CANCER THERAPY

Behind the Headlines of Endostatin's Ups and Downs

A year-long effort to replicate remarkable tumor-shrinking experiments in the glare of publicity has produced confusing results

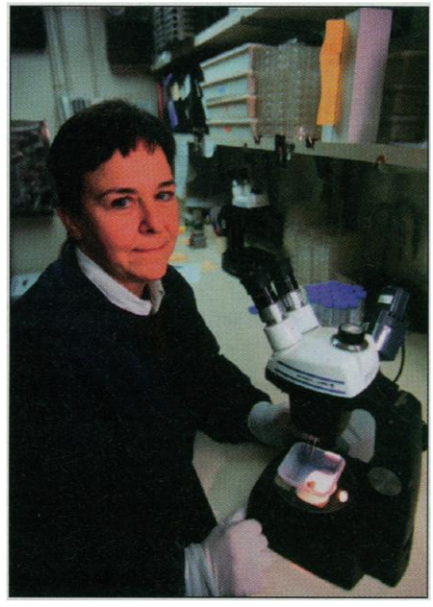
Melinda Hollingshead was "furious" when she read a story in the 11 February *Boston Globe* that said scientists from her National Cancer Institute (NCI) lab had been able to "reproduce" the world's most celebrated—and questioned—cancer experiment in mice. This feat, the *Globe* reported, paved the way for a promising new drug called endostatin to move into human trials. Three months before, Hollingshead was just as annoyed—by stories saying that she and other researchers had failed to reproduce the original results.

The remarkable results Hollingshead has been trying for more than a year to duplicate come from Judah Folkman and co-workers at Boston's Children Hospital. In mouse experiments, Folkman's group had found that endostatin hampered the growth of blood vessels—a process called angiogenesis—that feed tumors, making cancer disappear. The results touched off a media frenzy last spring when they were featured on the front page of *The New York Times*, only to be deflated in the fall when many media reported that researchers, including Hollingshead, couldn't repeat them.

Now, the *Globe* had reported—correctly—that scientists from Hollingshead's lab, working side-by-side with researchers in Folkman's lab, had arrived at the astounding results that Folkman first published in the 27 November 1997 *Nature*. But to Hollingshead, both the positive and negative newspaper coverage has missed the complexity of the endostatin story. The earlier failures didn't mean that the strategy was hopeless, and she qualifies her latest success: Repeating an experiment, she says, means doing it independently. "I don't feel we really verified or repeated anything," says Hollingshead. For cancer patients, she says, the coverage has been a brutal roller-coaster. "People are clinging to any little thread of hope they can catch hold to, and their fingers bleed from trying to climb."

Behind the headlines of angiogenesis inhibitors being a "miracle cure" one day and a "failure" the next lies a scientific saga that emphasizes how small differences in techniques, reagents, and assays can foil attempts by one lab to repeat the work of another. It shows that replication, a cornerstone of the scientific process, means different things to different people. And it also helps clarify why this media-driven frenzy about endostatin, fueled by a potent mix of medical and commercial promise, has been so confusing and frustrating to the public and scientists alike.

To Folkman, the father of angiogenesis, the media spotlight has been more than frustrating. With all the attention, "it is not possible" to conduct science, says Folkman, noting that he rarely gives scientific presentations any more and has turned down 2300 interview requests since last May's front page *New York Times* article. NCI similarly has been besieged. "What is unusual is not the drug—it's our attempt to respond to the unbelievable interest in this drug," says NCI director Richard Klausner.



Incomplete repeat. Melinda Hollingshead agrees that endostatin works in mice in Folkman's lab; but she hasn't repeated the work in her lab.

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The making of a Folkman hero

The "unbelievable interest" began with publication of the *Nature* paper. An accompanying "News and Views" by University of Toronto cancer researcher Robert Kerbel called the work "unprecedented" and "startling." Kerbel cautioned that success with a cancer drug in mice often doesn't translate to humans, but said angiogenesis inhibitors "could herald a new era of cancer treatment."

