

Realistic Rodents? Debate Grows Over New Mouse Models of Cancer

In laboratories and conference rooms across the vast U.S. cancer research enterprise, scientists are conducting a high-stakes debate over which mouse models of cancer should be used to screen new drugs before they enter human trials.

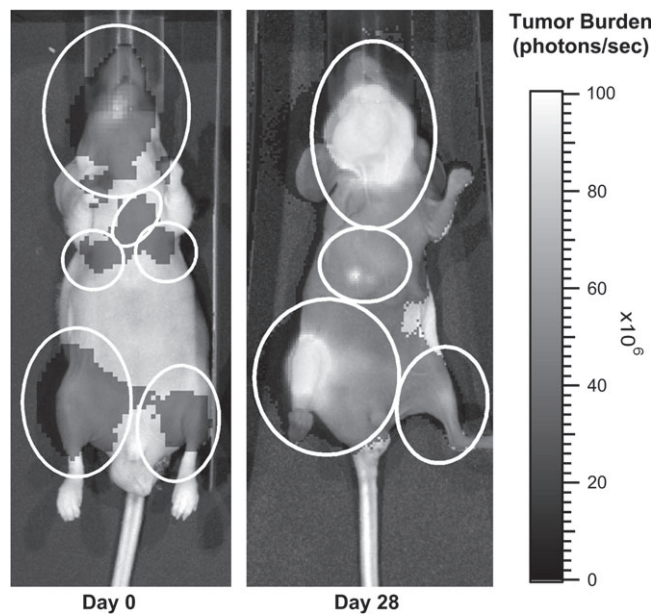
Change is needed. Thirty years of experience with subcutaneous xenografts, human tumors implanted under the skin of the mouse, have satisfied few because so many drugs that cure cancer in these mice fail to help humans. A 2004 analysis in the *Journal of the American Medical Association* showed that only 3.8% of patients in phase I cancer drug trials between 1991 and 2002 achieved an objective clinical response—and the response rate is declining. Almost all drugs tried in humans work against subcutaneous xenografts in mice.

“How many more negative data do you want? It’s very depressing,” said Isaiah Fidler, Ph.D., of the University of Texas M. D. Anderson Cancer Research Center in Houston.

Advocates for mouse alternatives are each claiming superiority. The orthotopic xenograft model transplants human tumors to the equivalent mouse organ, instead of under the skin (*see sidebar*). The transgenic mouse model uses genetically engineered mice that express cancer genes or mutate tumor suppressor genes (or do both) so that these genetic defects give rise to mouse tumors.

“These genetically engineered models, although unproven, are one of the best ways that people may have to test the efficacy of single agents and, even more importantly, of rational [drug] combinations,” said Kevin Shannon, M.D., a cancer researcher at the University of California in San Francisco.

Although these newer mouse models are technically impressive, no one yet knows whether they will perform any better. The debate is “highly opinionated,”



These images of a bone metastasis model mouse show the cancer’s progression over 28 days using bioluminescence imaging performed at MIR Preclinical Services.

Courtesy of Patrick McConville

said Wilbur Leopold, Ph.D., president of MIR Preclinical Services in Ann Arbor, Mich., “and the opinions are driven by suggestive but not conclusive science.”

Still the Standard

Both the orthotopic and transgenic camps can muster strong theoretical arguments for their respective models. But for all their faults, subcutaneous xenografts remain the standard for cancer drug screening in the pharmaceutical industry. These models are relatively easy to make and simple to use, since tumor growth on the mouse flank can be measured with just a pair of calipers. The U.S. Food and Drug Administration considers a drug’s effectiveness against xenografts sufficient for clinical trial approval.

And panels of xenografts are not worthless. In 2001, the National Cancer Institute reported testing 39 anticancer drugs that had completed phase II clinical trials in multiple subcutaneous xenograft models. Only lung cancer xenografts predicted a drug’s activity in lung cancer patients. But 45% of drugs

that showed activity in at least one-third of the xenograft models also worked in people—although usually on a tumor in a different organ. The National Cancer Institute of Canada reported similar results in 2003.

