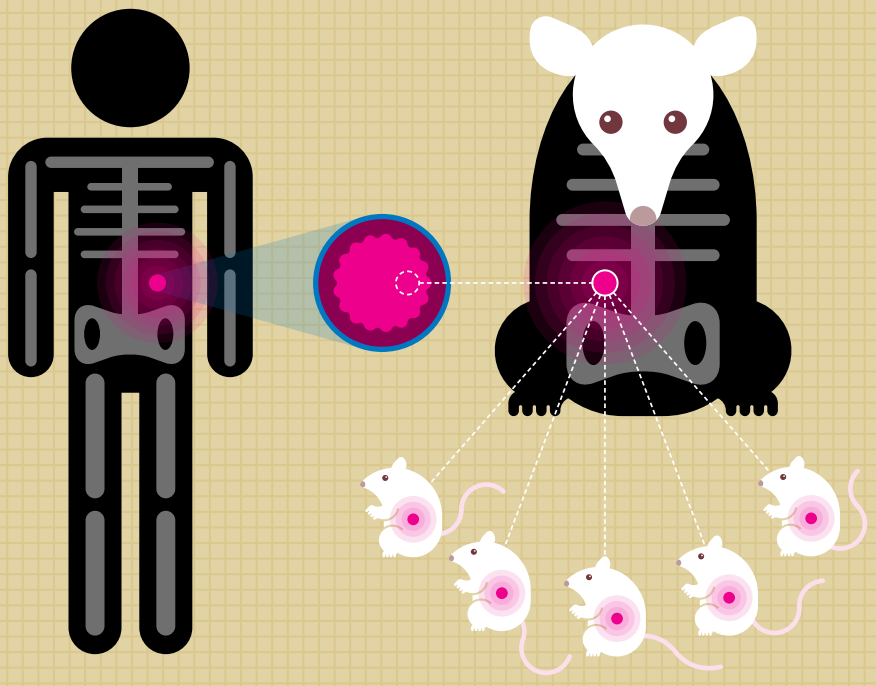
Even if the grafting challenge is overcome, the cost of developing individualized PDX mice still stands as the reigning barrier to their widespread use. First, a single xenograft mouse is not enough. To make a model system, a piece of a human tumor is implanted and grown in several immunocompromised mice, then harvested, fragmented, and implanted again in a larger set of mice that are treated with a variety of drugs and drug combinations. The cost of creating and maintaining the mice, plus doses of expensive drugs, can put the price tag of a PDX experiment at tens of thousands of dollars—for just a single patient's tumor.

Even using PDX mice in government-funded basic research can be cost-prohibitive. Bult, of the Jackson Laboratory, recognizes that mice are not always the right option. The Jackson Laboratory maintains a resource of more than 300 PDX mice developed from a range of cancers. The mice are used in numerous studies at the laboratory as a preclinical platform to test novel therapeutics against specific tumor types, but in some cases the expense is not appropriate for a study. "We are looking at cell-based and 3-D culture–based methods that would allow us to get to the same endpoint for some drugs faster and cheaper," says Bult.

But while 3-D cell cultures are good at predicting a tumor's drug resistance, notes Fiebig, whose company, Oncotest, offers such testing in addition to xenograft mice, they are not as sensitive to detecting what

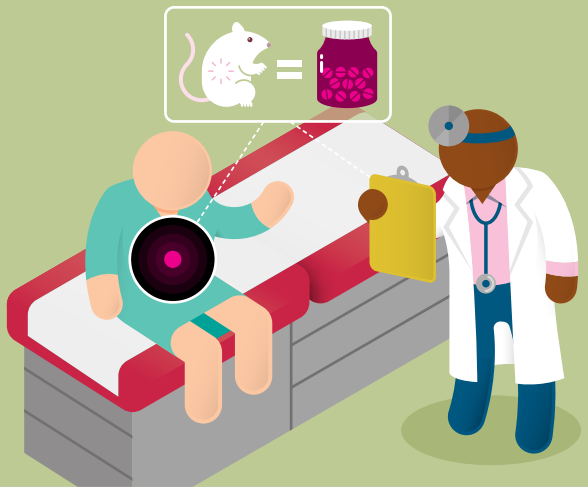
CANCER AVATARS

Patient-derived xenograft (PDX) mice provide arguably the closest model to human cancer available without using humans themselves. PDX mice are derived from donated human tumor tissue and may be used for biomarker-driven cancer research, preclinical drug testing, or to predict the drug responses of a specific patient's tumor.

