

The Scientist

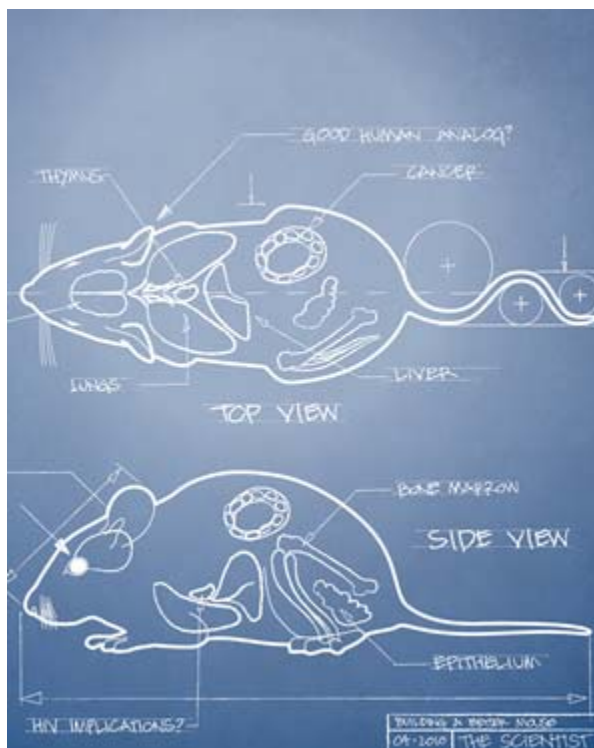
Volume 24 | Issue 4 | Page 34

By Edyta Zielinska

Building a better mouse

A notoriously poor proxy for the human experience of cancer, mouse models are now undergoing a major renovation.

She sits in the tiny consult room, with her husband at her side, fingering the stitches on her purse as her oncologist describes what he sees in her CT scans. “There’s no change,” in the lung cancer from the last time she was scanned. This is good news, he tells her, and she nods her head, but her fingers, still working, betray her lack of confidence in his response. She has already been on five different therapies, all of which failed to stop the spread of the disease. As the oncologist starts describing the clinical trial she is about to enter, in which she will try a new drug designed specifically for her type of cancer, however, her fidgeting begins to ease.



Illustrations by Andrew Meehan

On the evening news, she had heard about a promising new therapy for lung cancer. It appeared to be extremely effective, but only for patients with a specific translocation of the anaplastic lymphoma kinase (ALK) gene, a mutation that’s involved in a number of cancers. The oncologist, Jeffrey Engelman from the Dana Farber Cancer Institute, was recruiting patients for a clinical trial of the drug, which had skipped Phase II, and gone directly to Phase III

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because of the efficacy it exhibited. She asked her oncologist to contact Engelman and after testing, she proved to be positive for the *ALK* mutation.

But this isn't the story of a woman trying yet another targeted therapy that might or might not work. This is the story of her mouse—or, more specifically, the group of mice that will shadow her as she undergoes the experimental treatment.

The mice, which will also shadow several other lung cancer clinical trials, will be treated the same as patients: receiving the same drugs and undergoing the same scans to test for tumor shrinkage in tiny mouse-sized MRI and PET machines. Many of the mice have been specially engineered for this purpose, and researchers hope to see the same outcomes in both groups. Specifically, they hope that patients and mice that receive the same treatment will show similar responses to it, with similar chances for survival and similar progressions of disease.

These are not just any mouse models. Most of the time, in order to test a cancer therapy, researchers simply transplant human cancer tissue into a mouse. But those experiments rarely predict how a human will respond to the same treatment. In this trial, however, principal investigator Pier Paolo Pandolfi and others have engineered the mice to develop cancers that carry mutations similar to those seen in cancer patients—mutations scientists suspect may explain why some patients respond to a particular treatment and some don't.

