

A FOUR-DECADE QUEST TO SHRINK TUMORS:

The establishment scorns one man's discovery // Research languishes for lack of funding // Momentum is lost with human-trial failures // A life-changing drug cocktail arrives at last.

Turning Off Cancer

■ BY ANITA SLOMSKI // ILLUSTRATIONS BY LEIF PARSONS



In 2004, free of cancer for five years, Chicago undercover detective Jim Smith thought he'd beaten the odds. But a year later, at age 53, Smith could think only of death. His colon cancer, treated with surgery, radiation and chemotherapy in 1999,

had returned, metastasizing to his lungs with 14 new tumors.

Smith agreed to more chemotherapy. This time his doctor added a drug designed to disrupt the tumors' fuel lines, the blood vessels supplying oxygen and nutrients. By the fourth treatment, a CT scan showed the tumors were shrinking. But the best news came a few months later, after a car accident landed Smith in the hospital. "I was getting another CT scan, and I told the trauma doctor he would see spots on my lungs," says Smith. "He said, 'What spots?' There weren't any."

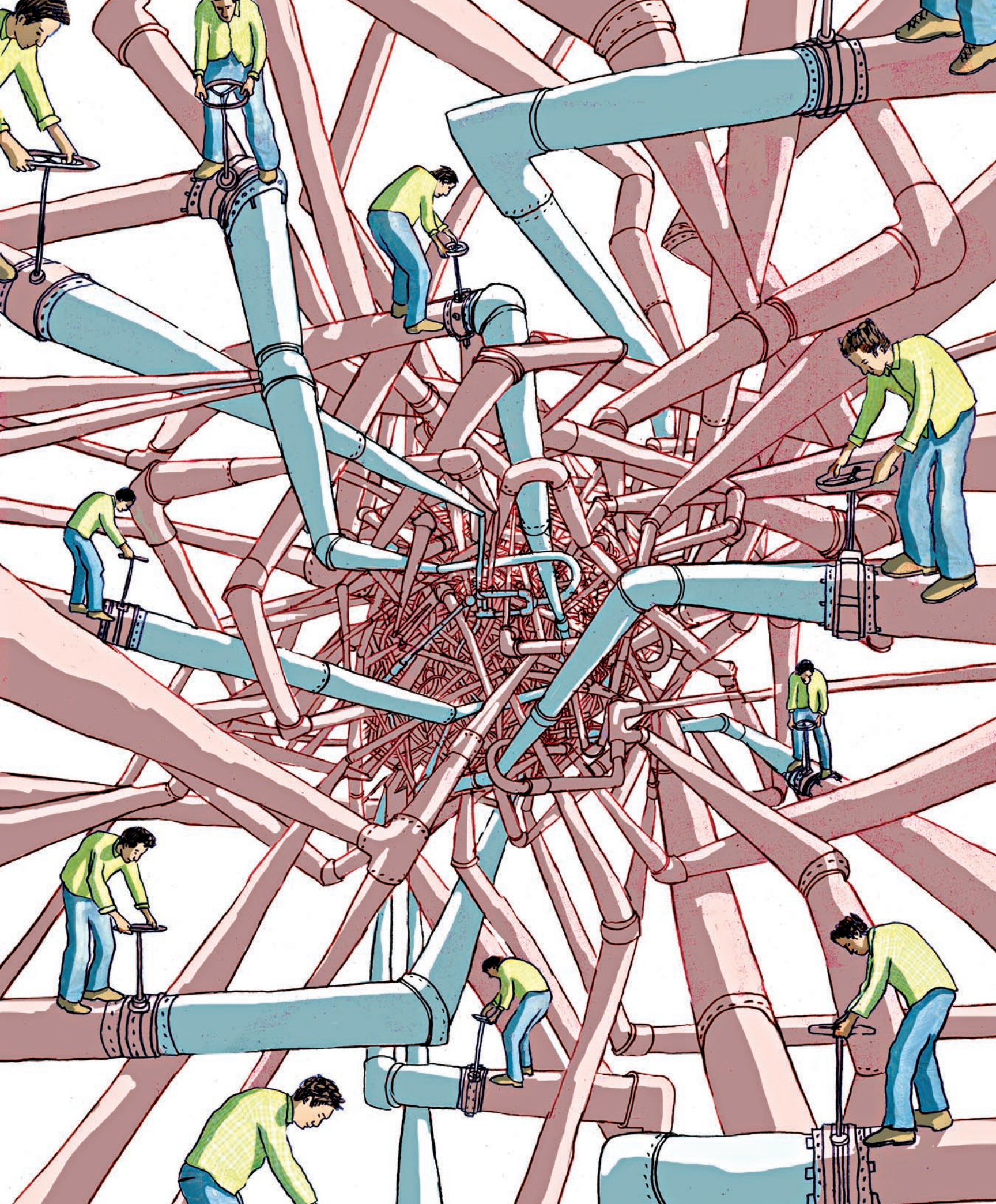
With Smith's metastatic cancer in full remission, his doctor, Mark F. Kozloff, director of oncology at Ingalls Hospital in Harvey, Ill., stopped the chemotherapy. But Kozloff is keeping Smith on a \$7,000-a-month maintenance dose of Avastin (bevacizumab), a drug that targets the tumors' vascular infrastructure. That should help keep the cancer at bay for a while. "Adding Avastin has stretched survival time for metastatic colon cancer to well over 20 months, from 17 months with chemotherapy alone," Kozloff says.

