From Bench Top to Bedside

More than 2 decades of research on oncogenes and tumor suppressors may finally pay off in a new generation of more specific, less toxic, cancer drugs

In the 25 years since then-President Richard Nixon declared the "War on Cancer," researchers have learned a great deal about the enemy. In particular, they have uncovered a host of genetic blunders that can drive cells to become cancerous and grow out of control. They have learned that the balance of power shifts within many cancer cells, as genes called oncogenes, whose protein products foster cell growth, become overactive, while so-called tumor-suppressor genes, whose products normally act to keep cell growth in check, are disabled. So far, these intelligence efforts have yet to accomplish the war's objectives: better treatments that can vanquish this dread disease. But that could be changing.

Researchers in both academe and industry, including a host of new biotech companies (see p. 1039), are developing an arsenal of drugs aimed at counteracting the genetic changes leading to cancer. Some of them have already yielded promising results in cell culture studies and in animals, and are moving into tests in humans—and more are on the way. Indeed, Ivan Horak, director for oncology at Janssen Research Foundation, a subsidiary of Johnson & Johnson in Titusville, New Jersey, predicts that in the next few years there will be an "explosion" of new therapeutic strategies, with "attacks on every gene that people feel plays a significant role in carcinogenesis."

The research efforts of the last 20 years have laid out a host of targets for these new drugs. For example, several oncogenes make cell surface receptors through which growth factors exert their effects, and researchers are working on antibodies and on small-molecule drugs that block the activity of those receptors. Other drugs seek to block oncogene products that transmit growth stimulatory signals inside cells, like the protein made by the *ras* oncogene. Still others aim to make up for oncogene or tumor-suppressor gene mutations that impair cells' ability to initiate a form of suicide, known as programmed cell death or apoptosis. Researchers hope that these new therapies will be more specific than current chemotherapeutic drugs and thus kill cancer cells more effectively, with less harm to normal cells.

There is no evidence yet that these treatments are going to fare any better than other, more general treatments, like interferon, that failed to meet high expectations set by animal experiments and early clinical trials. But cancer researchers are upbeat, in part because the treatments are so logical—rooted as they are in decades of basic research on the genetic basis of cancer. "We are at last beginning to move into blocking some of the signaling pathways that we now know are overactive in many cancers," says Alex Bridges, a chemist with Parke-Davis in Ann Arbor, Michigan. "I am very optimistic that we will get very useful agents out of this." But, Horak counters with a note of caution, "we don't have any proof for that optimism in our hands yet."

SOME DRUGS IN CLINICAL TRIALS

Drug Type	Goal	Company Developing	Cancers
Antibody	Block HER2 growth factor receptor	Genentech	Breast
	Block EGF receptor	ImClone Systems	Kidney, prostate, breast, head and neck
Tyrosine kinase inhibitor	Inhibit PDGF receptor	Sugen	Glioma
	Inhibit EGF receptor	Zeneca	Various tumors
	Inhibit EGF receptor	Oncogene Science/Pfizer	Various solid cancers
Farnesyl transferase inhibitor	Prevent Ras activity	Janssen	Various tumors
CDK inhibitors	Block cell cycle	Hoechst Marion Roussel	Various tumors
bcl-2 antisense	Restore apoptosis	Genta	Lymphoma, various solid cancers
Virus with *p53*	Restore lost *p53* tumor suppressor	Introgen Therapeutics	Lung, head and neck
		Canji/Schering-Plough	Lung, ovarian, liver
Modified adenovirus	Selectively kill *p53*-lacking cells	Onyx Pharmaceuticals	Head and neck, gastrointestinal, pancreatic, ovarian

Block that receptor

Drug designers' first line of attack—dating back 15 years—is directed at the oncogenes that make growth-factor receptors, molecules embedded in the membranes of cells that receive growth signals from outside the cells. In some cancers, these receptors are either produced in greater than normal amounts or have mutations that cause them to be overactive. Either way, the cell receives a revved-up growth signal. In the early 1980s, researchers began exploring whether they could shut off the receptors with antibodies that bind to them.

