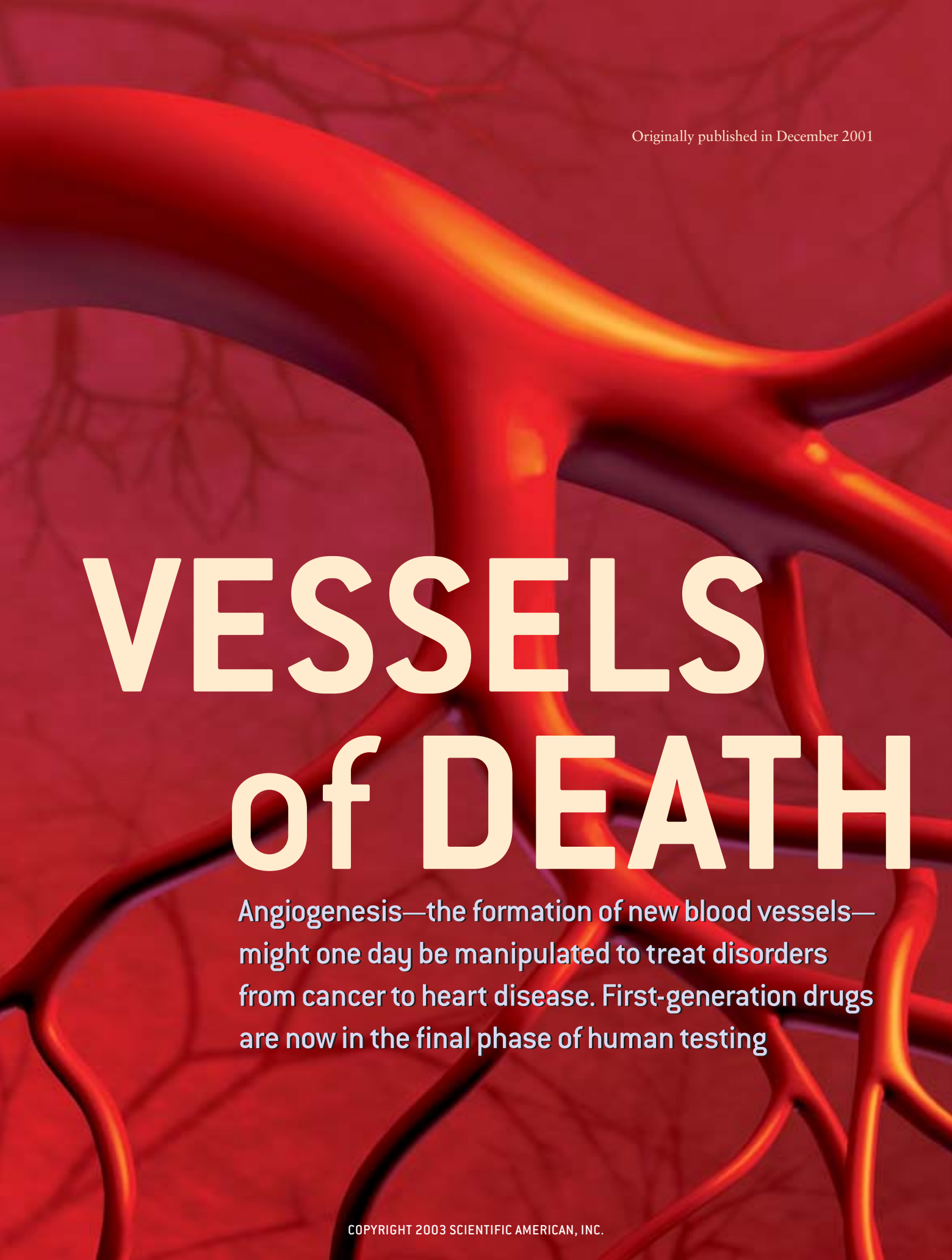

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VESSELS of DEATH

Angiogenesis—the formation of new blood vessels—might one day be manipulated to treat disorders from cancer to heart disease. First-generation drugs are now in the final phase of human testing