Many newspapers, including *The New York Times*, wrote about the exciting findings in similarly hopeful but tempered tones. As the *Nature* paper detailed, the researchers first injected cancer cells into the flanks of healthy mice, which developed what's called a Lewis lung carcinoma, an especially difficult tumor to treat with chemotherapeutic drugs. After an injection with endostatin, Folkman's lab found that the tumors shrank, and they stopped the treatment. When the tumors returned, they again injected the mice with endostatin, and the tumors regressed. The drug continued to work after six cycles, indicating that no resistance had developed. More remarkable still, after disappearing for the sixth time, the tumors had not returned more than 5 months later, at which point they ended the experiment. These results were much more striking than had been obtained with other compounds that inhibit angiogenesis.

Even Michael O'Reilly, the Children's Hospital oncologist who discovered endostatin and was last author of the *Nature* paper, had trouble believing his own results. "The first thing I thought when the tumors didn't come back is that it was just that one experiment, it was a fluke," says O'Reilly. So before submitting his paper to *Nature*, he repeated the entire experiment and also tested the drug against two other types of mouse tumors. The data held up.

NCI—which 3 months earlier had begun a project to develop endostatin with EntreMed, a biotech company in Rockville, Maryland, that had licensed the compound from Boston's Children Hospital—wanted to confirm the results before moving the drug into humans. "It's very unusual for us to sponsor a clinical trial where we haven't seen activity ourselves or the overwhelming preponderance of evidence hasn't shown that it's reproducible," explains Edward Sausville, associate director of NCI's developmental therapeutic branch and Hollingshead's boss. Folkman, too, liked the idea of replicating the data. "You must have evidence in mice before you move into humans," says Folkman. "That's basically the philosophy of the NCI, and we agreed with that."

Then on 3 May, the *Times* ran its glowing article, which quoted Nobelist James Watson comparing Folkman to Charles Darwin and saying "he is going to cure cancer in 2 years." EntreMed's stock skyrocketed. Two hundred journalists a day requested interviews with Folkman. And enormous pressure began to build at NCI to move endostatin into the clinic.

NCI's Hollingshead was "appalled" by the article, in part because she already had run into difficulties trying to repeat the experiment. Hollingshead had sent five scien-

tists to Boston for 2 days in February 1998 to learn techniques from Folkman's lab, where they were welcomed with open arms, she says. "Injecting mice and taking care of mice sounds very simple, but it has many, many little pitfalls," says Folkman. But despite the training, when Hollingshead injected the mice with endostatin in her lab, it didn't work.

Then again, this batch of endostatin turned out not to work in Folkman's lab, either. The problem, the Folkman team decided, was that O'Reilly's first experiments used endostatin he had made in small amounts by stitching the gene for the protein into the bacterium *Escherichia coli*. NCI, in contrast, hired a company to make large amounts of *E. coli*–derived endostatin, which they hoped to share with the research community at large. But something in the scale-up, apparently, had ruined endostatin's "activity." Later batches of endostatin, which NCI made at its own plant in Frederick, Maryland, using both an *E. coli* expression system and another one that relied on mammalian cells, fared no better when Hollingshead tested them.

A major stumbling block in trying to produce active endostatin was that the researchers had no test tube assay to assess the activity of a given batch of material. With many other angiogenesis inhibitors, researchers can assess activity by putting a solution of the compound on endothelial cells—which line blood vessels—and determine whether the cells stop growing or migrating around a dish. But the *E. coli*–derived endostatin was insoluble. "It's kind of like pouring sand or slime onto your cell surface," explains Hollingshead.

So O'Reilly made a small batch of material in his lab, tested it in mice for the ability to shrink tumors—the only assay he had—and sent it to Hollingshead by Federal Express, packed on dry ice. It, too, failed. O'Reilly wondered whether the shipping process was somehow responsible, so he suggested to Folkman that they mail some of their product to themselves. Don't waste the money, Folkman said—put some on dry ice in your car and drive around with it. "Sure enough ... it didn't work, either," O'Reilly says. "The protein is great if you don't try to ship it."