Antibodies against one such receptor, the product of the *HER2* oncogene, are being tested in large-scale clinical trials by Genentech Inc. of South San Francisco as a treatment for breast cancer. Interest in *HER2* began in 1986 when Dennis Slamon's group at the University of California, Los Angeles, reported that 25% to 30% of breast tumors overproduce the HER2 protein, suggesting that it helps drive the growth of the cancers. And these were the nastiest tumors: "Patients who had this genetic alteration had a very poor clinical outcome," says Genentech senior scientist Mark Sliwkowski. An encouraging sign came, though, when experiments in several laboratories showed that antibodies to the receptor could block the growth of breast cancer cells that overexpress *HER2*.

Buoyed by those findings, Genentech began a series of small trials in 1992. In one, tumors shrank by more than 50% in five of 44 women treated with the antibodies alone. In another study, 36 women with advanced breast cancer that had not responded to chemotherapy were given the antibodies in combination with a standard anticancer drug, cisplatin. The tumors of nine of the women shrank by more than 50%. That 25% response rate, Sliwkowski says, is much higher than expected for cisplatin alone.

The antibody had minimal side effects, and so Genentech moved on to a large-scale trial, including 650 women, in which it is

testing the antibodies alone or with cisplatin. The results are expected by the middle of next year, and if the antibodies again prove effective, they might be tried against other cancers that also overproduce HER2, such as ovarian cancer.

The epidermal growth factor (EGF) receptor also seems to make mischief in cancer; its gene—one of the first oncogenes to be identified in the early 1980s—is overactive in one-third of all cancers of epithelial origin, including breast, lung, and bladder cancer. John Mendelsohn, president of the University of Texas's M. D. Anderson Cancer Center in Houston, has developed monoclonal antibodies against this receptor, which block its activity and stymie tumor cell growth in lab and animal tests. The New York biotech company, ImClone Systems, has begun early-stage human trials to test the antibodies, either alone or in combination with traditional chemotherapy, on kidney, prostate, breast, and head and neck cancers (which include cancers of the tongue, soft palate, and upper airway, but not of the brain).

Treatment Marks Cancer Cells for Death

Tumor-suppressor genes are an obvious target for cancer treatment, because they are lost or inactivated in many cancers. Most efforts to exploit these genes have taken a straightforward approach: trying to replace them or mimic their function with some other molecule (see main text). But one candidate cancer treatment tries instead to turn the absence of a tumor suppressor—the *p53* gene—into an advantage.

In order to infect and kill cells, the human respiratory virus, adenovirus, has to disable *p53*, because the gene's activities include preventing viral DNA replication. Reasoning that an adenovirus unable to disarm the gene would only be able to infect cells in which *p53* was nonfunctional, researchers at Onyx Pharmaceuticals of Richmond, California, removed the viral gene that disables *p53*. Subsequent tests with cultured cancer cells and tumors growing in mice confirmed that the modified virus specifically kills tumor cells lacking *p53* (*Science*, 18 October 1996, p. 342).

Since then, the company has completed early human trials. The modified virus has to be injected directly into tumors, as the immune system would eliminate it if it were infused into the bloodstream. In 32 patients with head and neck cancers, these injections produced no problematic side effects, says Frank McCormick of the University of California, San Francisco, Cancer Center, and former vice president of research at Onyx. Even more encouraging, the virus caused the tumors to shrink in 12 of the patients, in some cases by as much as 90%. McCormick says Onyx has begun a second effectiveness trial for head and neck cancer and safety trials for additional cancers.

It "is a very clever approach," says Allen Oliff, executive director for cancer research at Merck Research Laboratories in West Point, Pennsylvania. But he notes that the need to inject the virus into tumors will limit its use for metastatic cancer. "It is not going to be penicillin for cancer," he says, but for certain tumors it "still could be very successful." –M.B.