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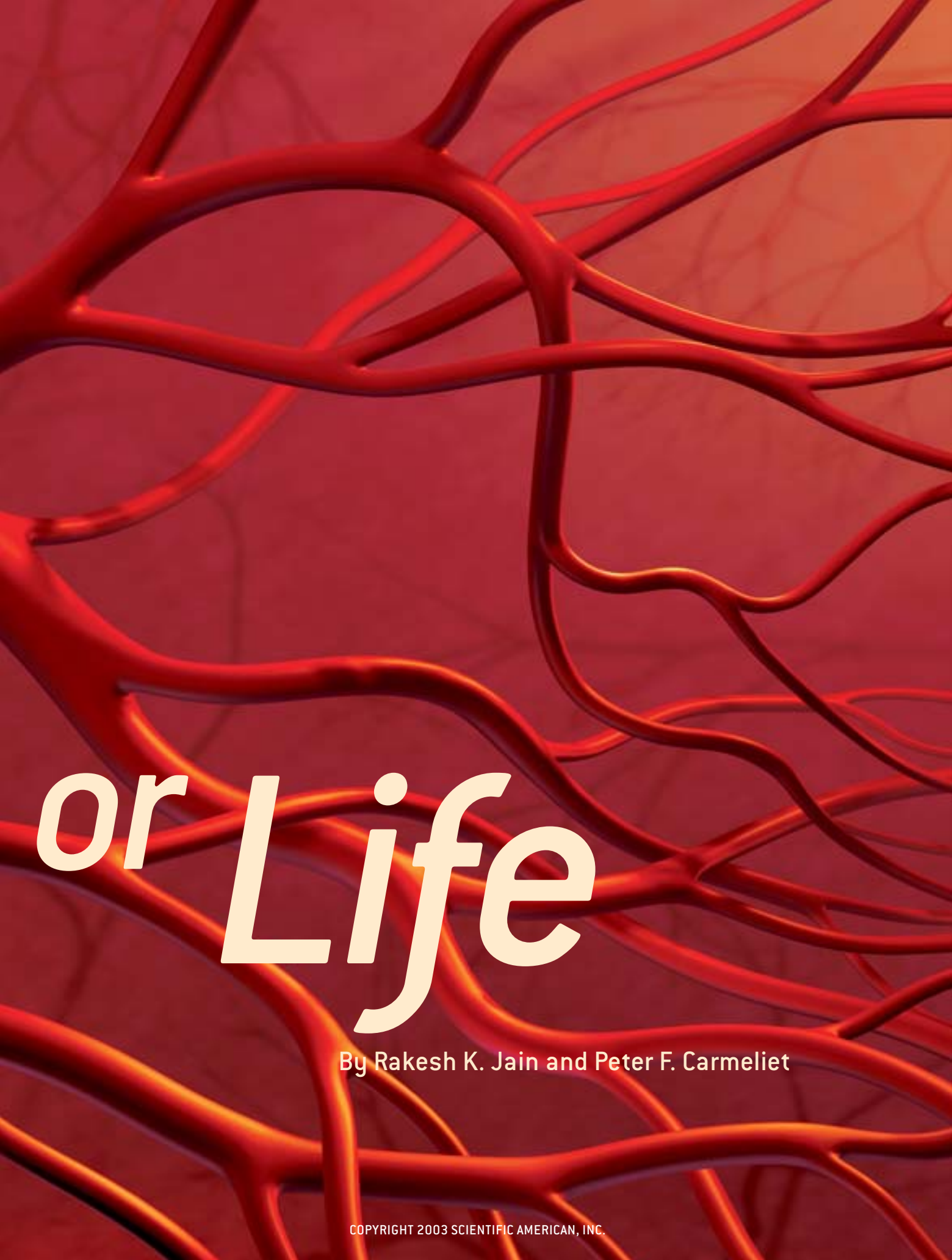

or Life

By Rakesh K. Jain and Peter F. Carmeliet

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They snake through our bodies, literally conveying our life's blood, their courses visible through our skin only as faint bluish tracks or ropy cords. We hardly give them a thought until we cut ourselves or visit a clinic to donate blood. But blood vessels play surprisingly central roles in many serious chronic disorders.