A drug that extends life by mere months may not sound like a big deal. But to many oncologists, Avastin's potential seems

huge. "You could say, 'What is all the hype about? Avastin simply delays the ability of tumors to kill,'" says Mark W. Kieran, director of pediatric medical neuro-oncology at the Dana-Farber Cancer Institute in Boston. "Or you could say, 'My God, we've finally found a new way to take us beyond the dent we've made in cancer with chemotherapy and radiation alone.'"

Though only approved by the Food and Drug Administration for first-line treatment of metastatic colorectal cancer in combination with chemotherapy, Avastin is proceeding through more than 100 clinical trials involving thousands of patients with many kinds of cancer. Researchers hope to prove it can keep cancer from recurring after an initial round of successful treatment. "With surgery, radiation and chemotherapy, you try to get out all of the cancer, but there may be stray tumor cells," says Patricia M. LoRusso, professor of medicine at the Barbara Ann Karmanos Cancer Institute in Detroit. Those cells are potential metastases. "If you can keep the cells dormant by preventing them from establishing a blood supply, you may be able to keep the cancer from ever coming back."

In that scenario, patients likely would have to stay on a lifetime regimen of Avastin (or some combination of related compounds), much like the cocktails that have helped transform AIDS into a treatable chronic disease. That's the potential of Avastin and an emerging class of similar drugs—that cancer, too, could become manageable. It would be the ultimate validation of a long-ridiculed theory first proposed 40 years ago.



Once scorned, surgeon Judah Folkman has seen his 1960s observation about tumor blood supplies blossom into a promising cancer treatment.

As a young surgeon during the 1960s, Judah Folkman, now director of the vascular biology program at Children's Hospital in Boston and professor of pediatric surgery and cell biology at Harvard Medical School, noticed a peculiar distinction between the large cancerous tumors he excised from patients and the microscopic tumors in dog thyroid tissue he was studying in the lab. The large ones were entangled in wild nests of blood vessels, while the minuscule ones had no blood supply and were apparently dormant. Folkman wondered whether the active tumors were secreting something that generated the profusion of blood vessels. And if so, whether there was a way to inhibit the process.

The creation of blood vessels, known as angiogenesis, was only cursorily understood. It occurs during the menstrual cycle to rebuild the uterus, and during pregnancy to form



The large tumors were entangled in wild nests of blood vessels, while those without a blood supply were small and apparently dormant.

the placenta, and children's bodies depend on it to nourish growing tissues and organs. Get a deep cut, and your body produces angiogenic growth factors, proteins that activate normally quiescent endothelial cells lining blood vessel walls. The vessels form capillaries to ferry oxygen and nutrients to the damaged tissue. Once healed, the body somehow turns off its angiogenesis switch.

When Folkman proposed that angiogenesis might play a crucial role in cancer, most researchers were focused on the possibilities of chemotherapy. Folkman's 1971 publication of a paper in the *New England Journal of Medicine* outlining his hypothesis was greeted with considerable ridicule. Many colleagues felt it was premature, and basic scientists noted that Folkman was a surgeon, not one of them. Few wanted to work in his lab, and at first he found it difficult to obtain grants.

But Folkman and a small group of believers were undeterred. Despite scant funding, their understanding inched forward.

Tumors put into rabbits' eyes grew in the iris but not in the cornea, which has no blood vessels. Through windows cut into the shells of fertilized chicken eggs, scientists watched blood vessels sprout toward tumors. Yet while evidence mounted to support his basic hypothesis—that angiogenesis is crucial to tumor growth—progress in isolating a chemical that promoted angiogenesis was excruciatingly slow. Folkman's laboratory finally isolated a tumor protein that could stimulate angiogenesis and published its findings in *Science* in 1984.