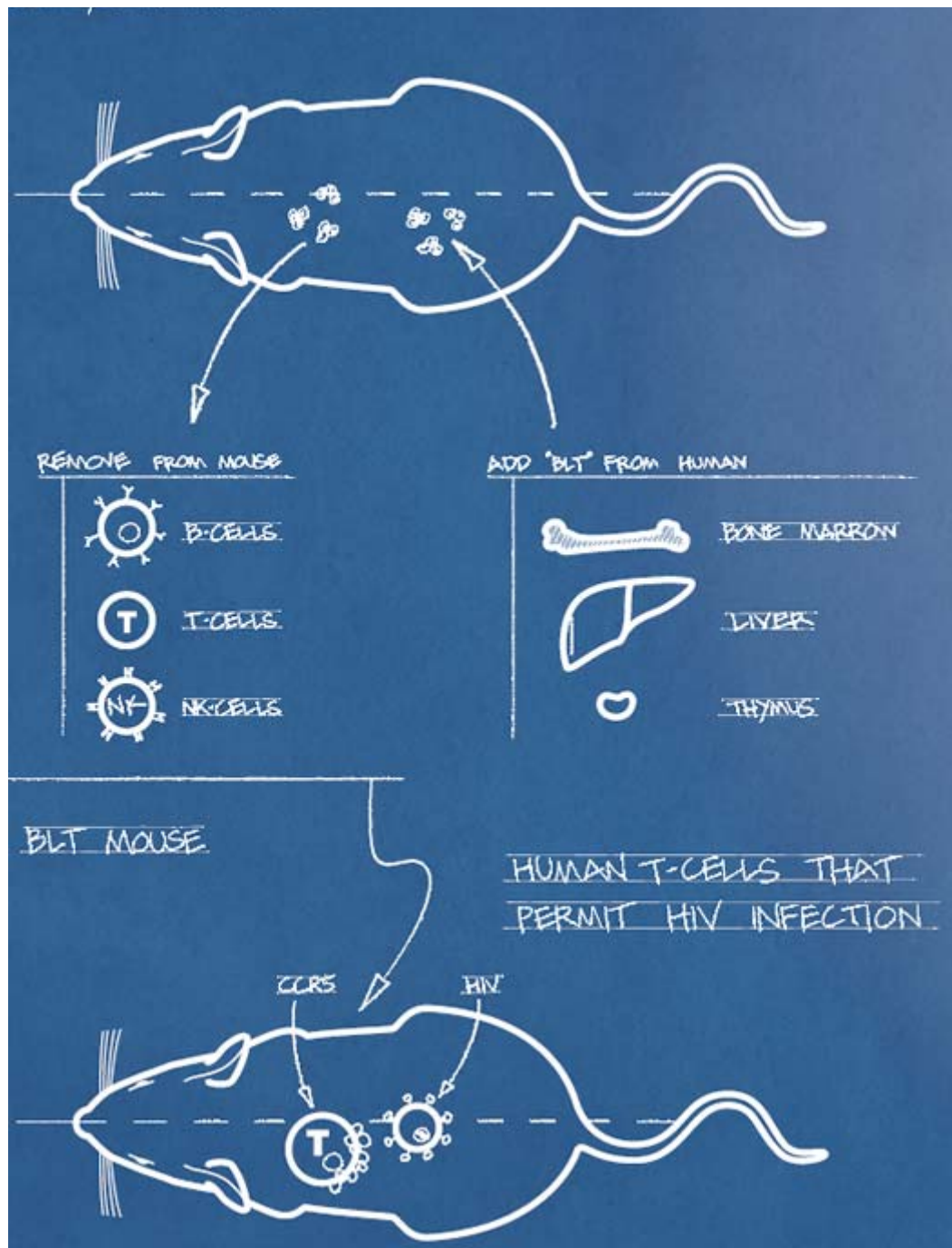
All of this, in the end, is for one purpose: to find a better mouse model for cancer, one that can successfully predict how a human cancer will progress and respond to treatment before the drug is ever tried in humans.

The goal: Find a mouse that can predict how a human will progress and respond to an experimental cancer drug.

Mouse models that use transplants of human cancer have not had a great track record of predicting human responses to treatment in the clinic. It's been estimated that cancer drugs that enter clinical testing have a 95 percent rate of failing to make it to market, in comparison to the 89 percent failure rate for all therapies.

Pandolfi's trial may help resolve the discrepancy between cancer mouse models and cancer patients, but to what extent remains unclear. The National Cancer Institute awarded the trial more than \$4 million in stimulus funds, but Cheryl Marks, the director of the NCI's division of cancer biology, isn't entirely convinced that these genetically modified mice will prove more predictive than the standard human transplant model. "This [trial] is the first thing that would make a believer out of you," she says. "To me, the jury is out."