New growth of the body's smallest vessels, for instance, enables cancers to enlarge and spread and contributes to the blindness that can accompany diabetes. Conversely, lack of small vessel, or capillary, production can contribute to other ills, such as tissue death in cardiac muscle after a heart attack. Accordingly, we and other scientists are working to understand the mechanisms that underlie abnormal vessel growth. This effort will help us develop and optimize drugs that block vessel growth—or improve vessel function.

The study of small vessel growth—a phenomenon referred to generally as angiogenesis—has such potential for providing new therapies that it has been the subject of countless news stories and has received enthusiastic interest from the pharmaceutical and biotechnology industries. Indeed, dozens of companies are now pursuing angiogenesis-related therapies, and approximately 20 compounds that either induce or block vessel formation are being tested in humans. Although such drugs can potentially treat a broad range of disorders [*see box on page 39*], many of the compounds now under investigation inhibit angiogenesis and target cancer. We will therefore focus the bulk of our discussion on those agents. Intriguingly, animal tests show that inhibitors of vessel growth can boost the effectiveness of traditional cancer treatments (chemotherapy and radiation). Preliminary studies also hint that the agents might one day be delivered as a preventive measure to block malignancies from arising in the first place in people at risk for cancer.

Results from the first human tests of several compounds that block blood vessel growth were announced earlier this

Overview/*Angiogenesis*

- More than 20 compounds that manipulate angiogenesis—either by stimulating new blood vessel growth or by blocking it—are now in human tests against a range of disorders, from cancer to heart disease.
- Angiogenesis inhibitors are generally safe and less toxic than chemotherapeutic drugs, but they are unlikely to treat cancer effectively on their own. Instead physicians will probably use angiogenesis inhibitors in conjunction with standard cancer treatments such as surgery, chemotherapy and radiation.
- The blood vessels of tumors are abnormal. Surprisingly, angiogenesis inhibitors appear to "normalize" tumor vessels before they kill them. This normalization can help anticancer agents reach tumors more effectively.

HURD STUDIOS (*preceding pages*)

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year. Some observers were disappointed because few of the patients, who had cancer, showed improvement. But those tests were designed solely to assess whether the compounds are safe and nontoxic, which they appear to be. Human tests of efficacy are under way and will be a much better judge of whether angiogenesis inhibitors can live up to their very great promise.

The Genesis of Angiogenesis

THE TERM "angiogenesis" technically refers to the branching and extension of existing capillaries, whose walls consist of just one layer of so-called endothelial cells. In its normal guise, angiogenesis helps to repair injured tissues. In females it also builds the lining of the uterus each month before menstruation and forms the placenta after fertilization. The development of blood vessels is governed by a balance of naturally occurring proangiogenic and antiangiogenic factors. Angiogenesis is switched on by growth factors such as vascular endothelial growth factor (VEGF) and is turned off by inhibitors such as thrombospondin. When the regulation of this balance is disturbed, as occurs during tumor growth, vessels form at inappropriate times and places.

Cancer researchers became interested in angiogenesis factors in 1968, when the first hints emerged that tumors might release such substances to foster their own progression. Two independent research teams—Melvin Greenblatt of the University of Southern California, working with Phillipe Shubik of the University of Chicago, and Robert L. Ehrmann and Mogens Knoth of Harvard Medical School—showed that burgeoning tumors release a then unidentified substance that induces existing blood vessels to grow into them. Such proliferation promotes tumor growth because it ensures a rich supply of blood loaded with oxygen and nutrients. In 1971 Judah Folkman of Harvard proposed that interfering with this factor might be a way to kill tumors, by starving them of a blood supply. What is more, Folkman later posited that blocking the factor could slow cancer's spread, a process called metastasis, because cancer cells must enter blood vessels to travel to other parts of the body.