While Folkman focused on malignant tumors, others were increasingly interested in isolating the growth factors at work in benign human tissue—to learn how organs develop as a step toward bioengineering new ones. For Napoleone Ferrara, an Italian-born obstetrician/gynecologist, this quest led to the pituitary glands of cattle, whose follicular cells caused blood vessel cells to multiply profusely.

Hired in 1988 by the biotechnology company Genentech in San Francisco to develop a drug to hasten labor, Ferrara was most passionate about this other research. He spent nights and weekends grinding cow pituitaries into a soup containing thousands of proteins. Early the next year, he finally isolated a protein with an amino acid sequence that matched that of no known substance. Ferrara called the protein vascular endothelial growth factor, or VEGF. Further testing determined that it was crucial for tumor angiogenesis, and scientists now know that more than half of the 220 kinds of human cancer produce VEGF to signal endothelial cells to start sprouting blood vessels to nourish tumors.

Meanwhile, Folkman and his colleagues were searching for substances that would turn off angiogenesis. Their discovery of the first, interferon alpha, was published in *Science* in 1980, followed by tetrahydrocortisol, in 1985, and ultimately, by nine other antiangiogenic compounds. Yet in the early 1990s, despite the lab's groundbreaking findings, drug manufacturers had no interest in making angiogenesis inhibitors. Finally, Entremed, a year-old biotechnology company, agreed to produce endostatin, angiostatin, thalidomide and 2-methoxyestradiol for clinical use. (Thalidomide and 2-methoxyestradiol are now in clinical trials for cancer.)

In Folkman's lab, the job of finding an antiangiogenic compound fell to a persistent researcher, Michael O'Reilly. After months of experimenting, O'Reilly found a lung cancer that would kill a mouse in three weeks. Yet the tumor's metastases remained dormant until he excised the tumor. That essential characteristic corresponded to the phenomenon Folkman had long observed, that tiny metastases would grow only after all visible evidence of cancer had been cut out.

Folkman suspected that the tumor was emitting both angiogenesis inhibitors and stimulators, and that the stimulator was cleared out of the bloodstream rapidly while the inhibitor lingered. Once the tumor was removed, he surmised, the inhibitor disappeared from circulation, which permitted remote metastases in other organs to begin forming blood vessels.

After a year of analyzing vats of mouse urine, O'Reilly and Folkman finally discovered a substance that appeared to inhibit

angiogenesis. It was a fragment of a protein molecule called plasminogen, produced by the liver to regulate blood clotting. Folkman and O'Reilly called the fragment angiostatin.

In 1994, O'Reilly planted the deadly lung tumors on the backs of mice. Three weeks later he removed the tumors and gave one group of mice daily injections of angiostatin, while giving a control group a saline solution. When O'Reilly killed the mice and examined their lungs, those on angiostatin were free of cancer, while the controls were riddled with it.

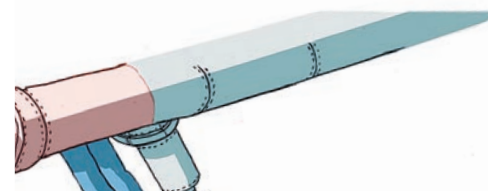
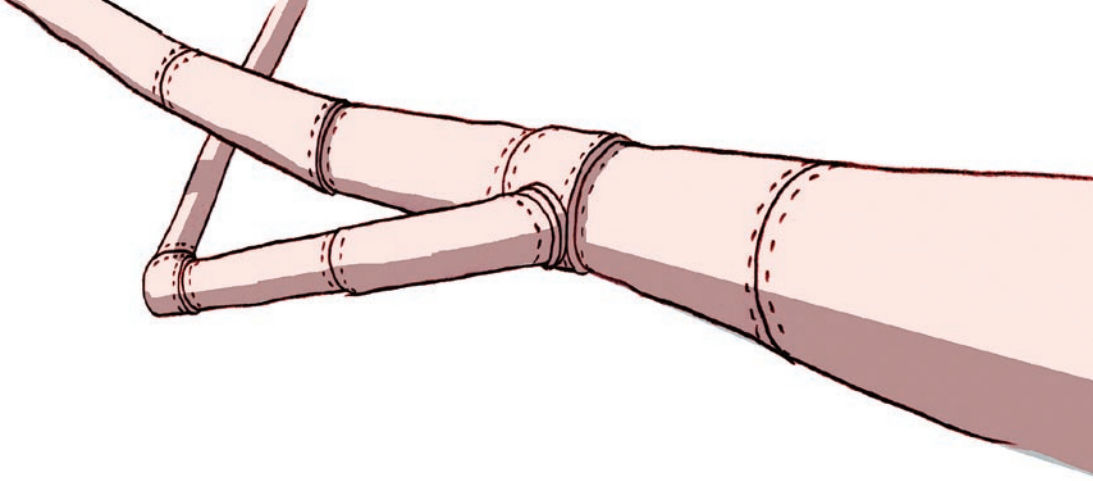
A year later O'Reilly found a second inhibiting molecule: endostatin. This time he gave daily doses to mice while the primary tumor was in place. The cancers disappeared, but they returned when he halted the endostatin. Combining angiostatin and endostatin worked better, though the tumors still returned after the drugs were halted.

