

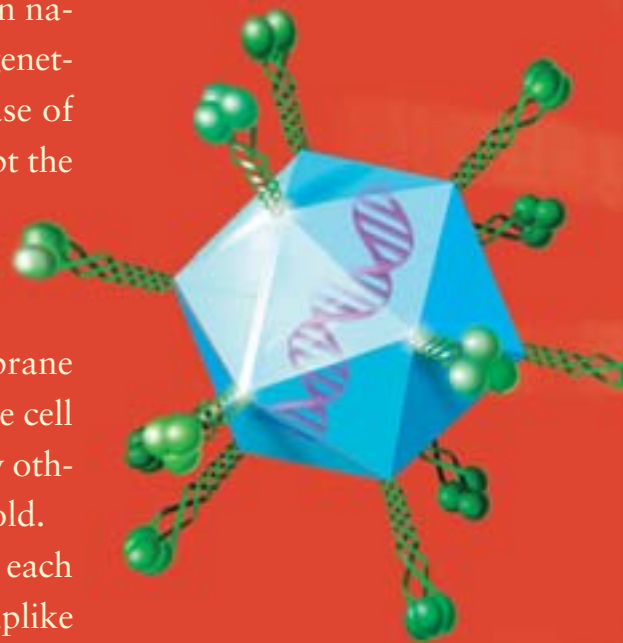
TUMOR-BUSTING

A new technique called virotherapy harnesses viruses, those banes of humankind, to stop another scourge—cancer

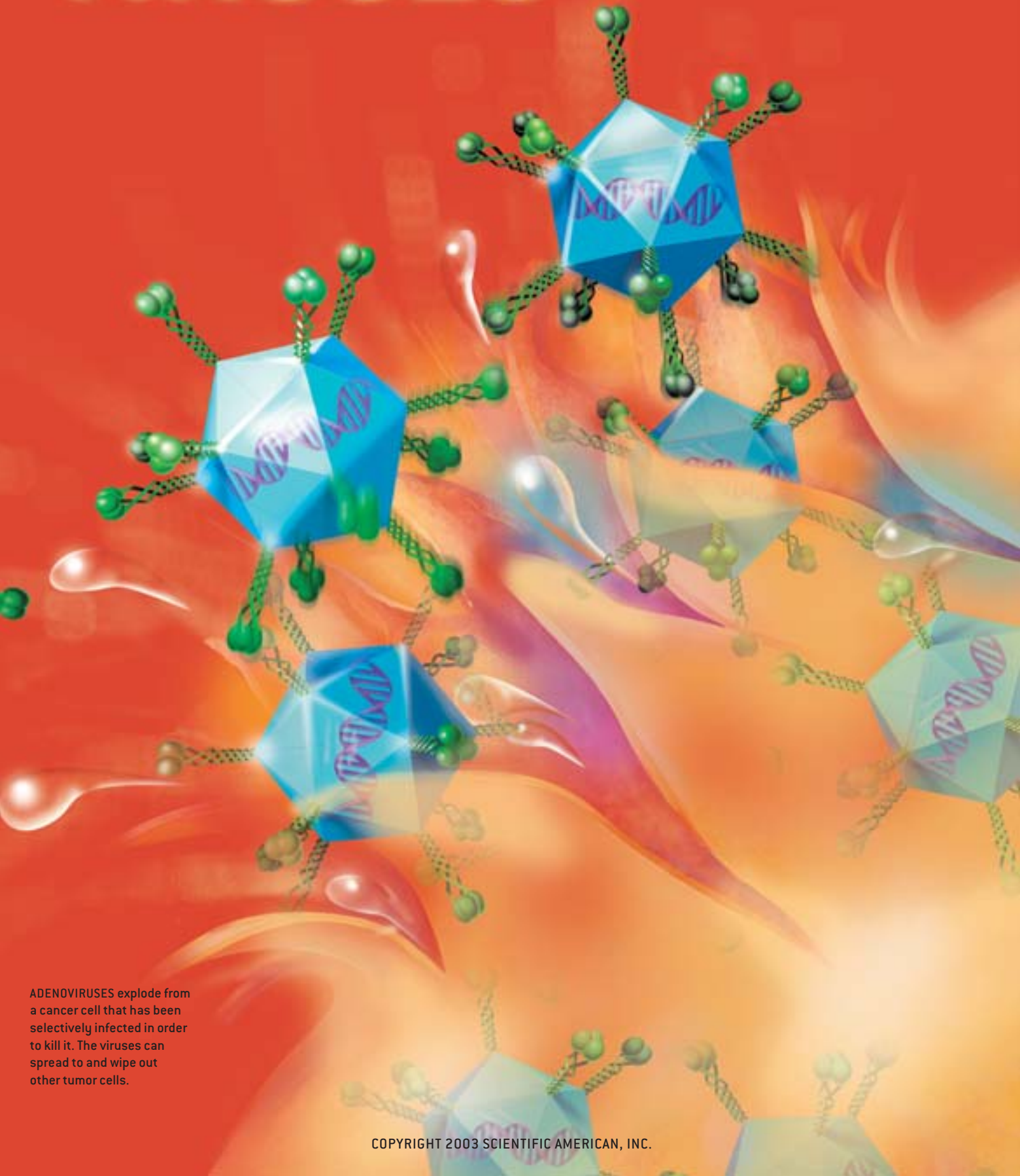
By Dirk M. Nettelbeck and David T. Curiel