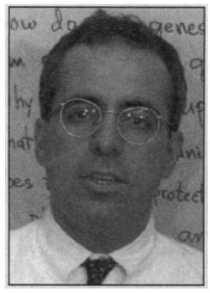
Bad news

By the fall of 1998, several labs had tried to engineer endostatin or received it from Folkman, but no one could make it work. (Another promising angiogenesis inhibitor, angiostatin, was apparently proving equally fickle for Bristol-Myers, which announced earlier this month that it was dropping work with the compound because it was having trouble producing it reliably.) On 12 November, *The Wall Street Journal* broadcast this problem in a front page article that catalogued several of Folkman's previous findings that others supposedly had trouble replicating.

Folkman, who has a stellar reputation for his ethics and scientific rigor, saw the article as "destructive" to him and people with cancer. "It's hard for the public and media to understand that when something doesn't work, it's not scientific manipulation, it's the way science is," he says. "All of our papers for 30 years have been reproduced, but they all took time, and it usually was 1 to 2 years." Ironically, Folkman notes, on the day the *Journal* article came out, Vikas Sukhatme of Beth Israel Deaconess Medical Center gave a talk at Harvard describing how he had suppressed the growth of tumors in mice with endostatin.

"The real proof is going to be if this works or not in patients."

—Michael O'Reilly



Sukhatme took a different tack from O'Reilly, however. He manufactured his mouse endostatin in yeast, which yielded a soluble protein that he could evaluate in the various test tube assays before giving it to mice. He then tested the compound in "nude" mice that had a renal cell carcinoma, a different tumor and mouse system from the one Folkman's lab used. So this work extended, but did not replicate, the findings in the *Nature* paper.

Bjorn Olsen of Harvard Medical School, who consults for EntreMed, now has positive results from yet another system: soluble human endostatin made in human kidney cells. Further confusing the picture, EntreMed hopes to conduct human trials with yet another variation: soluble human endostatin made in yeast. Olsen cannot compare his in vitro data to EntreMed's because they use different migration assays. And although NCI's Sausville says EntreMed's human endostatin "does not reach the same [activity] level" as mouse endostatin when tested in mice, Olsen cautions that nobody has yet compared mouse and human endostatin both made in yeast.

Kerbel of the University of Toronto points out that all these variables make news reports about endostatin all the more frustrating, because very few experiments are directly comparable. "None of these factors are being discussed," says Kerbel.

Repeat performance

While others explored these new tangents, a technician from Hollingshead's lab stood next to scientists in Folkman's lab from 18 to 26 January and conducted parallel experiments that aimed to reproduce the original results published in *Nature*. The endostatin worked, and NCI announced the accomplishment in a press release that simultaneously revealed plans to launch human studies.

"If you push us to the wall, have we replicated the experiment from soup to nuts? We haven't," says Sausville. "Have we put ourselves in the shoes of people who've done it? Yes. We agree there is a phenomenon to observe." Hollingshead, who is now planning to repeat the experiment with Folkman's workers in her lab, agrees. "We saw effects they observed with their mice, their tumor, their equipment, with the one exception being that our personnel were doing the injections of endostatin," she says. "All that really states is our people know how to inject endostatin."

That, in itself, may be a critical skill. Folkman says it took him years to perfect his techniques, which rely on factors such as the amount of material in the syringe, the gauge of the needle, where you inject the mice, and the temperature of the room that houses the animals. Douglas Hanahan, a cancer researcher at the University of California, San Francisco, confirms that he had much trouble with endostatin until he visited Folkman's lab and learned these subtleties. "Since then, we've had very reliable results," Hanahan says.

Hanahan, who co-chairs an NCI advisory subgroup on angiogenesis, says trying to repeat the experiment is important because it may help researchers figure out why endostatin is so fickle. "It would be a big shame if we moved into the clinic prematurely and the results were negative," he says.

NCI director Klausner says, however, that the accumulation of new data from EntreMed scientists, Olsen, and Sukhatme was enough to justify moving into clinical trials. "The decision to support going forward was made at a meeting about a month ago, and it was before I had seen the results from Boston," says Klausner.

NCI and EntreMed currently plan to begin small human trials of endostatin by the end of the year. Folkman and O'Reilly say they're excited to see how endostatin will work in the clinic. "Regardless of whether the media likes this stuff or doesn't, the real proof is going to be if this works or not in patients," says O'Reilly. You can be sure those results will get a blast of publicity—and spin.

–JON COHEN