In 1997 an estimated 1.38 million Americans will be newly diagnosed with cancer. Sadly, the main treatments currently available—surgery, radiation therapy and chemotherapy—cannot cure about half of them. This sobering fact has spurred serious efforts to develop additional strategies for treating the disease—ones based on the biology behind it. To that end, scientists are turning toward gene therapies, which involve introducing into the body genes that can potentially combat tumors.

Researchers initially explored gene therapies for remedying conditions caused by defective genetic instructions, or mutations, passed on from one generation to the next. Most cancers are not inherited in this way but instead result from acquired mutations, produced by external factors such as tobacco smoke or high doses of radiation—or just pure bad luck. These mutations accumulate in cells over time, ultimately rendering the cells unable to control their own growth—an inability that leads to cancer [see “What You Need to Know about Cancer,” special issue of SCIENTIFIC AMERICAN; September 1996].

Gene therapies in general deliver instructions—in the form of DNA sequences—to diseased cells so that they will produce a therapeutic protein of some kind. This type of therapy is possible because viruses, bacteria, plants and people all share the same genetic code. Researchers have learned a great deal in little time about how certain genes govern the fundamental processes of life and how they contribute to disease. And because genes from one species can be read and understood by another, experimenters can transfer genes between cells and species in their efforts to devise treatments.

For treating cancer, experimental gene therapies take varied forms: some involve imparting cancer cells with genes that give rise to toxic molecules. When these genes are expressed (that is, used by cells to make proteins), the resulting proteins then kill the cancer cells. Other designs aim to correct or compensate for acquired genetic mutations. Still others attempt to activate the processes by which such defects are normally repaired. And a host of ideas are coming from insights into how tumors evade recognition and destruction by the immune system, how they spread away from their sites of origin, how they gain a new blood supply and how they accomplish other feats that allow them to endure and spread.

Most of these approaches have yet to pass even the most preliminary clinical tests demonstrating their overall safety and efficacy, but these ideas may lead to

BRAIN TUMOR (white) appears in six MRI images of sequential slices taken before (left) and two weeks after (right) the patient received an experimental “suicide” gene therapy. The author and his colleagues inserted a gene for an enzyme called thymidine kinase (tk) from a herpesvirus into the patient’s affected brain cells. They then gave the patient ganciclovir. The tk enzyme converts this otherwise harmless drug into a toxic metabolite, capable of killing not only the cancer cells containing the tk gene but even some surrounding cancer cells as well. The computer models (bottom two images) reveal how quickly this same tumor (red) responded: a single treatment brought about the dramatic reduction shown.
BYSTANDER EFFECT, shown schematically, is critically important to the success of some gene therapies. On the left, gap junctions connect the tumor cells. As a result, toxin generated in one gene-modified cell (orange) can spread to neighboring “bystander” tumor cells (yellow), which have not themselves been altered. The tumor on the right lacks gap junctions and cannot be cured unless the therapeutic gene reaches each individual cancer cell—an unlikely bet for now.

Gene Vaccinations

In terms of treatment, scientists have for more than three decades tried to find ways to sic the immune system on cancer—a tactic termed immunotherapy or vaccine therapy. And with good reason. Because immunity is a systemic reaction, it could potentially eliminate all cancer cells in a patient’s body—even when they migrate away from the original tumor site or reappear after years of clinical remission. The problem with this strategy has been that the immune system does not always recognize cancer cells and single them out for attack. Indeed, many tumors manage to hide themselves from immune detection.

Recently, however, research in basic immunology has revealed means for unmasking such cancers. In particular, it now seems possible to tag cancer cells with certain genes that make them more visible to the immune system. And once awakened, the immune system can frequently detect even those cancer cells that have not been tagged.