Viruses are some of the most insidious creations in nature. They travel light: equipped with just their genetic material packed tightly inside a crystalline case of protein, they latch onto cells, insert their genes, and co-opt the cells' gene-copying and protein-making machinery, using them to make billions of copies of themselves. Once formed, the new viruses percolate to the cell surface, pinch off inside minuscule bubbles of cell membrane and drift away, or else they continue reproducing until the cell finally bursts. In any case, they go on to infect and destroy other cells, resulting in diseases from AIDS to the common cold.

Different viruses cause different diseases in part because each virus enters a cell by first attaching to a specific suction-cuplike receptor on its surface. Liver cells display one kind of receptor used by one family of viruses, whereas nerve cells display another receptor used by a different viral family, so each type of virus infects a particular variety of cell. Cancer researchers have envied this selectivity for years: if they could only target cancer therapies to tumor cells and avoid damaging normal ones, they might be able to eliminate many of the noxious side effects of cancer treatment.



VIRUSES



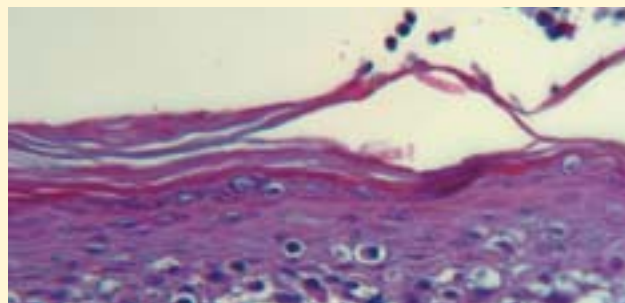
ADENOVIRUSES explode from a cancer cell that has been selectively infected in order to kill it. The viruses can spread to and wipe out other tumor cells.

TARGETING MELANOMA

THE SKIN CANCER melanoma is one of the most lethal cancers unless detected early; it arises from the uncontrolled growth and spread of pigmented cells in the skin called melanocytes.

Scientists are using the new approach of virotherapy to selectively kill melanoma cells while leaving healthy cells alone.

One technique for studying melanoma involves combining



Some scientists, including ourselves, are now genetically engineering a range of viruses that act as search-and-destroy missiles: selectively infecting and killing cancer cells while leaving healthy ones alone. This new strategy, called virotherapy, has shown promise in animal tests, and clinical trials involving human patients are now under way. Researchers are evaluating virotherapy alone and as a novel means for administering traditional chemotherapies solely to tumor cells. They are also developing methods to label viruses with radioactive or fluorescent tags in order to track the movement of the viral agents in patients.