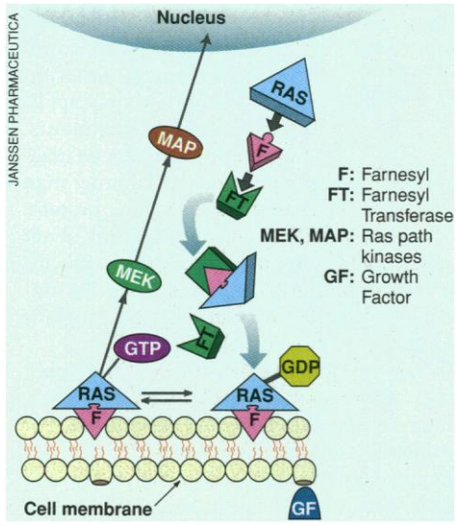
Small is beautiful

Antibodies are expensive, because they have to be made in animals and purified. Because they are proteins and would be digested if given by mouth, they must be injected. It may be, Mendelsohn says, that the advantages of antibodies, most notably their specificity for their targets, which should minimize toxicity, may outweigh their disadvantages. But, he says, "if everything else were equal ... cheaper, smaller molecules certainly would be desirable."

Drug companies agree. And many are looking for small molecules that can block growth-factor receptors. In contrast to the antibodies currently being tested, which react with the portion of the receptor that projects out of the cell, the small-molecule inhibitors now being considered act on the other end of the receptor molecule, the inside part that transmits the growth signals to the molecules of the internal signaling pathways. For most cancer-causing growth-factor receptors, these inside segments are tyrosine kinases, enzymes that activate the signaling proteins by adding phosphate groups to them. And a small compound stuck in the right place can often shut an enzyme down.

Companies have already found a variety of tyrosine-kinase inhibitors that work by wedging into the enzyme's binding site for the phosphate-donating molecule, ATP. Because hundreds of kinases bind ATP, though, researchers worried that these compounds would interfere with many normal enzymes, leading to crippling side effects. "The conventional wisdom held that you could never fashion a specific [inhibitor] going after the ATP binding pocket," says Alex Matter, director of oncology research at the Swiss drug company Novartis.

Defying the conventional wisdom, however, drug developers have tried to take advantage of small differences in the structure of the ATP pockets of the kinases to design inhibitors with the desired specificities.



Fatty anchor. Ras must be linked to a fat group such as farnesyl in order to stick to the cell membrane where it can be activated by growth signals from outside the cell. Active Ras then turns on kinase enzymes that take the growth signal to the genes in the cell's nucleus.

And in animal trials of these compounds, they got better selectivity than they expected, as the tyrosine-kinase inhibitors shrank tumors but produced few side effects. "These compounds appear to be very active in doses [that don't produce] obvious toxicity," says Dick Leopold, senior director of cancer biology at Parke-Davis Pharmaceutical Research, a division of Warner-Lambert in Ann Arbor, Michigan.

On the basis of the promising animal results, several companies have taken the inhibitors to human trials. Sugen of Redwood City, California, was first, with Su-101, a compound that selectively inhibits the receptor for platelet-derived growth factor (PDGF). The drug passed initial safety tests on 150 patients, showing low toxicity at moderate doses, says Peter Langecker, vice president of clinical affairs at Sugen. What's more, those doses showed some effects against glioblastoma, a pernicious brain cancer whose growth depends on PDGF. "There are patients ... who were expected to be dying soon, and who had failed all other treatments ... who have now been stable for over a year," says Langecker. Sugen is about to go to the U.S. Food and Drug Administration (FDA) with a proposal for a large-scale trial of Su-101 for glioblastoma.

Su-101—which must be administered intravenously because it breaks down in the stomach—is only the first of a long line

of tyrosine-kinase inhibitors headed for the clinic. Orally active ones, as well as ones that have gone through more rounds of engineering for specificity, are on the way. The British drug company Zeneca and Oncogene Science of Cambridge, Massachusetts, both have small-molecule inhibitors of the EGF receptor in early human trials for a variety of cancers, and Novartis plans to begin trials within the year of an inhibitor of the Abl tyrosine kinase, which is activated in several types of leukemia.