Ferrara, meanwhile, was proceeding with his own animal studies. After locating the gene that produces VEGF, he created an antibody that shrank tumors in mice by as much as 95%. Two years of additional animal testing later, Genentech created Avastin, a monoclonal antibody that binds to VEGF and blocks tumor angiogenesis.

Trials on patients with advanced breast and colorectal cancer began in 1997. The breast cancer trials failed; although tumor size shrank somewhat, survival time didn't increase. "We were all very gloomy," says Ferrara. But trials involving metastatic colorectal cancer prolonged lives by as much as 50%. In February 2004, the FDA approved Avastin for treating colon cancer, and the drug racked up almost \$1 billion in sales in 2005.

Folkman and O'Reilly also tried to translate laboratory gains into clinical advances. When word got out about their successes with mice, expectations soared, and newspaper stories predicted a cure for cancer within years. But clinical trials of endostatin and angiostatin, begun in 1999 and 2000, respectively, did not live up to the hype.

Though both drugs proved nontoxic to humans, their tumors didn't show the dramatic regression seen in mice. One reason may have been the nature of the trials, says O'Reilly,



now assistant professor of radiation oncology at Houston's M.D. Anderson Cancer Center. "The mouse tumors increased in size before the inhibitors could overcome the angiogenic stimulators," he says. "Used alone in patients with advanced cancer, the drugs may not have been able to get over that initial delay." Patients died before tumors had a chance to shrink.

The age of antiangiogenic cancer treatments now seems to be in full swing. New Avastin trials involving patients with advanced breast, kidney, lung and colorectal cancer continue to produce dramatic results. In a Phase III lung cancer trial, though median survival rates have increased by only two months, some patients are alive after two years, living with a disease that kills most victims within 10 months. About 50 other antiangiogenic drugs are in trials or under development.

For now, Avastin and some other angiogenesis inhibitors are more effective when used in conjunction with lower-than-normal doses of chemotherapy. Rakesh K. Jain, director of the Edwin L. Steele Laboratory of Tumor Biology at the Massachusetts General Hospital and professor of tumor biology

at Harvard Medical School, believes Avastin may convert a tumor's chaotic vascular tree into more normal blood vessels and reduce fluid pressure inside the tumor, thus providing a more efficient conduit for the chemotherapy.

Meanwhile, a report presented at an American Society of Clinical Oncology meeting in spring 2005 detailed a Chinese study in which 493 people with non-small-cell lung cancer received chemotherapy plus a Chinese formulation of endostatin called Endostar. "Although it wasn't clear how advanced the lung cancer was, the reported doubling of survival rates is very impressive," says O'Reilly. "We need to expand on that trial and see if the data are as robust as they seem."

Combinations of drugs, including those pegged for diseases other than cancer, are also proving able to thwart angiogenesis. "Many molecules do the job quite efficiently," says Kieran. "It's almost a game now, to discover that yet another drug we've used for years has an antiangiogenic effect."

In a Phase I trial begun in June 2001, Kieran combined the arthritis drug Celebrex with thalidomide, the notorious anti-nausea treatment that caused birth defects during the 1950s.

Too Many Blood Vessels—Or Too Few //

Some 60 diseases are linked to the excessive or restricted formation of blood vessels. Here is a sampling of research, at various stages, into flipping the angiogenesis switch on or off.

