# THE MICE THAT GROW HUMAN TUMOURS

*They were supposed to be ideal models of disease. Now researchers are discovering the limits of patient-derived xenografts.*

BY CASSANDRA WILLYARD

Lindsey Abel takes an anaesthetized mouse from a plastic container and lays it on the lab bench. With a syringe, she injects a slurry of pink cancer cells under the skin of the animal's right flank. These cells once belonged to a person with tongue cancer, a former smoker whose disease recurred despite radiation and surgery. The mouse is the second rodent to harbour them, creating a model for cancer known as a patient-derived xenograft (PDX). The tumour that grows inside will provide cells that can be transferred to more mice.

Abel has performed this procedure hundreds of time since she joined Randall Kimple's lab at the University of Wisconsin–Madison. Kimple, a radiation oncologist, uses PDX mice to carry out experiments on human tumours that would be impractical in people, such as testing new drugs and identifying factors that predict a good response to treatment. His lab has created more than 50 PDX mice since 2011.

Kimple's lab is not the only one doing this; PDX mice have exploded in popularity over the past decade and are beginning to supplant other techniques for modelling cancer in research and drug development, such as mice implanted with cancer cell lines. Because the models use fresh human tumour fragments rather than cells grown in a Petri dish, researchers have long hoped that PDXs would model tumour behaviour more accurately, and perhaps even help to guide treatment decisions for patients. They also allow researchers to explore the vast variety of human tumours. PDXFinder, a catalogue launched earlier this year, lists more than 1,900 types of PDX mouse. But there are many more scurrying around in academic and industry labs — as many as 10,000 PDXs have been created, says Nathalie Conte, a bioinformatician at the European Bioinformatics Institute, in Hinxton, UK, who leads PDXFinder.

PDX models are not perfect, however — and scientists are beginning to recognize their

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shortcomings and complexities. The tumours can diverge from the original sample, for example, and the models cannot be used to test immunotherapies. Now, biologists are scrutinizing PDX mice and looking for creative ways to cope with the challenges. "Every model is artificial in some way," says Jeffrey Moscow, head of the investigational drug branch at the National Cancer Institute in Bethesda, Maryland. "The real question is how predictive are these models going to turn out to be."

## RISE AND FALL OF THE AVATARS

Scientists have been transplanting human cancers into mice for more than 50 years. In the 1960s, for example, researchers removed a tumour from a 74-year-old woman with colon cancer, minced it and injected the fragments under the skin of mice without immune systems. The tumours grew and were then cut up and transplanted into more mice. The approach didn't gain much traction, however. Instead, many researchers relied on mice implanted with human cancer cells that had been grown in a dish, because that is cheaper and easier than using fresh tumour fragments from biopsies.

But in the early 2000s, researchers began to worry that cell-line xenograft models might not be very representative of human cancers. They realized that drugs that worked in these mice rarely worked as well in people, in part because the cells change in culture over time. So researchers turned again to PDX models.

One early adopter was Manuel Hidalgo, a cancer researcher at Harvard Medical School in Boston, Massachusetts. In 2002, he began working with a woman who had bile-duct cancer. Hidalgo proposed injecting her tumour cells directly into mice and seeing which drugs worked best on them. Four years later, Hidalgo co-founded a company aimed at generating these mouse 'avatars' for many more patients. That company — now part of Champions Oncology in Hackensack, New Jersey — began offering these models to oncologists and patients as a tool for determining the treatments most likely to work. Some people predicted that personalized mouse models would become a routine part of cancer treatment.

But the approach didn't pan out the way the company had hoped, Hidalgo says. Last year, he and his colleagues published a study[1] that included 1,163 people who sought the services of Champions Oncology. Because not all tumours grow in mice, the company managed to generate PDX models for only half of them.

For many of these people, the mice came too late or physicians didn't follow up with avatar testing. Still, the models do seem to be predictive: the researchers identified 92 patients who received treatments based on testing in the PDX models, and found that the PDX predictions were accurate 87% of the time.