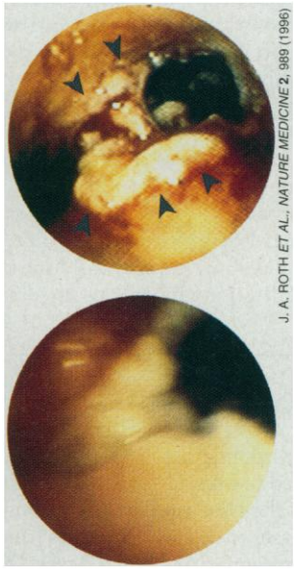
Ras-ional drug design

Tyrosine kinases are just the first step in an internal signaling pathway that triggers cell growth. Another key protein in that pathway is produced by one of the most commonly mutated oncogenes in human cancers—*ras*. *Ras* is activated by tyrosine kinases, and in turn draws other kinases to the cell membrane where they are activated and can then transmit the growth signal in the cell. There are three *ras* genes, and 25% to 30% of all cancers have mutations in one of them, making the Ras proteins obvious targets for drug design. Efforts to design Ras inhibitors have resulted in an unexpected bonus: the discovery of a class of drugs whose effects are not limited to tumors with *ras* mutations.

To block Ras activity, researchers took advantage of work done in the late 1980s showing that an enzyme called farnesyl transferase (FT) has to hook a 15-carbon fatty chain to Ras, before it can function. Researchers found drugs that inhibit FT, but then a possible obstacle to using them for cancer treatment emerged: K-Ras, the form of Ras that is by far the most often mutated in human tumors, can duck the farnesylation block and receive a fatty chain from an alternate enzyme. That discovery suggested that blocking FT shouldn't stop K-Ras–driven tumors.

But it does. Working with various cancer cell lines, researchers found that FT inhibitors block the growth of some tumors with a mutant *K-ras* gene, some with other *ras* mutations, and even some that have no *ras* mutations at all. "There is no correlation of the sensitivity of the [tumor] cell line with its mutant *ras* status," says Parke-Davis's Leopold. "That implies that there is another target besides Ras that is farnesylated and is very important for the growth of these tumors," adds Saïd Sebti, of the University of South Florida in Tampa, who worked with Andrew Hamilton at Yale University to develop FT inhibitors. Because FT modifies more than 20 proteins, researchers will have to sort through a lot of possibilities to find that key target.

Surprisingly, given their potential to affect many proteins, animal studies have shown that FT inhibitors are not very toxic. They also have "a great deal of activity" against tumors in animal models, says cancer biologist Neal Rosen of the Memorial Sloan-Kettering Cancer Center in New York City, who tested FT inhibitors on 50 different cultured tumor cell lines. "I'm very anxious to test [them] in patients."



Early promise. Therapy with a *p53*-carrying virus shrank this human lung tumor, which was viewed with a bronchoscope before *(top)* and after treatment *(bottom)*.

Janssen has the first FT inhibitor in clinical trials, although no results are yet available. Other companies, including Schering-Plough, Merck & Co., and Parke-Davis, plan to have FT inhibitors in patients soon. Says Leopold: "It is an example of using a very rationally selected fishhook and catching a type of fish you didn't expect to catch."

Putting on the brakes

Perhaps the biggest fish in the pond are the tumor-suppressor genes. That's because most cancers have inactivating mutations in one or more of these genes, which normally act to control cell growth, and some tumor-suppressor genes are inactivated by mutation in as many as 50% of all cancers. That means that ways to restore the genes' function would likely be widely applicable as cancer treatments. But compensating for an inactivated tumor suppressor is much harder than reining in an overactive oncogene protein. "It is a very difficult concept, to think about replacing the function of a large protein with a small-molecule drug," says molecular oncologist Kenneth Kinzler, of Johns Hopkins University Oncology Center.

But by looking downstream of the tumor suppressors at the proteins they influence, drug developers hope to find good targets. For example, the tumor suppressor *p16*, which is mutated in the skin cancer melanoma and other cancers, normally holds up cell division by blocking the activity of cyclin-dependent kinase 4 (CDK4), one of several related enzymes called CDKs that together propel cells through the cell cycle. A small-molecule inhibitor of CDK4 might therefore replace the function of *p16*, and many companies are working to develop such specific blockers.

