

From Human to Mouse and Back: “Tumorgraft” Models Surge in Popularity

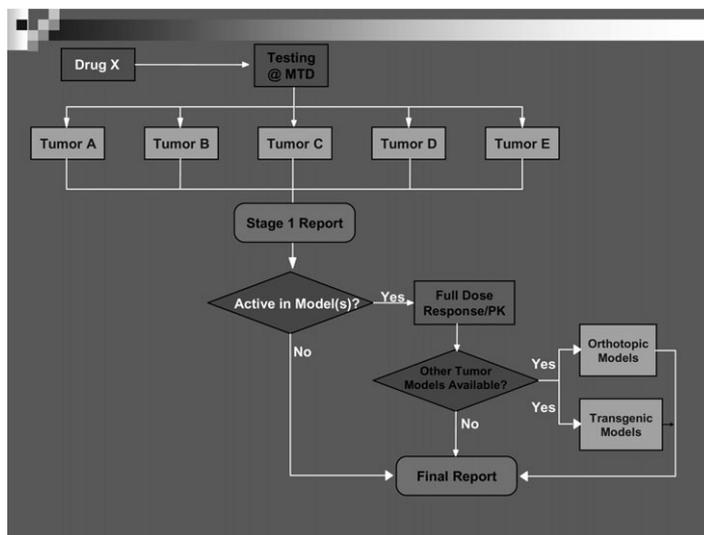
By Ken Garber

Mouse xenograft models of cancer, understandably, have a terrible reputation. Although researchers and companies routinely use these human tumors in mice for preclinical drug testing, individual models poorly predict how drugs will act in the clinic. Retrospective reviews published by the National Cancer Institute in 2001 and the National Cancer Institute of Canada in 2003 came to the same conclusion: Drugs that work against cancer in xenograft mice rarely work in people with the same tumor, with the exception of lung and possibly ovarian cancer.

“There’s this mantra: ‘Xenografts don’t predict for human effects,’” said Peter Houghton, Ph.D., a cancer researcher at the St. Jude Children’s Research Hospital in Memphis, Tenn.

But not all xenograft models poorly predict drug effects. In direct transfer, or “explant,” xenografts, recently dubbed “tumorgrafts,” tumors taken from patients are chopped into fragments slightly smaller than a pencil eraser and implanted directly into immunodeficient, or “nude,” mice. (Standard xenografts use permanent cell lines, not primary tumors, as the source of tumor material.) In several retrospective studies, drug effects in such mice closely mirror human effects when differences in drug activity between species are taken into account. “They work extremely well, as long as you factor in the drug systemic exposure,” Houghton said.

Now, these direct transfer xenografts, first used in the 1970s, are surging in popularity, as research groups and drug companies apply new molecular techniques to



New drug testing in mouse models by the NCI-supported Pediatric Preclinical Testing Program. The 3-year-old program, which has already sent several drugs into clinical trials, relies heavily on explant xenograft models.

these mice to begin creating personalized cancer treatments.

Of Mice and Men

Explant models are, to be sure, more tedious to create. Human tumors must be freshly implanted “[and] they’re hard to grow,” said David Sidransky, M.D., a cancer researcher at Johns Hopkins Hospital in Baltimore, at the 2008 American Association for Cancer Research (AACR) conference on molecular diagnostics in cancer therapeutic development. But “once growing, they’re very predictive of response.”

Tumorgrafts (Sidransky’s preferred term) are already being used in clinical settings. At Johns Hopkins, researchers take resected pancreatic tumors from newly diagnosed patients, implant them into multiple nude mice, and then test 10 approved and experimental treatments against these personal tumorgraft banks. Results are used to guide therapy when the patients relapse (*J Natl Cancer Inst* 2007;99:105–7.) At the AACR meeting, Sidransky described one case in which the mouse model predicted

resistance to gemcitabine, the standard therapy for pancreatic cancer, and sensitivity to mitomycin C, a rarely used treatment. The patient—who first failed gemcitabine therapy—received mitomycin C and has been in remission for more than 2 years.

The trial’s principal investigator, Manuel Hidalgo, M.D., Ph.D., stressed that tumorgrafts, at present, can be of only limited use for individualizing patient treatment because of the time and resources necessary to create tumorgraft banks. Many patients die even before their mouse models are ready for

drug testing. “All we’re trying to achieve here is proof of concept, that there is some prediction,” Hidalgo said. Tumorgrafts will be used mainly to screen new drug candidates for activity and to discover biomarkers that can predict drug effects, he said. For example, an April *Cancer Research* report by Hidalgo’s group described using pancreatic tumorgrafts to derive a 25-gene signature that successfully predicted mouse sensitivity to epidermal growth factor receptor inhibitors. A human trial prospectively testing the epidermal growth factor receptor biomarkers is pending.

Patient selection using such biomarkers is widely viewed as the future of cancer therapy, although few prospective biomarker trials have taken place. “If we really want to make progress, that’s the way to go,” Hidalgo said.

And for biomarker discovery, tumorgrafts are much easier to work with than primary tumor tissue because of the abundance and renewability of the tumor material. “You can go back and you can

repeat and repeat and repeat if you think you've found something that's real, as opposed to patient samples where you get basically one shot at doing something," said Houghton.