Viruses to the Rescue?

ONE OF THE FIRST INKLINGS that viruses could be useful in combating cancer came in 1912, when an Italian gynecologist observed the regression of cervical cancer in a woman who was inoculated with a rabies vaccine made from a live, crippled form of the rabies virus. Physicians first injected viruses into cancer patients intentionally in the late 1940s, but only a handful appeared to benefit. Twenty years later scientists found that a virus that causes the veterinary disorder Newcastle disease shows a preference for infecting tumor cells and began to try to enhance that tendency by growing the viruses for generations in human cancer cells in laboratory culture dishes. Although critics countered that such viruses could be exerting only an indirect effect against cancer by generally activating an individual's immune system and making it more likely to detect and kill cancer cells, reports continued to pop up in the medical literature linking viral infection and cancer remission. In the early 1970s and 1980s two groups of physicians described patients whose lymphomas shrank after they came down with measles.

The modern concept of virotherapy began in the late 1990s, when researchers led by Frank McCormick of ONYX Pharmaceuticals in Richmond, Calif., and Daniel R. Henderson of Calydon in Sunnyvale, Calif., independently published reports showing they could target virotherapy to human cancer cells grafted into mice, thereby eliminating the human tumors. (ONYX is no longer developing therapeutic viruses, and Caly-

don has been acquired by Cell Genesys in South San Francisco, Calif.) Both groups used adenovirus, a cause of the common cold that has been intensively explored for virotherapy. (Other viruses under study include herpes simplex, parvovirus, vaccinia and reovirus.) Adenovirus is appealing in part because researchers understand its biology very well after years of trying to cure colds and of using the virus in molecular biology and gene therapy research. It consists of a 20-sided protein case, or capsid, filled with DNA and equipped with 12 protein "arms." These protrusions have evolved over millennia to latch onto a cellular receptor whose normal function is to help cells adhere to one another.

Adenoviruses are distinct from the types of viruses usually used in gene therapy to treat inherited disorders. Gene therapy traditionally employs retroviruses to splice a functioning copy of a gene permanently into the body of a patient in whom that gene has ceased to work properly. Unlike retroviruses, however, adenoviruses do not integrate their DNA into the genes of cells they infect; the genes they ferry into a cell usually work only for a while and then break down. Scientists have investigated adenoviruses extensively in gene therapy approaches to treat cancer, in which the viruses are armed with genes that, for example, make cancer cells more susceptible than normal ones to chemotherapy. In general, tests involving adenoviruses have been safe, but regrettably a volunteer died in 1999 after receiving an infusion of adenoviruses as part of a clinical trial to test a potential gene therapy for a genetic liver disorder [see box on page 74].

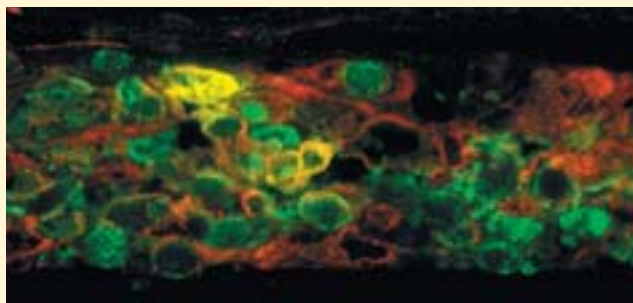
Gene therapists have been working to tailor adenoviruses and other viral vectors, or gene-delivery systems, to improve their safety and reduce the chances that such a tragedy might occur again. It is perhaps even more essential for researchers, such as ourselves, who are investigating virotherapy to develop safer, more targeted vectors, because virotherapy by definition aims to kill the cells the viruses infect, not just insert a therapeutic gene into them. Killing the wrong cells could be dangerous.