Nipping New Blood Vessels in the Bud

CURRENT TESTS of angiogenesis inhibitors against cancer employ several different strategies. Chief among these is interfering with the action of VEGF. This molecule, which was initially named vascular permeability factor when it was discovered in 1983 by Harold F. Dvorak and his colleagues at Harvard, appears to be the most prevalent proangiogenic factor identified to date. Scientists gained a tool for better understanding the function of VEGF in 1989, when Napoleone Ferrara of Genentech and his co-workers isolated the gene encoding the molecule. In 1996 groups led by Ferrara and one of us (Carmeliet) independently demonstrated the critical role of VEGF in vessel formation by generating mice that lacked one of the normal two copies of the VEGF gene. The mice, which made half the usual amount of VEGF, died in the womb from insufficient and abnormally organized blood vessels.

Researchers are exploring a number of ways to neutralize VEGF's angiogenic activity in patients. These include immune system proteins called antibodies that can bind specifically to and disable VEGF; soluble forms of the cellular receptors for VEGF, to act as decoys that sop up the growth factor before it can bind to cells; and small molecules that can enter cells and block the growth messages that VEGF sends into an endothelial cell's interior after binding to receptors at the surface. The compounds under study also include factors, such as interferons, that decrease the production of VEGF and substances, such as so-called metalloproteinase inhibitors, that block the release of VEGF from storage depots in the extracellular matrix, the "glue" that binds cells together to create tissues.

Although halving the amount of VEGF is lethal to mouse embryos, wiping out cancers in humans with such therapies will probably require the complete neutralization of all the VEGF protein present in a tumor, and that might be difficult to do. VEGF is a potent agent, and trace amounts could protect the endothelial cells from death. But even after all the VEGF is neutralized, a tumor could rely on other proangiogenic factors, such as basic fibroblast growth factor or interleukin-8.

Another widely studied approach for inhibiting angiogenesis in cancer patients is administering or increasing the natural production of antiangiogenic factors. The idea for this therapy emerged when Folkman learned that Noel Bouck of Northwestern University had identified a naturally occurring inhibitor—thrombospondin—in 1989. Surgeons already knew that removing a patient's primary tumor in some cases accelerated the growth of other, smaller tumors—almost as if the primary tumor had secreted something that kept the smaller tumors in check. They have never questioned the necessity of removing the primary tumor in most cases, because such tumors often obstruct the normal functions of organs and tissues, and leaving them in place would provide a source of cancerous cells for yet more metastases. But discovery of a natural angiogenesis inhibitor suggested to Folkman that the primary tumor's secretions might be harnessed as cancer drugs to suppress the growth of both primary and small metastases.

With this concept in mind, Folkman and his colleagues discovered two more of these naturally occurring antiangiogenic substances—angiostatin and endostatin—in 1994 and 1997, respectively. These inhibitors have received a great deal of attention. This is in part because of studies by Folkman's group showing that they can eradicate tumors in mice. A front-page story

THE AUTHORS

RAKESH K. JAIN and *PETER F. CARMELIET* bring complementary backgrounds to the study of angiogenesis. Jain, who is now the Andrew Werk Cook Professor of Tumor Biology at Harvard Medical School and director of the Edwin L. Steele Laboratory at Massachusetts General Hospital, started his career as a chemical engineer. He held posts at Columbia University and at Carnegie Mellon University before joining Harvard in 1991. Carmeliet is a professor of medicine at the University of Leuven in Belgium, where he also serves as adjunct director of the Center for Transgene Technology and Gene Therapy at the Flanders Interuniversity Institute of Biotechnology. He received his M.D. from Leuven in 1984 and his Ph.D. from the same institution in 1989.

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Therapeutic Angiogenesis

When making more blood vessels is good for the body

It's easy to understand how restricting the growth of new blood vessels could help kill tumors, but fostering vessel growth—a strategy termed therapeutic angiogenesis—could be useful against other disorders.

Researchers around the world are now evaluating whether the angiogenic substances they are trying to block to treat cancer might help heart attack patients—or those at risk for heart attack—grow new blood vessels in the heart. Those factors might also be used to treat people with vascular disorders in their feet and legs.