In a “point-counterpoint” review in the April 1 issue of *Cancer Research*, Ed Sausville, M.D., Ph.D., former associate director of the NCI’s developmental therapeutics program, argued for sticking with the established model. “Used intelligently,” wrote Sausville, who’s now at the University of Maryland in Baltimore, “mouse xenografts will remain the ‘gold standard’ in cancer drug development.”

The subcutaneous xenograft is clearly better than nothing, but its drawbacks are well known. The mouse has no functioning immune system, something rarely seen in human cancer, and the tumor is growing in an artificial site. Xenograft tumors almost never metastasize, which Fidler attributes to interferons in the skin. Finally, the tumor does not develop naturally in the mouse. Instead, it is transplanted from the cell line of a fully grown human tumor—another divergence from the human situation.

When you consider that drugs behave differently in mice than in humans, it’s not surprising that the subcutaneous xenograft is a poor predictor of success. In general, it’s much easier for a drug to shrink a tumor in such mice than in humans.

“Don’t do the easy thing because it’s easy,” advised Fidler, who favors orthotopic models. “It’s not getting us anywhere.”

Transgenic Mice Come of Age

In theory, the transgenic or genetically engineered model (GEM) solves many of these problems. The first cancer GEMs appeared in the early 1980s and were created by injecting DNA into oocytes (immature fertilized eggs), which were

implanted in foster female mice. The foreign cancer gene integrated randomly into the mouse genome—its expression driven by powerful viral promoters—and was present in every tissue, which is a poor model for the sporadic cancers that make up almost all human cases.

Then Mario Capecchi, Ph.D., of the University of Utah in Salt Lake City discovered that mouse somatic cells (cells not involved in reproduction) could perform homologous recombination, the seemingly miraculous pairing and integration of a foreign DNA sequence at its equivalent sequence in the mouse chromosome. By manipulating mouse embryonic stem cells, Capecchi created the first knockout mice using targeted gene replacement, and this technique promised mouse models of cancer much more faithful to cancer in humans.

Over the last 15 years, this promise has been largely realized. In the mid-1990s an “on-off” switch for genes controlled by altering the concentration of the antibiotic tetracycline in the drinking water of animals came into use, enabling researchers to orchestrate cancer gene activation (and tumor growth) in their mice. Around the same time, a system to inactivate tumor suppressor genes in specific tissues with the Cre-lox system, which uses the viral

enzyme Cre recombinase to cut out specific gene sequences, became available. In 2001 researchers in the MIT lab of Tyler Jacks, Ph.D., described a method for randomly activating oncogenes in mice by taking advantage of rare gene recombination events in dividing cells. Later that year, Harvard’s David Tuveson, M.D., Ph.D. (now at the University of Pennsylvania in Philadelphia), and Jacks reported using a recombinant adenovirus expressing Cre to control the timing, location, and number of new tumors in mice.

Taken together, these advances allow for precise control of cancer gene activation in mice, as well as a system for mimicking the random nature of sporadic human tumors. These new models are “light-years ahead of the traditional transgenic models,” Robert Weinberg, Ph.D., of the Whitehead Institute in Cambridge, Mass., said in an e-mail. “These improvements give great hope for the future.”

These GEMs have several theoretical advantages over xenografts. Instead of introducing a tumor from the outside, “you’re modeling independent events,” said Leisa Johnson, Ph.D., a senior scientist at Genentech in South San Francisco. She adds that, unlike subcutaneous xenografts, transgenic mice have intact immune systems, as well as a native tumor blood

vessel system and microenvironment—all important contributors to tumor growth. Finally, tumors in GEMs arise from cells that gradually become cancerous, like human tumors. When testing drugs, “we can ask, ‘[Do] early-, mid-, or late-stage disease have different responses to this agent?’” said Johnson. “You can’t ask that in a xenograft.”

Not Ready for Prime Time?

Nevertheless, GEMs have drawbacks, both theoretical and practical. Partly because of physiological and genetic differences between humans and mice, GEM tumors are often structurally different from their human equivalents. Johnson, however, points out that recent models of pancreatic cancer made by Tuveson and by Ronald DePinho, M.D., of the Dana-Farber Cancer Institute in Boston, are remarkably close to human pancreatic tumors. “These models are models,” warned Shannon. “The more complex the human tumor is, in terms of the different causes, the more difficult it’s going to be to model them.”