Adenoviruses bring with them characteristics that can make

Overview/*Anticancer Viruses*

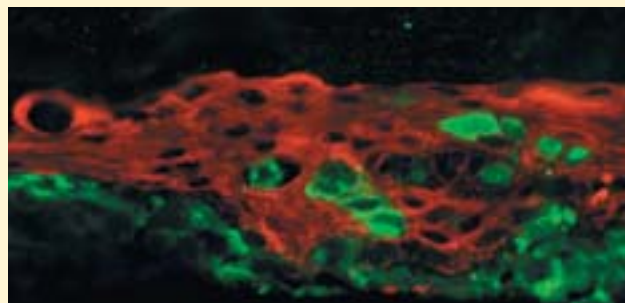
- Virotherapy is a new strategy to treat cancer by selectively infecting and killing tumor cells. Researchers are testing various approaches to target viruses—particularly adenoviruses—to cancer cells, leaving normal cells untouched.
- The viruses used in virotherapy can either kill tumor cells by bursting them open or deliver genes that make the cells more susceptible to traditional chemotherapies.
- The same types of viruses used in virotherapy can also be labeled with fluorescent or radioactive tags. Once delivered into the body, they home in on cancer cells. In the future, physicians might be able to use this imaging technique to detect the presence of tiny tumors.

melanoma cells (*dark dots in micrograph below left*) with normal skin cells called keratinocytes and collagen to make cancer-bearing artificial skin that can be grown in laboratory culture dishes. One of us (Nettelbeck) and colleagues have devised an adenovirus that can specifically reproduce in melanoma cells. In the center and right micrographs below, healthy keratinocytes



appear red; cells infected with the virus show up green. The center micrograph was made using viruses that were not specifically targeted to melanomas. The viruses were able to grow in healthy cells, making those cells look yellow. In contrast, the targeted virus (*below right*) did not replicate in healthy cells, so none of the cells are yellow.

—D.M.N. and D.T.C.



them riskier or safer, depending on the circumstances. Nearly everyone has been exposed at one time or another to adenoviruses, so almost all of us carry antibodies the immune system makes to target them for destruction. Accordingly, shots of adenoviruses as cancer therapies might cause severe, flulike symptoms if the body recognizes them as foreign and ramps up an immune response to eradicate them. (Wiping out the viruses would also squelch the therapy.) At the same time, recognition by the immune system ensures that the viruses do not reproduce out of control. Investigators are now designing various therapeutic approaches to optimize the efficacy of virotherapy and minimize the chances that adenoviruses will cause side effects. These strategies include giving immunosuppressive drugs at the time of virotherapy and modifying the adenoviruses so that they do not trigger a reaction by the immune system.

Homing In on the Target

VIROTHERAPISTS ARE DEVISING two main strategies to make sure their missiles hit their objectives accurately with no collateral damage. In the first approach, termed transductional targeting, researchers are attempting to adapt the viruses so that they preferentially infect, or transduce, cancer cells. The second method, called transcriptional targeting, involves altering the viruses so that their genes can be active, or transcribed, only in tumors [see box on next two pages].

Transductional targeting is particularly necessary because, unfortunately, adenoviruses bind more efficiently to the variety of normal tissues in the human body than they do to most tumor cells. We can reverse this pattern using specially generated adapter molecules made of antibodies that snap onto the arms of the virus like sockets on a socket wrench. By attaching carefully chosen antibodies or other molecules that selectively bind only to a specific protein found on tumor cells, we can render adenoviruses unable to infect any cells but cancerous ones. Once the antibody-bearing virus latches onto a targeted cell,

the hapless cell engulfs it in a membrane sac and pulls it inside. As the sac disintegrates, the viral capsid travels to a pore in the cell's nucleus and injects its own DNA. Soon the viral DNA directs the cell to make copies of the viral DNA, synthesize viral proteins and combine the two into billions of new adenoviruses. When the cell is full to capacity, the virus activates a “death gene” and prompts the cell to burst, releasing the new viruses to spread to other cells.