There are many types of cancer that behave very differently in



Recipe: A Mouse Model of HIV

Mice don't express the CCR5 receptor that HIV uses to enter cells, making it impossible to test HIV vaccines and therapies in mice. In order to engineer a mouse with an HIV-infectable T cell, researchers inserted human bone marrow (containing T-cell progenitors), liver (for fetal immune development), and thymus (for T cell maturation) into an immunodeficient mouse, enabling it to produce CCR5-expressing human T cells. Using this "BLT" mouse, researchers recently demonstrated that retrovirals could prevent HIV infection if they were administered before HIV exposure, rather than as a treatment (*PLoS One*, 5:e8829, 2010).



mice than in humans. So in order to coax mice to accept and grow

a more humanlike cancer, scientists had to tinker. They began with the first immunodeficient mouse—the nude mouse, so named for its lack of fur. The nude lacked the *FOX1* gene, preventing it from forming a normal thymus, critical for proper development of the immune system. Researchers showed that they could engraft human cancer cells (called xenografts) that the mouse would not reject, but not all cancers grew well.

The SCID mouse, with a mutation in a gene involved in immune cell maturation, had an even more deficient immune system. When Richard Bankert from the University of Buffalo engrafted cancer under the skin of a SCID mouse in the late 1980s, he found that it grew “floridly,” he says. Finally, scientists could study the progress of cancer in the mouse. The SCID mouse soon became a favorite of pharmaceutical companies; the model was simple, reproducible, and relatively inexpensive. All researchers would have to do was take an established cancer cell line and insert it under the skin of the SCID mouse, regardless of where the cancer would have normally grown, then test the mouse’s response to an experimental cancer drug. Xenograft studies still provide the most common proof-of-concept data submitted to the FDA for a new cancer therapy. Most cancer drugs released to date were likely originally tested on SCID mice.

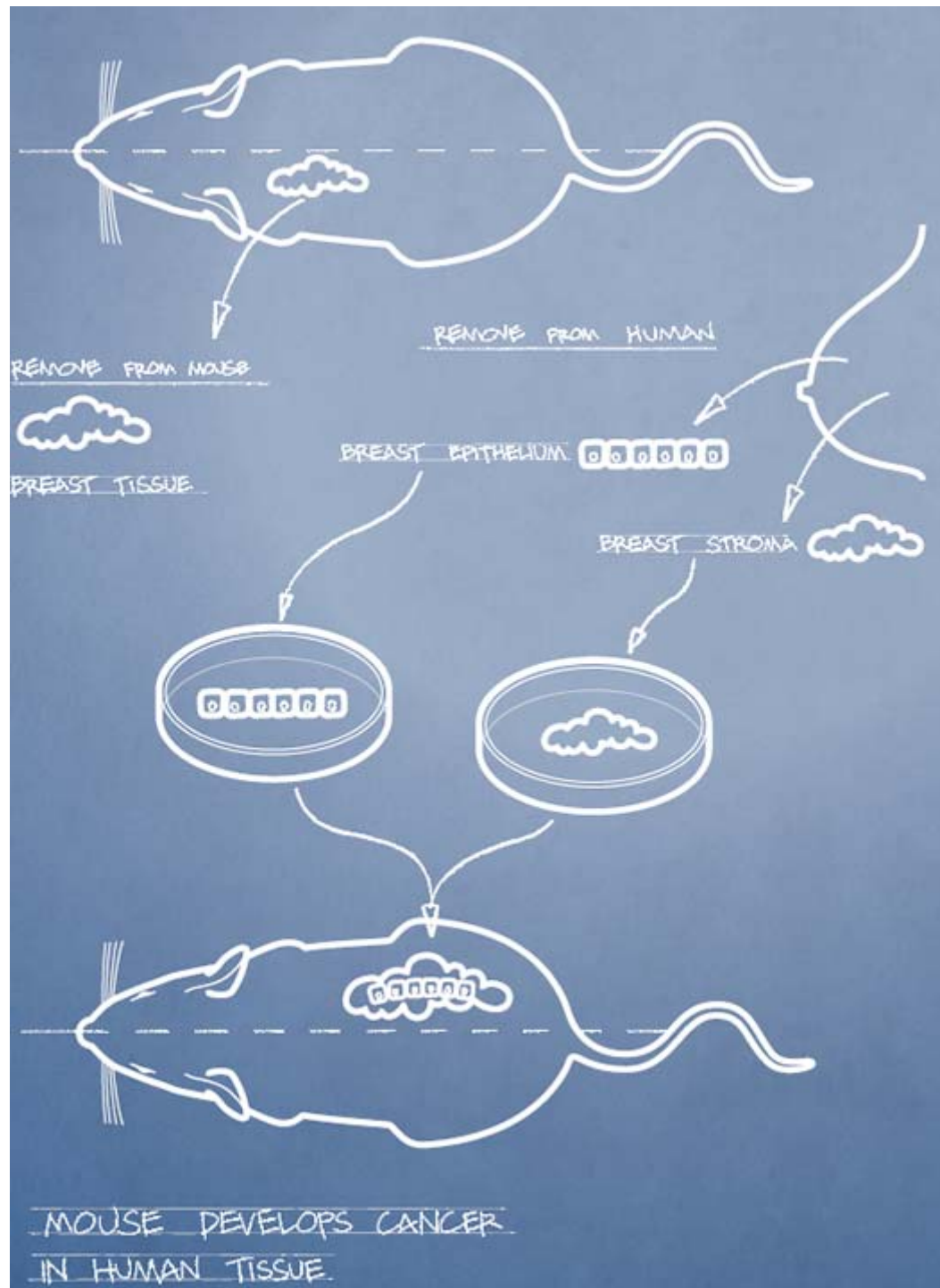
But it is a hugely flawed approach. Although the cancer cell lines are human in origin, they have been harvested years—sometimes decades—earlier and kept in culture, continually mutating (as cancers do) and adapting to their new plastic-dish environments. Plus, the tumor is not a homogenous tissue, but made up of different cells with different genetic mutations, of which scientists can insert only one small portion. “What you put in culture may not be the most representative clone. Every time you transplant you are misled by what you choose,” says Pandolfi.