A heart attack, properly called a myocardial infarction, occurs when a blood clot forms in one of the arteries that feeds the heart muscle, preventing part of the heart from receiving oxygen and nutrients, a condition known as ischemia. Unless the clot is dissolved or dislodged rapidly, the patch of heart muscle can die. In addition, many diabetics suffer from a lack of circulation in their extremities caused by occluded blood vessels; some require amputations.

Therapeutic angiogenesis can involve directly administering a vessel growth–promoting substance, such as vascular endothelial growth factor (VEGF). It can also be accomplished using gene therapy, administering to a patient genetically engineered viruses, cells or pieces of DNA that carry the gene encoding VEGF or another angiogenic factor.

Therapeutic angiogenesis with VEGF or fibroblast growth factor (FGF) has been explored for the past 10 years. In 1991 scientists led by Stephen H. Epstein of the National Institutes of Health studied the effects of FGF on the heart vessels of animals. A year later Paul Friedmann and his co-workers at Baystate Medical Center in Springfield, Mass., showed that FGF injections could prompt angiogenesis in the hind limbs of rabbits. In the mid-1990s several groups—including those led by Epstein, Michael Simons of Harvard Medical School, Jeffrey M. Isner of St. Elizabeth's Medical Center in Boston and Ronald G. Crystal of Cornell University Medical School in New York City—demonstrated that therapy involving angiogenic factors or the genes that encode them could stimulate angiogenesis in the hearts and limbs of animals.

Clinical trials aimed at evaluating the safety and efficacy of angiogenic factors in patients are now under way. Carmeliet and others are also testing the therapeutic potential of other promising molecules, such as placental growth factor, a relative of VEGF. Creating functional blood vessels appears to be a formidable challenge, however. Researchers are trying to find the best combinations of such proangiogenic agents as well as the optimal dose, administration schedule and delivery route for the drugs. They are also evaluating whether transplants of endothelial stem cells—the precursors of the endothelial cells that make up blood vessels—can augment the regeneration of blood vessels. Such stem cells can be isolated from the bone marrow of adults.

But potential risks accompany the promise of proangiogenic therapy. Therapeutic angiogenesis could increase a patient's risk of cancer by allowing tiny tumors that had been dormant in the body to gain a blood supply and grow. In addition, because the atherosclerotic plaques that underlie heart disease require their own blood supply as they become larger, therapeutic angiogenesis could backfire as a treatment for cardiac disease by stimulating the growth of plaques that had caused the individual's heart attack in the first place.

Human studies to evaluate the likelihood of these dire scenarios have only recently begun. We hope one day to be able to use genetic tests to evaluate a patient's natural balance of proangiogenic and antiangiogenic factors before beginning to treat them with proangiogenic drugs.

This information might also help us understand whether myocardial ischemia results from the insufficient production of angiogenic factors or from the excess production of angiogenic inhibitors. The results will undoubtedly aid in the development of more directed strategies for therapeutic angiogenesis.

—R.K.J. and P.F.C.

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heralding such successes in 1998 in the *New York Times* increased the visibility of the entire field of angiogenesis.

Clinical trials of angiostatin and endostatin are currently in early stages (experiments involving small numbers of patients to evaluate a potential drug's safety). Preliminary results reported at this year's American Society of Clinical Oncology conference, which were alluded to earlier, indicate that endostatin is safe and causes no side effects. We await the outcome of the various clinical trials of these and other angiogenesis inhibitors in the coming years.

Going after Established Blood Vessels

THE TWO APPROACHES described thus far interfere with the formation of new blood vessels. But what about preexisting vessels in a tumor? Is it possible to target those without disrupting the established vessels in healthy tissues and organs (an approach termed antivascular therapy)?

Luckily, it turns out that the blood vessels of tumors are abnormal. Not only are they structurally disorganized, tortuous, dilated and leaky, but the cells that compose them display certain molecules on their surfaces from a class known as integrins that are absent or barely detectable in mature vessels. Biologists have recently produced small proteins, called RGD peptides, that preferentially recognize the integrins on tumor vessels. These peptides can be linked to cell-killing drugs to target such therapeutic agents to tumors without damaging other tissues. They could also be used to clog the vessels that feed the tumor, by delivering molecules that cause blood clots to form.