Meanwhile, one nonspecific CDK blocker has already arrived in the clinic. The drug, flavopiridol, is a general kinase inhibitor, but researchers at the drug company Hoechst Marion Roussel found that the CDKs are most sensitive to its effects. Even though its broad effects on CDKs might seem to threaten normal cells as well as cancer cells, animal studies showed relatively few side effects, says Dagmar Oette, head of clinical research in cancer therapeutics at Hoechst. The drug has now been given to more than 100 cancer patients in clinical trials, again with few signs of unwanted effects. What's more, while the trials to date focused on safety testing, the tumors in a number of patients stopped growing or shrank, and there was one complete remission. Encouraged by those results, the company plans to begin effectiveness trials soon.

Restoring suicidal drive

While some mutations of oncogenes and tumor suppressors make their mischief by boosting cell growth, others, including those in the tumor suppressor *p53* and the oncogene *bcl-2*, impair another key process of normal cells: apoptosis, or programmed cell suicide, which serves among other things to remove cells whose DNA has been damaged. These mutations are particularly nasty because they pack a double punch. Not only do they allow damaged cells to avoid suicide and possibly turn cancerous, but they also make cancer cells resistant to the many chemotherapeutic drugs that work by triggering apoptosis. Researchers are now looking for ways to repair this self-destruct switch in cancer cells.

One of the most hotly pursued involves using gene therapy to replace *p53*, a tumor-suppressor gene that plays a role in apoptosis and is defective in about half of human cancers. First off the mark was Jack Roth, a thoracic surgeon at the M. D. Anderson Cancer Center, who decided 8 years ago to try using retroviruses and, later, the respiratory virus adenovirus to deliver normal copies of *p53* to lung cancer cells. Because the immune system would clear the viruses from the bloodstream if they were given systemically, they must be injected directly into the tumor or its surroundings, and that limits their potential for treating metastatic disease. But animal tests suggest that the injection strategy can shrink primary tumors, which can be the major causes of death in some types of cancer, including lung and head and neck cancers. And when the researchers injected the *p53*-bearing viruses into human lung tumors growing in mice, says Roth, "we saw rather substantial tumors regress."

Austin biotech company Introgen Therapeutics took Roth's viruses into early-stage clinical trials for lung and head and neck cancers, and Canji Inc., a San Diego subsidiary of

Schering-Plough, has a similar *p53*-carrying adenovirus in human trials. So far, neither the Schering-Plough nor the Introgen viruses have caused any troublesome side effects, and while Schering declined to comment on the effectiveness of its virus, Introgen reported that tumors have regressed or at least stopped growing in some patients treated with the virus alone or together with the drug cisplatin. Based on those results, Introgen, in collaboration with RPR Gencell, a division of the French drug company Rhône-Poulenc Rorer, has just begun a larger trial aimed at evaluating the treatment's effectiveness for head and neck cancers.

Other efforts focus on knocking out the function of the oncogene *bcl-2*, an inhibitor of apoptosis, which is overactive in about half of all human cancer types. "Cells with upregulated *bcl-2* ... are very difficult to kill with anything you throw at them," says cell-death researcher John Reed of the Burnham Institute in San Diego. Several companies are trying to develop drugs to inhibit the Bcl-2 protein, says Reed, while others are already testing a more direct assault on Bcl-2: antisense nucleotides designed to prevent the protein from being made in the first place.

In April, Andrew Webb of the Royal Marsden Hospital in Sutton, Surrey, in the United Kingdom, and his colleagues reported the results of the first human trial of anti-*bcl-2*, in nine patients with advanced non-Hodgkin's lymphoma. One patient had a complete remission and another had partial reduction of his tumors. Based on the promise of those results, medical oncologist Howard Scher of Sloan-Kettering in New York, along with the San Diego antisense biotech company Genta, which developed the drug, are waiting for FDA approval to begin testing it in 10 to 20 patients with a variety of solid tumors that overexpress *bcl-2*.

Once the roadblocks along the suicide pathway have been removed, says Reed, it may be necessary to trigger apoptosis by damaging the cells. As a result, he says, efforts to restrain *bcl-2* or replace *p53* may be most effective, he says, when used "as a sensitizer in combination with traditional cytotoxic therapies."

Although traditional therapies are likely to remain a part of the anticancer armamentarium, the new approaches could transform treatment strategies. If even a few of them pay off, they may mean a future in which doctors will screen tumors for the mutations they carry, then target the defects in the cancer cells directly to prime the tumor cells for killing. "It won't matter whether it is a breast, ovarian, or prostate tumor," says Peter Hirth, executive vice president for research and development at Sugen. "[The mutation] will be the target for therapeutic intervention."