“We have been interpreting the data badly.”

—Jeff Engelman

The next hit came when researchers realized that although the adaptive immune system (T and B cells) is mostly out of the picture, the innate immune system is still quite healthy in the SCID mouse. Specifically, natural killer (NK) cells, which have a particular talent for killing tumors, are abundant. Therefore, if the cancers in these mice regress, it would be unclear whether the regression stemmed from the therapy or NK cells attacking the tumor.

Scientists and companies compound the problem by putting too much stock in the results from these troubled models. “We have been interpreting the data badly,” says Engelman, who collaborates with Pandolfi. Researchers might see a statistically significant difference between the rate of tumor growth in mice treated with a cancer therapy and control mice. But if the cancer continued to grow in the treated mice, this should have nixed the treatment, since continued



Breast Cancer in Context

To learn how breast cancer develops in human tissue, researchers collected breast tissue from healthy women and separated it into epithelial cells and supporting tissue, or stroma. Researchers modified each cell type with genes that would cause or support cancer growth, mixed the cells and implanted them in a mouse whose breast tissue was removed. Like patients, only mice carrying the Her2 mutation responded to the Her2-mutation specific drug Herceptin (*PNAS*, 106:7022-7, 2009).

growth won't work in the clinic, says Engelman. "This is a bullshit result."

Indeed, “we had loads of models that were not predictive, that were [in fact] seriously misleading,” says NCI’s Marks, also head of the Mouse Models of Human Cancers Consortium, a network of cancer researchers (including Pandolfi) who set goals, meet regularly, and collaborate in their search for a better mouse model. In 2001, researchers at the NCI looked at the xenograft data from 39 cancer drugs that had already successfully completed Phase II trials in humans. Only one of the xenograft models showed a similar response to the cancer drug as the patients who received it (*Br J Cancer*, 84:1424-31, 2001).

Over the years, researchers made additional improvements to the SCID mouse, knocking out more genes to further deplete its immune system. They mated the SCID mouse with the nonobese diabetic (NOD) mouse, which removed most of the natural killer cells. A third advance was a knockout for the gene that produces IL-2 γ , a component of many immune cytokine receptors—now the most immune-compromised mouse to date. Researchers also began to transplant cancer cells directly from a patient’s biopsy, rather than an established cell line. Here, “you have a model of an individual patient,” says Marks. The benefit would be the specificity, but the cost is cost (a mouse per patient would be exorbitant), and even this mouse hasn’t proven predictive yet.

Meanwhile, while removing more and more of the mouse immune system, researchers were creating a blank slate, a vessel that could then receive any foreign tissue. From pieces of human liver, to a thymus, to beta cells for the study of diabetes, the immunodeficient mouse became more and more human with transplanted tissue. By inserting blood progenitors, researchers could even engraft a human immune system. But even this may not be enough.