Power To Predict

The drug industry, in the last few years, has recognized the potential utility of tumorgrafts, and drug candidate screening using tumorgraft models is now becoming routine. "Some of the large pharmaceutical companies have established very large panels of [tumorgrafted mice] in the particular cancer types that they're interested in ... to see how their drugs are working," Houghton said. For example, at the AACR meeting, Frederic de Sauvage, Ph.D., of Genentech in South San Francisco, Calif., described using such models to decipher how experimental inhibitors of the hedgehog signaling pathway target tumor connective tissue.

How strong is the evidence that tumorgrafts can accurately predict actual human response to drugs, given that standard xenografts have done so poorly? Heinz-Herbert Fiebig, M.D., Ph.D., a medical oncologist and cancer researcher in Freiburg, Germany, has been creating direct transfer xenograft models since the late 1970s and retrospectively testing their predictive power for human treatments. Fiebig has reported consistently good results. For example, in 2004 he reported in the *European Journal of Cancer* that his xenografts correctly predicted response in 90% of patients (19 of 21 tumors) and resistance in 97% (57 of 59).

The failure of standard cell line-derived xenografts to predict, said Fiebig, is caused by changes in culture. Tumor cells in culture tend to undergo selective pressure to become less differentiated and more homogeneous, so that the tumors they give rise to in mice no longer faithfully reflect the original tumor. Most tumorgrafts, on the other hand, closely mirror their parent human tumors micro-

scopically, he said. And Houghton's group has shown that they change minimally on the molecular level, when taking into account the absence of surrounding human connective tissue, or stroma. "They have maintained the [gene] expression profiles and genomic profiles very, very accurately," Houghton said.

Tumorgrafts, though, have their own problems, including the high transplantation failure rate and the labor required for multiple transfers, or "passages," from mouse to mouse (needed to propagate each human tumor in multiple mice). The overall "take rate" varies from 40% to 60% for non-small-cell lung cancer, colon cancer, and melanomas down to 12%–20% for breast cancer and just 3% for prostate cancer, said Fiebig. So tumorgrafts cost much more than conventional xenografts.

"There's this mantra: 'Xenografts don't predict for human effects.'"

Both Houghton and Fiebig stress the importance of limiting the number of mouse-to-mouse passages to prevent the tumors in the mouse host from gradually mutating away from the parent tumors. Frozen tumors make up a permanent repository that allows unlimited replenishment. "As a measure of precaution, we go back to the frozen master stock after 10–12 passages" to generate new mouse tumors, said Fiebig.

Mining the Model

The main knock against tumorgrafts is the need for more prospective trials showing that they predict drug activity in humans. Several such tumorgraft validation studies are ongoing or planned. But for many researchers, the evidence is already convincing, and use of the models is growing. In 2005, Houghton's group reported a clinical trial in pediatric neuroblastoma with the drug topotecan, guided by individual drug level adjustments and dosing schedules tested in a direct transfer mouse xenograft model. "The response rates in the clinic were exactly as predicted from the models—a 60% response rate," Houghton said.

On the basis of those results, the NCI is funding a multicenter drug development initiative, the Pediatric Preclinical Testing Program, which uses 60 mouse tumor models, with most of the tumors taken directly from patients rather than from cell culture. "Basically the drugs that work in human cancers, childhood cancers, work in the mice," said Houghton. "Now we're using those models to identify new drugs, which are being fast-tracked into pediatric trials."

Similar work is under way at Fiebig's company, Oncotest GmbH in Freiburg, Germany, which now has a bank of more than 200 tumors established directly from patients. Oncotest has also derived more than 40 cell lines from these tumors, which are used for biochemical and molecular studies. U.S. drug companies especially



Peter Houghton, Ph.D.

have shown "a huge interest" in these tools for drug development, said Fiebig, beginning 4 years ago when gene expression chips were first used to characterize these tumors on a

molecular level. Personalized therapy, based on such gene expression signatures, is the ultimate goal. Fiebig has identified signatures in his models that predict response to 14 cytotoxic drugs and two targeted agents, bevacizumab and cetuximab. Overall response rates for both targeted drugs and most of the cytotoxics were close to those of past clinical results. Fiebig is planning to validate these signatures further using samples from clinical trials that have been completed and for which the outcomes are known, and eventually in prospective clinical trials.

In 2006, Sidransky and Hidalgo also cofounded a company, BioMerk, to commercialize the tumorgraft platform. The following year, Champions Biotechnology in Arlington, Va., acquired BioMerk, later naming Sidransky chairman and Hidalgo chief scientific officer. Champions, in collaboration with South Texas Accelerated Research Therapeutics, a San Antonio company, is establishing

tumorgraft models in a wide range of cancer types, characterizing them molecularly, and testing a variety of drugs in these models. Drug industry customers include ImClone in New York and

Concordia Pharmaceuticals in Fort Lauderdale, Fla.

The most important test for tumorgrafts is taking place right now, as new drugs, selected using the model, make their way through

clinical trials. “The next few years are really going to tell us if this really does work as well as we anticipate,” Houghton said.

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