And, for systematic drug testing, GEMs are harder to use than xenografts, and they are much more expensive. Targeted gene replacement requires three generations of mice, so it takes more

A Better Xenograft Mouse?

The orthotopic xenograft model, like the subcutaneous xenograft (*see* main bar), involves growing human tumors in mice. But orthotopic (from the Latin for “correct place”) tumors are transplanted to the appropriate organ in the mouse. For example, human pancreatic cancer cells are injected into the mouse pancreas—not into the skin on the mouse’s back.

In theory, the tumors that arise there will more closely resemble human tumors because the nearby blood vessel system and other supporting tissues will better mirror the tumor’s microenvironment, realistically influencing its growth. Such models, unlike subcutaneous xenografts, readily metastasize—a huge theoretical advantage.

“Orthotopic models are absolutely essential,” said Isaiah Fidler of the University of Texas M. D. Anderson Cancer Center in Houston, a pioneer of the orthotopic approach. Effective therapies will need to target not only the tumor (the seed) but also its microenvironment (the soil), which orthotopic models better reproduce, he said. “If we can attack the seed and the soil, rather than focus only on the tumor ... our success is going to increase dramatically,” said Fidler.

Robert Hoffman, Ph.D., president of Anticancer Inc., a pre-clinical cancer testing company in San Diego, has refined the orthotopic approach. Hoffman grows human tumor cells under the skin of mice and then takes fragments of the resulting tumors and surgically implants them in the corresponding organ of mice lacking immune systems. That keeps the tumor structure intact.

“We feel very strongly that even when the tumor starts to grow, the structure’s terribly important,” said Hoffman. “So ... we implant the fragments.”

But orthotopic xenograft models are more expensive than standard subcutaneous models, and measuring tumor growth is harder, although new tumor imaging techniques are simplifying the task. Aside from anecdotal accounts of success in predicting drug activity in human cancer, there is no definitive proof that orthotopic models are superior to standard xenografts. But, to Fidler, the advantages are obvious.

“Is it important to target the seed and the soil?” he asked. “Absolutely yes, and to do that we need an orthotopic model.”

—Ken Garber

than a year to make a given GEM; xenografts can be created virtually overnight. Transgenic mice also develop tumors at different times.

“From the perspective of large-scale evaluation of drug candidates, it’s a very cumbersome system to try to use,” said Leopold.

Most importantly, there is no proof yet that GEMs can better predict drug activity in humans. “We’ve never seen a clear demonstration of benefit to justify the extra investment,” said Leopold.

“Would I include a transgenic model if I had one in my drug evaluation program? Yes. Would I rely on it exclusively? No.”

Shannon acknowledges that solid evidence for GEMs’ worth in drug screening remains scant. But he expects that to change. “Somebody’s going to do an experiment that’s going to show that you can take the data from a preclinical [GEM] model and that it’s highly predictive of the drug’s eventually working in a subset of human cancer patients,” he said. “As soon as that paper’s published, all the big pharmaceutical companies will now make a big investment in this. And until then they won’t.”

Meanwhile, many smaller companies are discouraged from using GEMs by Harvard’s “oncomouse” patents, which were exclusively licensed to DuPont. To commercialize drugs that were tested in genetically engineered mice, one must sublicense these patents. “It’s a costly license,” said Johnson. “I think it really hurts younger companies, companies that don’t have the resources, that are fighting to stay alive.”

Johnson expects that GEMs will enter widespread use to probe the mechanism of drugs that already work in humans or to model drug combinations use in the clinic, rather than to screen for new drugs. The intact immune system and microenvironment, she said, makes GEMs ideal for such studies. Meanwhile, she said, xenograft use should continue.

Will GEMs ultimately prove superior to subcutaneous xenografts for drug screening? Finding out will require massive effort—and a leap of faith. “We’re never going to know the answer until we do it,” Johnson said. “It’s going to take years ... but we’ve got to start.”

—Ken Garber

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