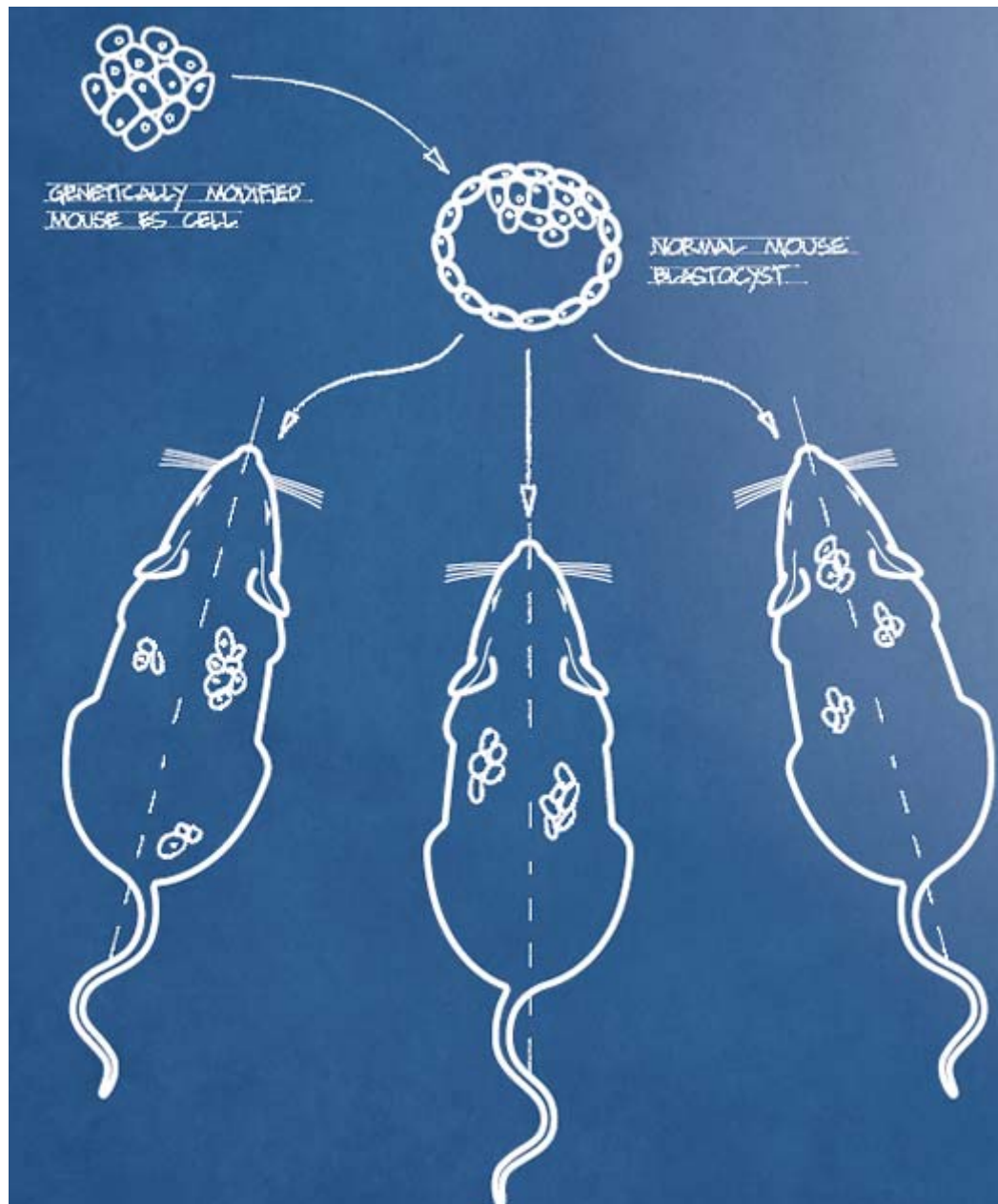


While knocking out a mouse immune system lets an experimenter introduce foreign tissue like cancer, it also fundamentally changes how that animal reacts to the cancer. Cancers are thought to interact with the immune system throughout their development (read “Cancer vs. the immune system” in our Nov. 2009 issue), which can change the course and outcome of the disease. One solution is to simply keep the mouse’s immune system intact, but genetically create a humanlike cancer by inducing the same genetic mutations in the mouse that cause cancer in humans.