The viruses can also be engineered more directly. In this regard, Curiel's group at the University of Alabama's Gene Therapy Center has designed adenoviruses that bind to cellular proteins called integrins. These molecules help cells stick to the network of connective tissue, called the extracellular matrix, that organizes the cells into cohesive tissues. Although integrins are also made by healthy cells, cancer cells produce them in abundance as they become metastatic and begin to squeeze through tissue layers and travel throughout the body. The University of Alabama research group has had encouraging results using the engineered viruses in mice bearing human ovarian cancers. The viruses homed in on the ovarian tumor cells

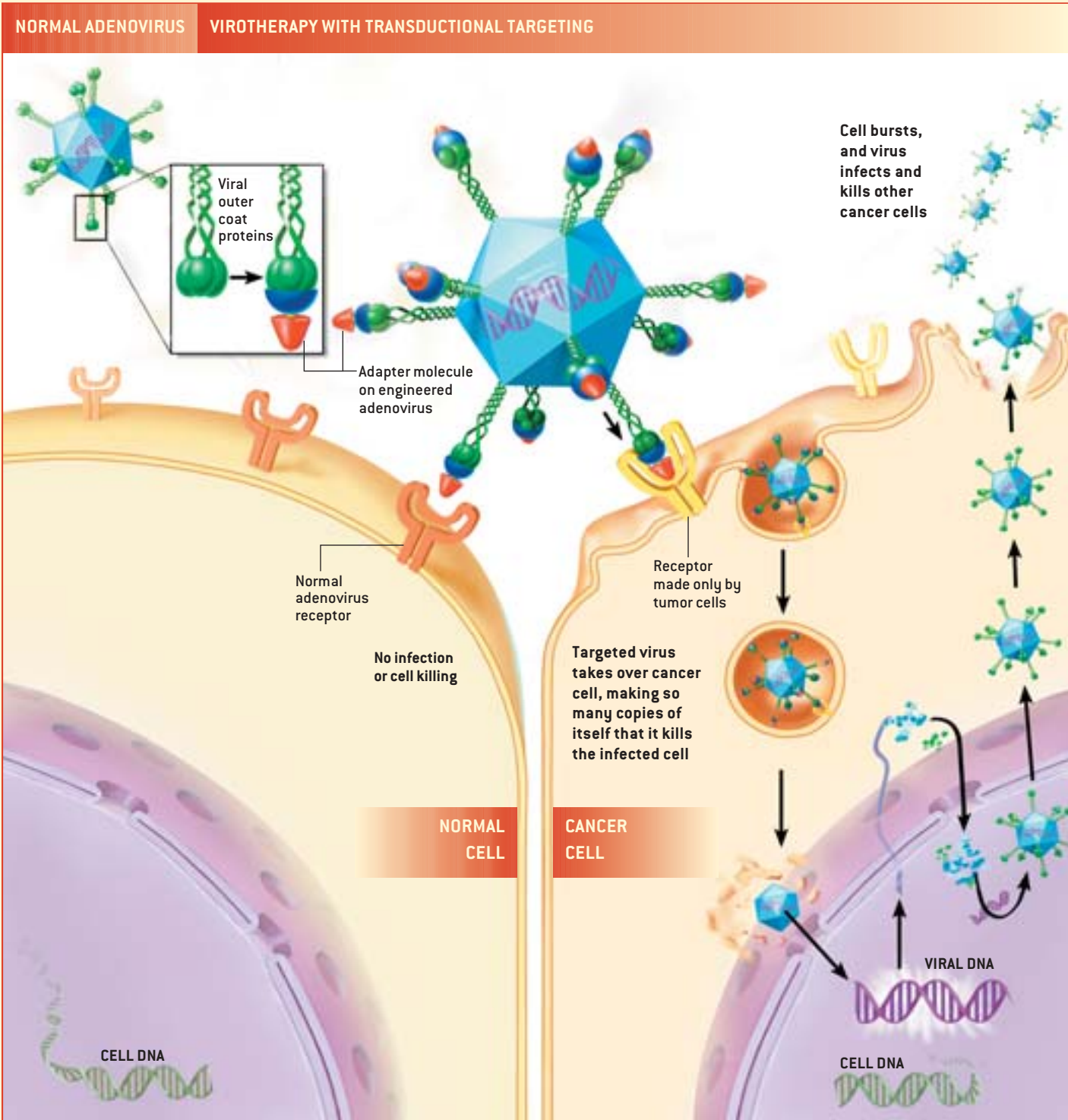
THE AUTHORS

DIRK M. NETTELBECK and **DAVID T. CURIEL** began their collaboration at the Gene Therapy Center of the University of Alabama at Birmingham (UAB), where Curiel is director of the division of human gene therapy. Curiel, who holds an M.D. and a Ph.D., is the Jeanne and Anne Griffin Chair for Women's Cancer Research at UAB and a professor of gene therapy at the Free University of Amsterdam. Nettelbeck—who is now heading a research group focusing on virotherapy for malignant melanoma in the department of dermatology at the University of Erlangen-Nuremberg in Germany—was a molecular biologist and postdoctoral fellow of the German Research Association at the University of Alabama from 2000 to 2003. He received his Ph.D. in 2000 from Philipps University in Marburg, Germany, and was honored with a graduation award from the Novartis Foundation for Therapeutic Research.

ZAPPING CANCER CELLS WITH VIRUSES

TWO MAIN STRATEGIES are being explored for virotherapy, which is the technique of using reproducing viruses to kill tumors. In the first method, dubbed transductional targeting (*below*), scientists are attempting to engineer viruses such as adenovirus—which normally causes respiratory

infections—to selectively infect and destroy only cells that have turned cancerous. They are attaching adapter molecules onto the viral outer coat proteins or directly modifying these proteins to try to prevent the viruses from entering normal cells and instead prompt them to home in