But it might not be so easy for any drug to zero in on all a given tumor's blood vessels. The individual cells that make up even a single tumor vessel can vary widely. Studies in one of our labs (Jain's) have found that 15 percent of the blood vessels in human colon cancers are mosaic: some have a particular protein on their surfaces, whereas others do not. If the proteins targeted by new drugs turn out to differ from one tumor to the next or to vary within a tumor during the course of its growth or treatment, this heterogeneity will make it difficult to get therapies that target blood vessels to work on their own.

Combine and Conquer

MOST LIKELY, surgery or radiation—or both—will continue to be used to attempt to eliminate the original tumor. Today chemotherapy is often administered before or after such therapy to shrink tumors and mop up undetectable malignant cells remaining in the body. Antiangiogenic drugs could well be combined with any of the other approaches to improve the success rate.

Following the pioneering studies of Beverly Teicher of Harvard in the 1990s, several groups have shown the benefits of such a combined approach. Recently Folkman, Robert Kerbel of the University of Toronto and Jain's group have found that combined therapy can produce long-term cures in mice.

Interestingly, antiangiogenic therapy appears to boost the effectiveness of traditional cancer treatments. This is surprising because chemotherapeutic agents depend on blood vessels to reach a tumor, and radiation kills only those cells that have an adequate supply of oxygen (it turns oxygen into toxic free radicals). Logic suggests that by compromising the blood supply of tumors, antiangiogenic therapy would interfere with the effectiveness of these standard treatments. But scientists have demonstrated that the delivery of chemotherapy—as well as nutrients and oxygen—improves during the course of some antiangiogenic therapies.

Indeed, researchers led by Jain have shown that antiangiogenic factors can "normalize" tumor vasculature before killing it by pruning excess, inefficient vessels while leaving efficient vessels temporarily intact. In studies of mice, the researchers found that angiogenesis inhibitors decreased the diameters of tumor

ANGIOGENESIS INHIBITORS NEARING THE MARKET

These potential therapies for cancer are in phase III testing, the last stage before Food and Drug Administration approval. Angiostatin and endostatin are in earlier phases of evaluation. Similar compounds are also in trials against the eye disease macular degeneration.

PRODUCT	DEVELOPER	DESCRIPTION	DISEASE TARGET
Avastin	Genentech	Monoclonal antibody that disables vascular endothelial growth factor (VEGF), a promoter of angiogenesis	Breast and colorectal cancer
BMS275291	Bristol-Myers Squibb	Synthetic compound having multiple effects	Nonsmall cell lung cancer
Interferon alpha	Roche, Schering	Protein that inhibits release of growth factors such as VEGF	Various tumors
Marimastat	British Biotech	Synthetic compound having multiple effects	Breast and prostate cancer
Neovastat	Aeterna	Naturally occurring inhibitor with a range of properties	Nonsmall cell lung and renal cancer
SU5416	Sugen	Synthetic compound that blocks the receptor for VEGF	Colorectal cancer
Thalidomide	Celgene	Organic molecule whose specific mechanism of action is unknown	Renal cancer and multiple myeloma

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blood vessels and made them less leaky, so they began to resemble normal vessels. If such studies pan out in humans, however, physicians will need to work out the optimal dosage and timing of administration.

As is true for many drugs, future generations of antiangiogenic agents are likely to be more effective than the first generation. To optimize future drugs, researchers will need to modify their investigation methods. Most preclinical studies, performed before a drug can be tested in people, are carried out on tumors that are artificially grown under the skin of animals such as mice. But few human tumors arise beneath the skin. To get a more realistic idea of whether a given cancer drug will work in people, researchers will need to study animals with spontaneously occurring tumors growing in more natural sites.