Researchers have been genetically engineering mice for decades to learn more about various biological processes, but because of the species differences, no one really thought these modified mice could predict human outcomes in the clinic. Then, as an undergraduate student in the early 1990s, Pandolfi was involved in cloning the genetic mutations and translocations that characterized a small subset of leukemias called acute promyelocytic leukemia (APL). He and other researchers showed that there were actually two types of APL, one involving chromosomes 15 and 17, and another involving 11 and 17.

Once he had his own lab, Pandolfi inserted the same genetic changes into mice. This wasn’t just a mouse that would develop cancer; it would develop a human

type of cancer, and develop it the way a human would—spontaneously, from mutations, not in a petri dish. Mice have been known to develop other leukemias and lymphomas, but “they never develop APL,” says Pandolfi. Not only did his mice spontaneously develop APL, but they also exhibited many of the secondary symptoms of the disease such as low white blood cell counts and hemorrhagic disorder.



Genetically Diverse Human-like Mouse Cancers

Even cancers of the same type from the same patient can differ genetically from one lesion to the next. To incorporate diversity of entire patient populations for cancer drug testing, researchers inserted mouse embryonic stem cells with a genetic predisposition to cancer into a normal mouse blastocyst that then developed into mice. The modified cancer genes could be turned on only in tissues specific for that cancer once the mouse was mature, but would acquire additional and unique mutations as the cancer progressed

(*Nat. Biotechnol.*, 28:71-9, 2010).

What was more remarkable was that Pandolfi's mouse models showed similar patterns of resistance to drugs as patients. The APL patients and mice with mutations on chromosomes 15 and 17 were initially responsive to treatment with retinoic acid (RA), a type of chemotherapy, but both the mouse and patient would quickly develop resistance. In the APL linked to chromosomes 11 and 17, neither patients nor mice responded to RA.

Using the mice to screen for more effective treatment combinations, they found that APL 15;17 mice could be cured of their leukemia if given a combination of RA and arsenic trioxide, another chemotherapy drug. The APL 11;17 mice, in contrast, responded to RA combined with a newer drug, phenylbutyrate, a histone deacetylase. Again, both predictions bore out in the clinic, turning a fatal form of leukemia into one with a 70-90 percent cure rate.

Since the success of the APL mouse, many others have tried to recreate human cancers in mice by knocking out tumor suppressors and knocking in oncogenes implicated in particular types of human cancers and resistance to therapies. A number of these models are now being tested in Pandolfi's lung and prostate cancer trials, along with more traditional models of immunosuppressed mice carrying grafts of tissue from some patients' biopsies. The 2-year project, supported by \$4.2 million in stimulus funds, is the most systematic test to date of how well genetically engineered mouse models can predict human outcomes in cancer.

Pandolfi and Engelman, along with colleagues such as Lewis Cantley and Kwok-Kin Wong, chose to shadow a handful of Phase III lung cancer trials and a handful of Phase III prostate cancer trials, all made up of patients with specific mutations in their cancers. The lung cancer trial will include one genetically engineered mouse and one of several xenograft models, created using cancer tissue taken directly from some of the patients, in order to directly compare a xenograft to a genetically engineered model.

The lung cancer trials include patients with a mutation in *ALK*, as well as mutations in *Kras* and *EGFR*—two genes that have been implicated in causing resistance to some therapies. For the prostate cancer trials, the patients receiving various therapies will be shadowed by mice that contain combinations of mutations in typical cancer genes like *p53*, and *ERG*, a recently discovered prostate cancer gene.

It can cost \$100,000 to generate a mouse from scratch, and \$100 to buy an immunodeficient mouse for xenograft studies. But, Pandolfi asks:

What if that \$100,000 could

Since the results from mouse testing will

be available much sooner than those from human trials, researchers will be able to identify mice that don't respond to the experimental therapy, which may help reveal the genetics behind that resistance. And in the roughly 15-20 mice used for each treatment group, they'll be able to test additional

therapies. The trial received the green light at the end of 2009; Pandolfi and his team are enrolling patients and setting up their mouse facility, and hope to publish some results from the mouse portion of the trial before the 2 years are up.