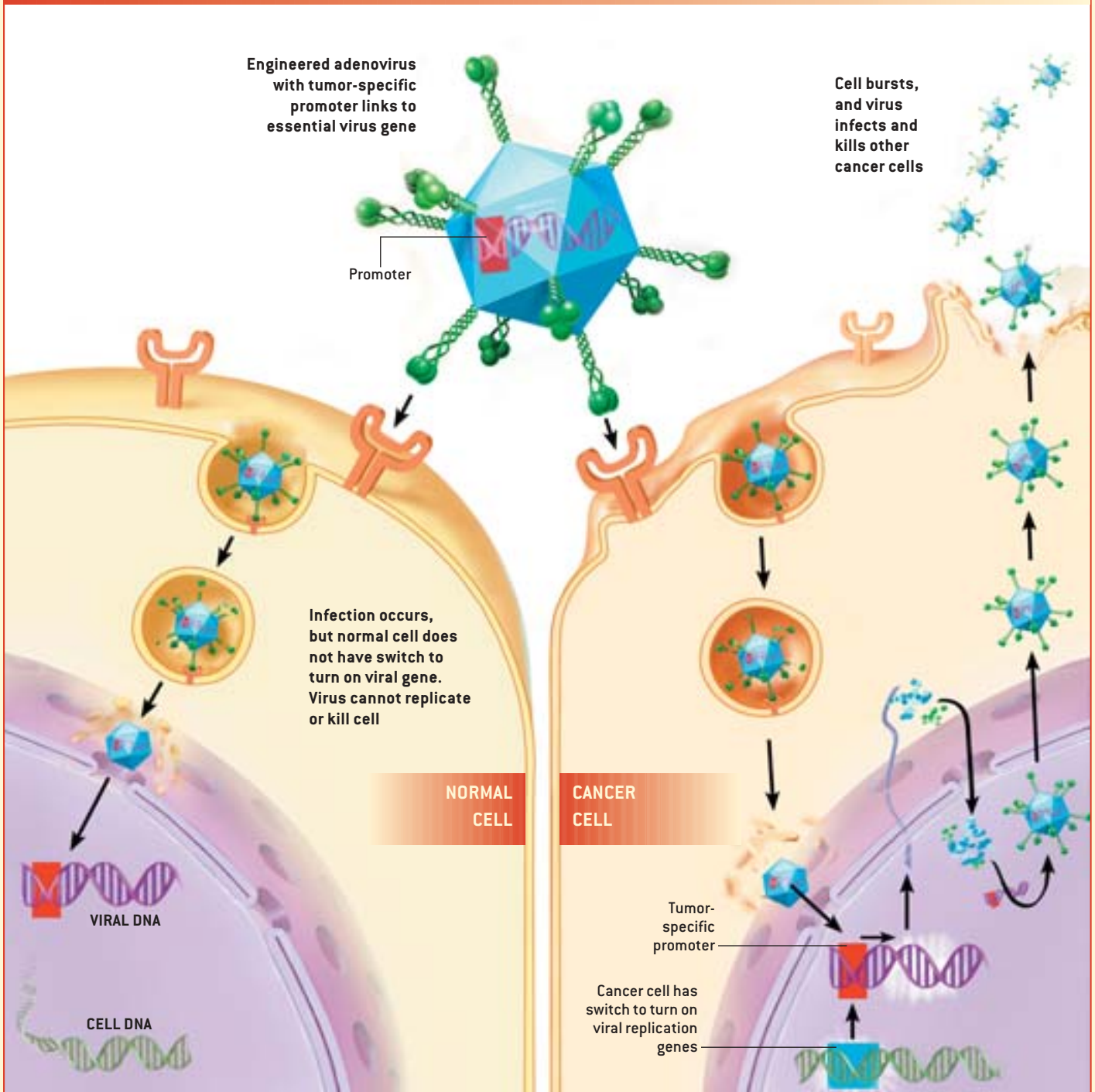


TERESE WINSLOW

on tumor cells. The second approach (*below*) involves placing a snippet of DNA called a tumor-specific promoter next to one of adenovirus's essential genes. The promoter acts as an "on" switch that permits the gene to function only in cancer cells. The engineered viruses

can enter normal cells, but they cannot reproduce and kill them. Once they enter cancer cells, however, the tumor-specific promoter lets them make millions of copies of themselves and ultimately burst the cancer cells. They can then spread to—and destroy—other tumors. —D.M.N. and D.T.C.

VIROTHERAPY WITH TRANSCRIPTIONAL TARGETING



and killed them, ridding the treated animals of the disease.

Transcriptional targeting generally takes advantage of genetic switches (promoters) that dictate how often a given gene is functional (gives rise to the protein it encodes) in a particular type of cell. Although each body cell contains the same encyclopedia of genetic information, some cells use different chapters of the encyclopedia more often than others in order to fulfill their specialized tasks. Skin cells called melanocytes, for instance, must make much more of the pigment melanin than liver cells, which have little use for the protein. Accordingly, the promoter for the key enzyme for making melanin gets turned on in melanocytes but generally is off in most other body tissues. In the deadly skin cancer melanoma, the gene encoding this enzyme is fully functional, making the tumors appear black. We, and others, have engineered adenoviruses that have a promoter for the enzyme adjacent to genes that are essential for the viruses' ability to replicate. Although these viruses might infect normal cells, such as liver cells, they can reproduce only inside melanocytes, which contain the special combination of proteins needed to turn on the promoter.

Researchers are currently tailoring adenoviruses with a variety of promoters that limit their activity to particular organs or tissues. In liver cancers, for example, the promoter for the gene α -fetoprotein—which is normally shut down after fetal de-

velopment—becomes reactivated. Adenoviruses containing that same promoter hold promise for eradicating liver tumors. Scientists led by Jonathan W. Simons at Johns Hopkins University have tested the approach in men whose prostate cancer recurred following treatment with radiation. The researchers used adenoviruses that had been engineered by Cell Genesys to contain the promoter for prostate-specific antigen, a protein made in abundance by prostate tumors. They administered the virotherapy to 20 men who received varying doses of the adenoviruses. In 2001 Simons and his colleagues reported that none of the men experienced serious side effects and that the tumors of the five men who received the highest doses of the virotherapy shrank by at least 50 percent.