DISEASE/CONDITION	ROLE OF PRO/ANTIANGIOGENESIS THERAPY	TREATMENT STATUS
Cancer	Attempts to block essential growth factors that many cancers need to establish the blood supplies that allow them to grow and proliferate.	Approved drugs include Avastin, for colorectal cancer; Endostar (in China) for lung cancer; Iressa, for lung cancer; Velcade, for multiple myeloma; Sorafenib, for kidney cancer.
Wet macular degeneration	Halts development of abnormal blood vessels that leak blood and fluid into the eye, causing scarring and loss of vision.	Macugen is approved to treat macular degeneration; Lucentis, pending approval; Avastin, being used "off label."
Diabetic retinopathy	Could decrease excess growth and restore normal permeability of weak capillaries in danger of rupturing or depositing damaging proteins in the retina.	Eventually, Macugen and Lucentis may be tested for treatment of diabetic retinopathy.
Psoriasis	Could slow the growth of blood vessels that appear to exacerbate the excessive production of scaly skin, which characterizes psoriasis, and other diseases that involve chronic inflammation.	Both MEDI-522 and Curcuminoids C3 Complex, derived from turmeric, are in Phase II trials to inhibit blood vessel formation.
Coronary artery disease and peripheral arterial disease	Stimulates collateral blood vessel growth to feed oxygen-starved tissue, the result of coronary arteries narrowed by plaque. In early-stage coronary disease, antiangiogenic therapy might even help prevent plaque growth.	In lab studies, endostatin has blocked 85% of atherosclerotic plaque in mice.



From a cattle protein to mouse tumors to an almost-magic bullet, the research of Genentech's Napoleone Ferrara culminated in the blockbuster cancer drug Avastin.

Though he worried that most of the participants, 20 children with terminal cancer, might die before the end of the planned six-month trial, he instead faced a very different problem—forcing unwilling patients to stop taking the drugs after two years because of concerns about long-term toxicity.

Tumor growth stabilized in 40% of the patients; one in six saw tumors shrink by at least half. Many patients regrew their hair and regained lost weight, and five were still alive three years later. A Phase II trial under way at 15 medical institutions will test which tumor types respond best to this therapy.

Yet as excited as most cancer experts now are about the potential of antiangiogenic treatments, they are also realistic. Most tumors can make at least four stimulators of angiogenesis, and Avastin, the best drug available, targets just one—VEGF. Moreover, tumors that start out making only VEGF can eventually begin to overproduce other angiogenic growth factors as they progress to late-stage disease. (Broad-spectrum angiogenesis inhibitors are in development, but have not yet received FDA approval.)

“Tumors develop a lot of bypass mechanisms to survive, and my concern is that eventually they may grow through Avastin,” as they have done with other drugs, says LoRusso. “There are too many different kinds of cancer with too many

different pathways and alternative mechanisms they can turn on to think that one drug will be a magic bullet,” she adds.

Moreover, Avastin's extreme expense could discourage some physicians and their patients from using it. A year's treatment for colon cancer costs about \$50,000, according to Genentech, and the higher doses recommended for patients with lung or breast cancer come with proportionately steeper price tags. Even with insurance coverage, patients may face copayments of \$10,000 to \$20,000 annually.

Yet with angiogenesis—either too much or too little—now thought to contribute to as many as 60 disorders, including cancer, macular degeneration, stroke and coronary artery disease, the journal *Nature* recently declared that the \$4 billion already invested in angiogenesis research and drug development “will probably change the face of medicine in the next decade, with more than 500 million people worldwide predicted to benefit.”

For antiangiogenesis to finally reach its potential for managing cancer, says Folkman, it will take some time as physicians become accustomed to the principles of antiangiogenic therapy, which differ from those of conventional chemotherapy. This process, though, is bound to be much shorter than the time it took for Folkman's hunch about tumors' blood supply to enter the medical mainstream. “In 1971 there were only three research papers published on angiogenesis; two were ours, and one was criticizing our work,” Folkman says. “Today there are 25,000 papers with *angiogenesis* in the title, with about 70 new papers published each week.” ■

→ DOSSIER

1. *Dr. Folkman's War: Angiogenesis and the Struggle to Defeat Cancer*, by Robert Cooke [Random House, 2001]. A highly readable account of the obstacles and skepticism Judah Folkman faced in proving his theory.
2. “Normalization of Tumor Vasculature: An Emerging Concept in Antiangiogenic Therapy,” by Rakesh K. Jain, *Science*, vol. 307, Jan. 7, 2005. Beautifully illustrated article on the response of tumors to antiangiogenic therapy.
3. “Insight: Angiogenesis,” *Nature* [<http://www.nature.com/nature/supplements/insights/angiogenesis/index.html>]. Special supplement on the role of angiogenesis in disease and medicine.