The immune response involves many different cells and chemicals that work together to destroy in several ways invading microbes or damaged cells. In general, abnormal cells sport surface proteins, called antigens, that differ from those found on healthy cells. When the immune system is activated, cells called B lymphocytes produce molecules known as antibodies. These compounds patrol the body and bind to foreign antigens, thereby marking the antigen bearers for destruction by other components of the immune system. Other cells, called T lymphocytes, recognize foreign antigens as well; they destroy cells displaying specific antigens or rouse other killer T cells to do so. B and T cells communicate with one another by way of proteins they secrete, called cytokines. Other important accessory cells—antigen-presenting cells and dendritic cells—further help T and B lymphocytes detect and respond to antigens on cancerous or infected cells.

One gene therapy strategy being widely tested at the moment involves modifying a patient’s cancer cells with genes encoding cytokines. First the patient’s tumor cells are removed. Into these tumor cells, scientists insert genes for making cytokines, such as the T cell growth factor interleukin-2 (IL-2) or the dendritic cell activator called granulocyte-macrophage colony-stimulating factor (GM-CSF). Next, these altered tumor cells are returned to the patient’s skin or muscle, where they secrete cytokines and thereby catch the immune system’s attention. In theory, the altered cells should solicit vigorous immune cell activity at the site of the reinfected tumor. Moreover, the activated cells, now alerted to the cancer, could circulate through the body and attack other tumors.

In certain instances, these gene-modified tumor vaccines do seem to awaken the immune system to the presence of the cancer, and some striking clinical responses have been observed. All these
clinical studies, however, are preliminary. In most cases, patient responses to these treatments have not been carefully compared with responses to conventional treatments alone. Also, the response patterns are not predictable, and they are not consistent from one tumor type to another or among patients who have the same type of cancer.

Another problem with these studies is that nearly every person tested so far has had widely disseminated terminal cancer. Usually these patients have previously received intensive anticancer therapy, which has weakened their immune systems. Thus, even if gene vaccines did activate immunity in these individuals, the responses might not be easily noticeable. Gene-modified tumor vaccines are most likely to prove beneficial in patients with minimal tumor burdens and robust immunity. Testing patients in this category, though, must wait until researchers are finished testing more seriously ill patient groups and have established the risks associated with the treatment. As this research so well illustrates, the development of new cancer therapies is a very complex and lengthy process.

A related gene therapy involves antigens that are found predominantly on cancer cells. During the past three to four years, scientists have made remarkable progress in identifying antigens produced by tumor cells. In addition, they have uncovered the genes that encode these tumor-associated antigens, particularly those on the most serious form of skin cancer, malignant melanoma. Now that at least some of these antigens have been described, it might be possible to develop a vaccine to prevent cancer, much like the vaccines for preventing tetanus or polio. The approach might also help treat existing tumors.

**Preventive Immunizations**

As with the cytokine vaccines, these antigen-based cancer vaccines require gene transfer. They work best when administered to cells that are readily accessed by the immune system. For example, Philip L. Felgner of Vical in San Diego and Jon A. Wolff of the University of Wisconsin and their colleagues observed that injecting a DNA fragment coding for a foreign antigen directly into muscle triggered a potent immune response to the antigen in mice [see “Nonviral Strategies for Gene Therapy,” by Philip L. Felgner, on page 102]. The explanation for this reaction is simple: a bit of the foreign DNA enters the cells of the muscle or other nearby cells and directs them to produce a small amount of its protein product. Cells containing this newly synthesized foreign protein then present it to roving antibody-producing B cells and T cells. As a result, these sensitized immune components travel the body, prepared to attack tumor cells bearing the activating antigen.

The same basic strategy is revolutionizing the development of vaccines for preventing many infectious diseases. When these DNA immunizations are tested against cancer, the genes for newly identified tumor antigens are delivered directly into the body by way of vaccinia or adenovirus particles that have been rendered harmless or by such nonviral gene delivery systems as naked DNA. At present, the tests involve patients with widely spread cancer. It is clearly too late in these cases for DNA vaccines to prevent disease, but the studies should demonstrate whether the antigens can meet the essential requirement of eliciting a defensive response in the human body. Further, the studies offer a sense of whether DNA vaccines might have any merit for treating existing cancers. Given how sick many of these patients are, though, the results so far are difficult to interpret.