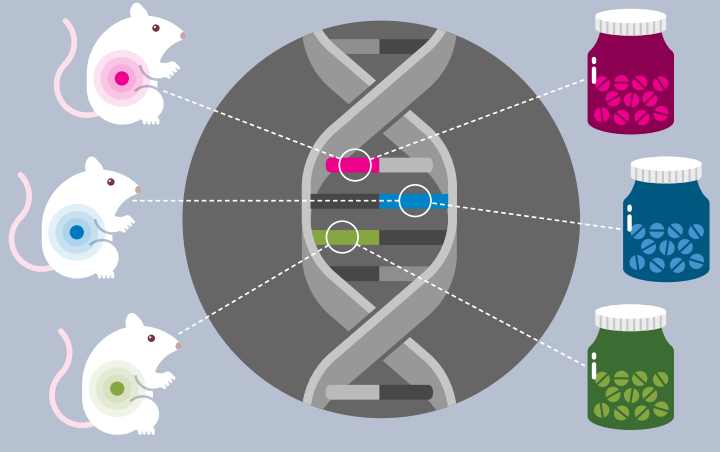


To develop a PDX mouse model, a researcher grafts a bit of a tumor into a handful of mice. Tumor sections from a successful graft expand, then are harvested, divided, and regrafted into many more mice, providing a large number of animals on which to test a variety of drugs or drug combinations.

For individualized mouse avatars sold by Champions Oncology directly to patients, treatments that show success in shrinking the mice's tumor may then be attempted in the patient, with the guidance of an oncologist. This strategy will soon be tested in clinical trials to see if drug predictions from the mice have a positive impact on patient outcomes.



More commonly, academic and pharmaceutical researchers use donated tumor tissue to create PDX mice with a variety of cancers for preclinical drug screening. In this case, researchers analyze donated tumor tissue using whole genome or exome sequencing, then test a drug in question against mice engrafted with that tissue. This research is helping to guide early-stage drug development for specific subtypes of cancer based on genetic markers.



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drugs will kill a tumor in the first place; 3-D assays are around 60 to 70 percent accurate for predicting responsiveness, as compared with 90 percent for PDX mice.

The patient's Champions

For those who can afford them, PDX mice are commercially available from Champions Oncology, which boasts a much higher graft-acceptance rate than academic labs—ranging from 25 percent for less aggressive cancers up to 90 percent for highly metastatic cancers, according to the company. Johns Hopkins University's David Sidransky, chairman of the company's board of directors, attributes that success to optimization of the grafting process, including restricting the amount of time between surgery and implantation in the mouse. The company has labs around the world, from London to Israel to Singapore, to reduce the amount of time a given tumor sample spends on a plane, for example.

The growing process takes three to six months, says CSO Paz, and the xenografts, which the company has dubbed "TumorGrafts," are routinely analyzed using exome and RNA sequencing to confirm that they match the genetic profile of the original tumor. "We see they are nearly identical," says Paz. "It definitely keeps a high level of fidelity; . . . you can trust them to reflect sensitivity to drugs."

In a handful of anecdotal successes, the TumorGrafts have demonstrated their value. Fifty-year-old Evan Rose thought he had beat cancer in 1985, when he was successfully treated for metastatic testicular cancer. But 22 years later, ear pain led his doctors to discover a large mass in his neck. He was eventually diagnosed with a rare sarcoma called ossifying fibromyxoid tumor. Only about 200 cases have ever been recorded.

With such a rare cancer, there was no standard drug therapy for Rose's condition. "I don't know how many different chemos I went on. We'd start one, a couple months later do a CT scan, see it wasn't working, and start another," recalls Rose, an urban designer and architect living in Brooklyn with his wife and young son. "For almost three years—you name it, I tried it." None of the drugs shrank his tumors, and Rose was constantly ill due to toxins coursing through his body.

Tired of being a guinea pig in the search for remission, in August 2012 Rose sent his excised larynx tumor to Champions. "Fortunately, we had the resources to be able to do that," he says, estimating the mice initially cost between $30,000 and $40,000, plus another $20,000 for a second round.

After a seven-month wait, Rose got results from his TumorGraft mice in February 2013. By that time, he had become very sick, and his doctor didn't expect him to make it through the year without a successful therapy. The TumorGraft results pointed to one promising combination, which Rose began in March. By April, a scan showed an unprecedented image—his tumor had shrunk. "My doctors were literally jumping up and down," says Rose.

That drug combination worked for about a year before Rose's cancer became resistant in April 2014. He moved onto a second regimen predicted by his mice, but this time, the drugs did not work. Rose is currently awaiting results from a new round of mice in development using newer tumor cells. "We'd like results from Champions again," says Rose. "We're not out of options yet."