–**Marcia Barinaga**

BIOTECHNOLOGY BUSINESS

On the Biotech Pharm, a Race To Harvest New Cancer Cures

More than 1400 biotechnology companies make their homes in North America, according to the investment newsletter *Biotech Navigator*, yet fewer than 50 biotechnology products have been successfully commercialized to date. With statistics like that, it's easy to see why biotech has gained a reputation as "one of the worst investments on the street," in the words of David Tomei, founder and chief executive officer (CEO) of the Richmond, California, firm LXR Biotechnology Inc. Yet the former Ohio State University pharmacologist is among a cadre of scientist-entrepreneurs who believe they can reverse that reputation—by developing drugs that remedy the genetic and molecular defects behind most cases of cancer.

world's eight top-selling anticancer drugs, four—the prostate cancer drugs Casodex, Eulixin, Lupron, and Zoladex—are merely palliative, yet have combined annual sales of $1.7 billion, while sales of the breast cancer drugs tamoxifen and taxol are approaching $500 million and $800 million, respectively.

But will tapping into this revenue stream be any easier for these new firms than for many of their predecessors in biotech? Tomei and others say yes, because companies such as LXR aren't merely applying ideas developed by academic scientists, but are generating many of their own advances in basic cancer biology. That kind of innovation "will succeed in reversing the feeling that biotech was wishful thinking," says Tomei. Still, any

A SAMPLING OF MOLECULAR ONCOLOGY FIRMS

Company (Location)	Type of Product	Status
Calydon (San Francisco, CA)	Prostate cancer gene therapy	Preclinical testing
Canji/Schering-Plough (San Diego, CA)	*p53*, *RB* gene replacement therapy	Early clinical trials
IDUN Pharmaceutical (San Diego, CA)	*bcl-2* inhibition	Preclinical testing
Incyte (Palo Alto, CA)	Gene sequence databases	19 subscriptions sold
Introgen Therapeutics (Austin, TX)	*p53* replacement therapy	Early clinical trials
Genta (La Jolla, CA)	Antisense *bcl-2* inhibition	Early clinical trials
LXR (Richmond, CA)	Antiapoptotics	Advanced clinical trials
Mitotix (Cambridge, MA)	Cell cycle inhibitors	Preclinical testing
Myriad Genetics (Salt Lake City, UT)	Genetic testing	BRACAnalysis kit
Onyx (Richmond, CA)	*p53* gene therapy	Early clinical trials
Ribozyme (Boulder, CO)	Growth factor inhibition	Preclinical testing
Targeted Genetics (Seattle, WA)	*HER2* gene therapy	Advanced clinical testing

The goal of this new effort in "molecular oncology" is to devise drugs that correct the specific defects that cause cancer in the first place—the abnormal activation of growth-promoting oncogenes, for example, or loss of tumor-suppressor genes. The hope is that treatments will turn out to be more effective and have fewer side effects than conventional cancer chemotherapeutic drugs (see p. 1036). More than two dozen firms, with a combined capitalization in the hundreds of millions of dollars, are competing for leading positions in this emerging market.

The potentially huge profits available to the inventors of better cancer drugs explain their eagerness. The American Cancer Society estimates that 1.4 million new cases of cancer will be diagnosed in the United States in 1997, with the overall medical costs from cancer amounting to $35 billion. Of the

new molecular oncology company faces other hurdles that have tripped up many biotech companies before, leaving them cashless and without a product to sell.

To avoid this fate, a new company must have a scientific advance that promises a practical treatment. It must beat out competitors for precious start-up capital and find revenues to supplement that capital during the protracted process of preclinical and clinical testing. It must protect its intellectual property, and if it raises money by agreeing to share scientific advances with larger pharmaceuticals firms—a standard practice among young biotech firms—it must actually deliver the goods.

For the many cancer researchers who, like Tomei, have left academic posts to seek their fortunes in biotech, there's a clear message: Bucking the biotech trend won't be simple. "If you have a great idea, solid science, and