Although the company still creates avatars for people who want them, it shifted its focus away from the personalized models about three years ago, according to chief executive Ronnie Morris. They took too long to deliver answers, and they cost too much. "It was just a bad business for us," Morris says.

## SCIENTIFIC STAND-INS

Meanwhile, the popularity of PDX mice has soared in the research realm. Scientists have embraced the models to improve their understanding of tumour biology and to find new drugs. And yet questions remain as to whether they are better than previous models.

Todd Golub, head of the cancer programme at the Broad Institute in Cambridge, Massachusetts, and his colleagues analysed the genomes of hundreds of PDX models representing dozens of cancer types. They were looking at duplicated stretches in the genome and how they changed as the tumour cells passed through several live mice[2]. The tumours evolved quickly: by the fourth passage, 88% of the PDX models had at least one large chromosomal aberration, and a median of 12% of the genome had been affected.

Juliet Williams, head of oncology pharmacology at Novartis in Cambridge, says it has been clear for some time that genetic changes occur. "The question is, does that small amount of drift that you see matter functionally?" she says. In 2015, Williams and her colleagues put together a panel of 250 PDX models and used them to test more than 60 drugs and drug combinations, including a handful that had been approved[3]. They found that the PDXs responded to approved drugs just as human responses predicted. And all the data Williams and her colleagues have collected since then suggest that tumours in PDXs respond as they do in people.

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But when Golub and his colleagues reanalysed the data, they found three cases in which genome changes might have altered the outcome of the testing. Golub doesn't think that PDX mice should work any better than mice implanted with cell lines. "I just don't see the PDXs as being some magically different thing," he says.

Golub and a colleague have argued for an international effort to establish more than 10,000 cancer cell lines[4]. This would be a boon, says David Weinstock, an oncologist at Harvard Medical School, and might obviate the need for PDX mice. But there are fewer than 2,000 cell lines available right now, and generating new ones is tricky. And although xenograft mice from these lines could be valuable, researchers have had more success in skipping the cell-line step to make PDX mice directly. "We've made 350 leukaemia and lymphoma models in one laboratory with not that much money and not that much expertise," Weinstock says. "We can't make 350 cell lines."

## A MORE-HUMAN MOUSE

The real Achilles heel of PDX mice, however, is that to get the tumours to grow, researchers must use animals that lack an immune system. That makes it impossible to use PDXs to test immunotherapies. Several groups are now working to change that.

The Jackson Laboratory in Bar Harbor, Maine, takes stem cells from a human umbilical cord and injects them into mice that are a few weeks old. These stem cells differentiate and form some parts of the human immune system, mostly T cells. The researchers then transfer human tumours into those mice. "Nobody thought this would work," says James Keck, a cancer researcher at the laboratory, because the umbilical-cord donor doesn't match the tumour donor, so the T cells should attack the tumour. But the tumours have defence mechanisms to block the immune system, so "nine times out of ten, the tumour still grows", says Keck. That has allowed scientists to test immunotherapies in a mouse model with human immune cells.

And just like in humans, these therapies don't always work. For instance, Keck and colleagues have found that pembrolizumab, which ramps up the T-cell response, curbs bladder-cancer growth in mice carrying stem cells from one donor but not in mice carrying cells from another, even though both mouse types carried pieces of the same tumour[5]. "We're actually getting close to what everybody has been asking for: a mouse model that mimics what's going to happen in the clinic," Keck says.

Ideally, researchers would like to create mice with tumour and immune cells from the same person. Meenhard Herlyn, who studies melanoma at the Wistar Institute in Philadelphia, Pennsylvania, and his colleagues are trying to use skin or blood cells from a patient to generate induced pluripotent stem cells, which could then be used to create immune cells. The model is almost complete, Herlyn says.

But even these next-generation PDX models have drawbacks. For example, human connective and vascular tissues in the tumour transplants are gradually replaced by mouse equivalents as they pass between mice.

Still, Keck is excited about the possibilities. "This is not your dad's or mom's xenograft any more," he says. "These are models of complexity. We've now gone into a whole new level of oncology research." ■

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