Champions has recently been working to prove the utility of their method outside of anecdotal stories like Rose's. In a study published last April, Sidransky and colleagues created TumorGrafts for 29 patients with advanced metastatic sarcoma. Of the 22 patients whose tumors successfully grafted, six died before data from the mice were available, but in 13 of the remaining 16 cases, there was a positive correlation between mouse and human results.[2] In a second study, performed in collaboration with Manuel Hidalgo of the Spanish National Can-

MINI-ME MOUSE: Champions Oncology sells mouse avatars, in which a sample of a patient's tumor is grafted under the skin of immunocompromised mice. These patient-specific rodent models are then used to test treatments that might work on the patient's cancer.

A FLY ALTERNATIVE

The two greatest barriers to widespread use of mouse avatars are the time and expense required to breed and maintain mice engrafted with human tumor tissue. An obvious way to surmount those obstacles is to use a cheaper, faster-breeding organism.

Enter the humble fruit fly. Ross Cagan's laboratory at Mount Sinai Hospital in New York City has developed a method to recreate human tumors in *Drosophila*, which is believed to have functional homologs for some 75 percent of human disease-causing genes. First, the researchers analyze sequence data from an individual's tumor and identify four to 10 genes altered in the cells. Next, they genetically modify a fruit fly to dial up or down the activity of those genes, and, if possible, do so in the location where the original human tumor was found. For example, to mimic a colorectal cancer, the team alters genes in cells of the fruit-fly gut. The result is a fly tumor: cells that proliferate, invade nearby tissues, and even metastasize to other parts of the body.

The tumor-riddled flies are then used in a unique high-throughput drug-screening process. A total of 960 flies are grown in a plate with 96 wells. Ten embryos are hatched per well, each of which contains food laced with a different drug or drug combination. The young cancerous flies are very sick, but begin eating the food. The flies that survive are those treated with drugs that were effective against the tumor but not so toxic as to kill the fly. "We set up the model so the flies are [almost] dead, if you will, and screen for drugs that bring them back to life. Anything moving—you're in," says Cagan.

The team creates and screens about 100,000 fly avatars, as Cagan fondly calls them, per patient in about six months, and for a fraction of the money it takes to make a PDX mouse. And while personalized fly models are not yet available to cancer patients, the process has proven useful to human disease. In 2005, Cagan's team created a general fly model of a human thyroid tumor caused by mutations in the Ret receptor tyrosine kinase gene, then screened a panel of drugs including a kinase inhibitor called vandetanib that suppressed the tumor (*Cancer Res*, 65:3538-41, 2005). Based on data that included those from the fly, AstraZeneca took the drug into human clinical trials, and in 2011, vandetanib became the first drug approved for late-stage medullary thyroid cancer.

But fly avatars won't work for every type of cancer, Cagan notes, as fruit flies do not share hormones similar to ours, so hormone-dependent diseases, such as prostate cancer, cannot be recapitulated. But we may be seeing more of these flies soon. Cagan's lab recently received approval from the US Food and Drug Administration to begin a clinical trial in colorectal and medullary thyroid carcinoma to see if fly avatars can successfully help guide patient care. More than a dozen cancer patients have already applied to participate in the trial.

cer Research Center, the team found that 6 of 13 patients with advanced solid tumors who were treated based on results from personalized PDX mice had partial tumor remissions, even in cases where genetic sequencing of the tumor showed no actionable mutations.[3]

Champions would like to conduct a prospective controlled study in which physician recommendations will be compared side by side with the recommendations derived from mouse avatars, says Paz. A head-to-head comparison would go a long way toward showing that PDX mice are not just a nice model for human cancer, but can actually improve patient care.

Such data will be needed to convince a largely skeptical community of cancer researchers and physicians. Today, doctors have clear guidelines to treat tumors with specific drugs, and they are unlikely to deviate from those established procedures unless all else fails, says Fiebig.

Rose acknowledges that the mice aren't right for everyone, but for him, they made a difference. "So far it bought me at least two years of life," says Rose. "It seems like money well spent."

Unfortunately, stories like Rose's are few and far between. While PDX mice used for basic research have helped identify drug candidates, personalized mouse avatars have yet to demonstrate clear changes in the course of a person's disease, says Edward Sausville, an oncologist at the University of Maryland School of Medicine. Because of that, he says, "I would categorically never recommend it to my patients."

"This could be a fruitful area in the future, but we have to show there is value," agrees Goetz.

In the end, many oncologists believe that PDX mice are a powerful means to an end—achieving successful, individualized treatment plans for cancer patients based on the genetic makeup of their tumor—but not the end itself, as developing PDX mice for every cancer patient is simply not practical. If researchers can use the mice to gather information about specific tumor responses to specific drugs, then they can bank that information to be used in the general population.

"We're looking to jump over this need to have an individual patient have an avatar," says Bult. "We want to make this approach of tailored cancer therapy faster and cheaper for everyone." ■

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