Yet another gene immunotherapy for cancer currently being tested in patients and in the laboratory involves antibodies. Thanks to highly variable regions on individual antibodies, these molecules are exquisitely specific. They can distinguish the slightest differences between foreign or mutated and very similar self-antigens. As it turns out, specific antibody molecules exist naturally in the outer membranes of some cancer cells—such as lymphomas that develop from B cells, which are committed to producing antibody molecules. Because a single lineage or clone of cells produces one specific antibody, all cancers of these cells will contain the same specific membrane molecule. This antibody then provides a unique molecular marker by which the cancer cells might be differentiated from similar but noncancerous antibody-producing cells.

Occasionally scientists have managed to produce antibodies to the antibodies found on cancer cell membranes. And some patients treated with these so-

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**Gene Therapies Being Studied in Cancer Patients**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Number of U.S. Trials Approved since 1988 or Awaiting Federal Approval*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisense therapy (to block synthesis of proteins encoded by deleterious genes)</td>
<td>4</td>
</tr>
<tr>
<td>Chemoprotection (to add proteins to normal cells to protect them from chemotherapies)</td>
<td>7</td>
</tr>
<tr>
<td>Immunotherapy (to enhance the body’s immune defenses against cancer)</td>
<td>58</td>
</tr>
<tr>
<td>Pro-drug, or suicide gene, therapy (to render cancer cells highly sensitive to selected drugs)</td>
<td>21</td>
</tr>
<tr>
<td>Tumor suppressor genes (to replace a lost or damaged cancer-blocking gene)</td>
<td>6</td>
</tr>
<tr>
<td>Antibody genes (to interfere with the activity of cancer-related proteins in tumor cells)</td>
<td>2</td>
</tr>
<tr>
<td>Oncogene down-regulation (to shut off genes that favor uncontrolled growth and spread of tumor cells)</td>
<td>2</td>
</tr>
</tbody>
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*This table was up-to-date as of April 1997. It includes only those trials requiring approval by the federal government.
called anti-idiotypic antibodies have responded exceedingly well. Unfortunately, producing anti-idiotypic antibodies is laborious. Thus, even though the approach can sometimes provide an effective treatment, it has seen only limited use. More recently, gene transfer techniques have offered other options. Because antibodies are gene products, scientists have been able to prepare anti-idiotypic DNA vaccines that include the DNA encoding the critical cancer marker (the idiotypic). This DNA sequence has then been linked with a gene encoding the cytokine GM-CSF. So far this double-whammy cancer vaccine has been tested only in laboratory animals, but it shows exciting promise.

Another double-whammy therapy in the works couples antibodies and T lymphocytes. Some rare patients have cancers that their T cells do recognize. But the T cells from these patients usually attack only their own tumor cells or those from a small fraction of cases with the same type of cancer and tissue type. Also, people rarely produce antibodies to tumors. In contrast, mice immunized with human cancers do make antibodies that react strongly to those same human cancer cells. In some cases, the mouse antibodies bind to nearly all the tumor cells of one cancer in a test tube—even if they have been taken from many different individuals with the same kind of cancer. The mouse antibodies, though, are usually not effective in killing the cancer cells in humans. Even if the murine antibodies do have cancer-killing activity in a patient, the response is usually very short-lived because the patient soon produces inactivating antibodies against the mouse antibodies.

Therefore, oncologists have long hoped to find some way to combine the targeting ability of the murine antitumor antibodies with the killing ability of human T cells. Recombinant DNA technology offers the necessary tools. Researchers have successfully isolated the antitumor antibody genes from mouse cells and recombined parts of them with gene segments encoding the receptor that killer T cells use to recognize their targets. The modified receptor gene redirects killer T cells, which often do not recognize cancers, to see what the less discriminating mouse antibodies see. Indeed, killer T cells rearmed with chimeric T cell receptors kill cancer cells in a test tube quite efficiently. Early clinical experiments using this strategy are now under way in cancer patients, as well as in those infected with HIV, the AIDS-causing agent [see box on these two pages], and other pathogens.