Another limitation of preclinical studies is that they are time-intensive and costly, so researchers usually halt them when tumors begin to shrink but before they can be sure a treatment being tested will actually eradicate the cancers. Because tumors can recur from even a very small number of surviving cancer cells, scientists should follow treated animals for longer periods to better determine the promise of new drug candidates. In addition, investigators tend to begin administering experimental drugs to animals before tumors are fully established, at a time when the cancers are vulnerable—possibly tilting the scales in the drug's favor. Animal tumors also tend to grow more quickly than those in people, and drugs that kill such fast-growing cancers might not be effective against slower-growing human tumors.

Researchers also need to study combinations of antiangiogenic drugs. Cancer cells are masters of evasion. Each tumor produces different combinations of angiogenic molecules that may vary or broaden as they grow. Administering an antiangiogenic drug that blocks only one molecule, such as VEGF, can simply prompt tumors to use another proangiogenic substance to attract a blood supply. In the end, optimal antiangiogenic therapy might consist of a cocktail of several angiogenesis inhibitors.

An Ounce of Prevention

IF ANGIOGENESIS INHIBITORS fulfill their early promise against cancer, patients will probably need to take them for a long time. The drugs might also be administered as cancer preventatives to people with a high risk of particular cancers—an approach initially suggested in 1976 by Pietro M. Gullino of the National Cancer Institute. Consequently, they must be shown to be safe over the long term. (The drug interferon, an indirect antiangiogenic agent, has been given for years with no side effects to pediatric patients with hemangiomas—benign blood vessel tumors.) The existing human trials will not address this question; they are designed to evaluate safety for just a few months. Animal studies hint that some antiangiogenic compounds might not be safe enough for the long-term administration required to prevent growth or relapse of cancer. Mice that have been genetically manipulated to reduce their production of VEGF can develop neurological defects after a prolonged period, for example, as shown in experiments by Carmeliet.

Insufficient angiogenesis can also impair the heart's recovery from ischemia, tissue starvation stemming from a poor supply of blood. During a heart attack, a blood clot lodges in an artery that supplies the heart muscle, killing a part of the organ. Indeed, researchers are testing agents that spur angiogenesis as treatments for ischemic heart disease. Accordingly, antiangiogenic cancer treatments might increase a patient's risk of ischemic heart disease. As with any therapy, then, physicians and patients will have to carefully weigh the risks and benefits of using angiogenesis inhibitors.

Nevertheless, the burgeoning understanding of angiogenesis has changed our thinking about how to attack cancer. Current treatment with radiation and chemotherapy halts many cancers, but too often the existing treatments bring about only a temporary symptom-free period before the tumor shows up again, spreads throughout the body and kills. Part of the problem is that physicians and pathologists lack reliable, sensitive, cheap and easy-to-use tests that can identify characteristics about each patient's cancer that indicate the best treatment strategy. Genetic analyses of tumors and patients promise to improve the accuracy of diagnoses as well as the efficacy and safety of treatments in the future, but we suspect that within the next 10 or 20 years, better visualization of abnormal vessel structure and function will help as well.

Antiangiogenic approaches have already shown benefit in patients with hemangiomas. As knowledge of tumor angiogenesis progresses, cancers may be detected through elevated levels of angiogenic molecules in the blood—long before clinical symptoms. Physicians may begin to examine patients regularly using molecular tests and new imaging techniques to determine an individual's profile of proangiogenic and antiangiogenic factors.

Based on such tests, doctors will be able to devise treatment plans that, along with other therapies, incorporate a mix of angiogenesis inhibitors appropriate for that individual's tumor. Tests that detect the presence of abnormal vessels will allow doctors to detect possible relapses at an early, potentially treatable stage. Perhaps, as safe oral antiangiogenic drugs are developed and become available, cancer patients will be able to take "a pill a day to keep the cancer away." If so, forms of cancer that are currently untreatable will be reduced to chronic health problems similar to hypertension or diabetes, and many more people will be able to live long, satisfying lives. SA

MORE TO EXPLORE

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The National Cancer Institute Web site provides updates on cancer trials that are using angiogenesis inhibitors: **www.cancertrials.nci.nih.gov**

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