**save a company from
spending hundreds of
millions of dollars on a failed
product?**

By the end, Pandolfi hopes to identify the mouse models that best mimic human cancers—the way they respond to drugs, the way they progress in the clinic, and the mutations that help them escape death by a cancer drug. But even if he does, the problem is far from solved. For he'll only have answers for the small subset of lung and prostate cancers he's testing. And his approach is far from widely feasible, since once key genes for a cancer are identified—a sizable effort in its own right—it can take about a year to generate an appropriate mouse, and then another year or more to characterize its phenotype and determine if its cancer behaves similarly to those in humans. With investigator time counted, it can cost some \$50,000-\$100,000 to generate a mouse from scratch, versus the roughly \$100 it takes to buy an immunodeficient mouse for xenograft studies. It's a hefty price tag, Pandolfi admits, but adds: What if \$100,000 could save a company from spending hundreds of millions of dollars on a failed product?



Not everyone is convinced that genetically engineered models will produce better mouse models for cancer. For one, “The sheer number of genetic changes that you need to make a mouse cancer and a human cancer are different,” says Charlotte Kuperwasser at Tufts University. “You actually require fewer [mutations] in mice than you do in humans,” a fact that could make these induced tumors in engineered mice easier to cure than in humans. Another potential caveat for genetically engineered models is that “we do see some phenotype drift,” says Marks—in other words, a mutation can get passed on, but not necessarily produce the same phenotype in each generation, potentially reducing the reproducibility of some of the models. “Both [xenografts and genetic engineering] have strengths and weaknesses,” adds Kuperwasser.

And not every xenograft model is a simple engraftment of a human cell line or patient sample into an immunocompromised mouse. For Kuperwasser's model of breast cancer, she takes normal human breast tissue samples from women and inserts genes that cause cancer in vitro, then inserts the tissue into the breast area of a NOD-SCID mouse. The mouse then grows a human cancer that's “histologically indistinguishable” from human breast cancer, she says. While the goal of her model is to study the changes that occur in breast cancer rather than

undertake drug screening, she has been able to show that her models containing the *Her2* mutation respond to Herceptin, a drug designed for women who overexpress *Her2*.

Another group is adopting a different approach to genetic modification to create a better system for screening cancer drugs. Scientists at AVEO Pharmaceuticals, based in Cambridge, Mass., start with a mouse embryonic stem cell, and manipulate the genes responsible for a particular cancer. Of course, changing a gene in an embryonic stem cell means every cell that comes from that original cell carries the mutation. So the researchers modify genes designed to cause cancer only in specific tissues (in the lung for lung cancer) and insert a few of those cells into an unaltered mouse blastocyst. The engineered cells integrate seamlessly into the blastocyst's developmental program and create a patchwork mouse with a combination of normal cells and cells with a ticking time bomb of cancer genes in the right organ. When researchers are ready (such as when the mouse reaches maturity), they can turn on oncogenesis. "We recognize that human tumors grow up in an organismal context," AVEO's senior vice president of oncology, Murray Robinson, said during an interview last fall. The company is now waiting on results from the ultimate test of the predictive value of their mice: results of their Phase III clinical trial that will allow them to compare how well their mouse model recapitulated the human response to the same therapy.

But will this model, or any other, predict human experiences of toxicity, recurrence, drug response, metastasis? "It's a very challenging question," says Marks.

A few years ago, when Pier Paolo Pandolfi's father was being treated for pancreatic cancer, he heard about a then-new drug, Avastin, that blocks angiogenesis. "There was little data about Avastin and pancreatic cancer," but researchers thought it looked promising. Then an MD and PhD at Memorial Sloan Kettering, Pandolfi was surrounded by oncologists, and "as a VIP patient my father got Avastin." But the drug did not work, and Pandolfi's father died a year after his diagnosis. This experience reinforced to him the importance of early data—if the drug had been carefully tested on a more predictive mouse model, perhaps it would have saved his father the trouble.

It's also one of the reasons why Engelman's patient will never know about the mouse that is shadowing her cancer and her treatment. If this sixth round of therapy fails to work for her, she won't know if there was a mouse with the ALK mutation that also failed the treatment—or, unfortunately, if researchers were able to locate an alternative drug that was effective for that mouse. She won't know, says Engelman, because the purpose of the trial is to pit mouse models against each other in a careful way, thus everything must be blinded. Researchers won't be able to answer which mouse was predictive and which was not until the very end. They don't want to make any assumptions. Because that, after all, is what led them to work with limited mouse models of cancer for decades, putting drug development—

and, crucially, patients—at risk.

Correction (March 29): The original version of this article stated that the results of AVEO's Phase III clinical trial are due later this year. In fact, enrollment for the trial has just started. The correction has been made and The Scientist regrets the error.



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