Other Gene Therapies

Immunotherapy aside, cancers can be battled on other genetic fronts. There has been intense interest in identifying the precise DNA defects that cause cancer. Some mutations, scientists have learned, are associated with specific types of cancer. Other mutations occur in many varieties. Furthermore, there are different kinds of mutations. Some activate genes, called oncogenes, that drive uncontrolled growth in cells. Other mutations—those in so-called tumor suppressor genes—result in the loss of a normal brake on uncontrolled cell growth.

One of the most commonly mutated tumor suppressor genes in human cancer is p53, a gene whose protein product normally monitors the DNA in a cell as it divides. If the DNA is flawed, the p53 protein may halt cell division until the damage can be fixed or may induce cell suicide (apoptosis). When a normal copy of p53 is reintroduced to cancer cells in tissue culture, those cells return to a more regular growth pattern or self-destruct. Either outcome would be useful in cancer treatment, and so a great deal of effort has gone into developing methods for inserting normal p53 genes into cancers growing in the body.

There are still major roadblocks: as Theodore Friedmann notes in the first article of this section on page 96, current technologies for delivering genes to specific organs or cell populations are inefficient. In addition, there are no perfected means for extending the effects of such locally delivered genes to other ar-
are capable of providing therapeutic genes to precisely those cells that most need them. A range of genes are being tested for their ability to disable HIV. One gene type, containing what is called a dominant negative mutation, generates inactive versions of proteins that HIV normally makes in order to replicate. When an infected, treated cell produces these useless look-alikes, the altered proteins trip up their ordinary cousins—either by binding to them or by taking their place in molecular reactions. Clinical testing of a dominant negative mutation of the HIV gene rev began in 1995.

Scientists are also evaluating the merit of delivering genes that would be transcribed into short RNA strands that mimic essential viral control RNAs. The hope is that these RNA decoys might bind to HIV regulatory proteins and block them from functioning. Genes transcribed into ribozymes (catalytic RNAs) capable of degrading viral RNA might similarly interrupt HIV replication. A related idea involves delivering genes encoding proteins that are made by the host cell and that interact with HIV particles. For instance, soluble forms of the protein CD4 might bind to HIV particles extracellularly, thereby keeping them from infecting T cells that display CD4 molecules on their outer surface.

Scientists are also exploring for HIV treatment so-called suicide genes, which are not unlike those being tested as cancer gene therapies. Because the gene would presumably get into any cell normally invaded by the selected vector, researchers want to be sure the suicide gene will be expressed only in the subset of recipient cells that harbor HIV infection. So they plan to attach the gene to control elements that become active, and switch on the gene, only in cells that are infected by HIV.

Another design borrowed from gene therapies for cancer relies on enhancing the ability of functioning helper T cells to recognize infected cells and orchestrate an immune response against them. For instance, a gene coding for part of the antibody molecule that recognizes and binds to the gp120 protein on HIV’s surface can be integrated with genes encoding the molecule, or receptor, on killer T cells that is normally responsible for recognizing diseased cells. The chimeric receptor that results from this mix takes better notice of HIV and thus redirects the T cells to destroy the infected HIV cell. A Phase 1 clinical trial (looking at safety) is currently under way.

Other therapeutic genes being scrutinized give rise to antibody fragments that act within infected cells. By binding to some newly made viral protein, these intracellular antibodies, or intrabodies, prevent virus particles from being assembled. Finally, clinical trials of gene vaccinations for preventing AIDS have been approved. As in other gene vaccinations, these immunizations supply to cells patrolled by the immune system genes coding for HIV molecules that distinguish the virus as a foreign invader. The immune system then reacts to these antigens by producing antibodies that wander through the body, ready to attack any cells presenting the antigens, should they ever appear.

—R.M.B.

Gene Therapy for Cancer

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