Other Strategies

VIROTHERAPISTS MIGHT END UP combining the transductional and transcriptional targeting strategies to ensure that the viruses kill only tumor cells and not normal ones. Adenoviruses engineered to contain the promoter for the enzyme that makes melanin, for instance, can also replicate in normal melanocytes, so on their own they might cause spots of depigmentation. And adenoviruses that are designed to bind to receptors on the surfaces of tumor cells can still invade a small proportion of healthy cells. But viruses altered to have several

But Is It Safe?

Many approaches to virotherapy use adenoviruses, which caused a death in a clinical trial of gene therapy four years ago

IN SEPTEMBER 1999 18-year-old Jesse Gelsinger died after receiving an infusion of adenoviruses into his liver. He had a mild form of an inherited liver disease called ornithine transcarbamylase deficiency (OTCD) and was participating in a clinical trial of a new gene therapy to use adenoviruses to ferry a corrected copy of the gene encoding OTCD into his liver cells. Unfortunately, four days after an infusion of the viruses, he died of acute respiratory distress syndrome and multiple organ failure, apparently caused by an overwhelming immune reaction to the large dose of adenoviruses he had been administered as part of the trial.

Although Gelsinger's death was part of a gene therapy trial, the tragedy also has ramifications for the new field of virotherapy. Gene therapy uses crippled versions of viruses such as adenovirus to introduce a new gene into cells; virotherapy employs actively replicating viruses (which may or may not contain added genes) to kill specific types of cells. Both, however, rely heavily on adenoviruses.



JESSE GELSINGER, who died in 1999 after receiving an infusion of adenoviruses, in a family photograph.

Gelsinger's autopsy showed that the engineered adenoviruses had spread to his spleen, lymph nodes and bone marrow, and an examination of his records revealed that his liver function was probably too impaired for him to be a volunteer in the trial. A number of scientists have also suggested that he might have mounted such an extreme immune reaction because he had previously been infected with a naturally occurring adenovirus.

Since Gelsinger's death, gene therapists and virotherapists alike have focused on refining adenoviruses to make them safer. But researchers are still unsure why Gelsinger reacted so violently to the adenoviral infusions: a second patient participating in the same clinical trial

tolerated a similar dose of the viruses. And dozens of other people worldwide have been treated so far with adenoviruses with no serious side effects.

A National Institutes of Health report generated in the aftermath of Gelsinger's demise recommends that all participants in such clinical trials be monitored closely for toxic reactions before and after the infusion of therapeutic viruses. It also stipulates that volunteers be screened for any predisposing conditions that would increase their sensitivity for the viruses.

—D.M.N. and D.T.C.

SELECTED COMPANIES INVOLVED IN VIROTHERAPY

Company	Headquarters	Virus	Diseases	Viral Modifications	Clinical Trial Status
BioVex	Abingdon, Oxfordshire, U.K.	Herpes simplex virus (HSV)	Breast cancer and melanoma	Carries the gene for granulocyte-macrophage colony stimulating factor, an immune system stimulant	Phase I/II
Cell Genesys	South San Francisco, Calif.	Adenovirus	Prostate cancer	Targeted to prostate cancer cells using prostate-specific promoters	Phase I/II
Crusade Laboratories	Glasgow	HSV	Glioma (brain cancer), head and neck cancer, melanoma	Has a gene deletion that restricts it to actively dividing cells such as cancers	Phase II for glioma and head and neck cancer; Phase I for melanoma
MediGene	Martinsried, Germany	HSV	Glioma and colon cancer that has spread (metastasized)	Harbors two gene deletions that prevent it from reproducing in normal cells	Phase II for glioma; Phase I for colon cancer metastases
Oncolytics Biotech	Calgary, Alberta, Canada	Reovirus	Prostate cancer and glioma	Able to replicate only in cancer cells bearing the activated oncogene <i>ras</i>	Phase II for prostate cancer; Phase I/II for glioma

NOTE: Phase I tests are designed to evaluate safety in small numbers of patients. Phases II and III are intended to determine the appropriate dose and efficacy, respectively.

fail-safe mechanisms would be expected to be less likely to harm normal cells. There are no results at present, however, to demonstrate that a combination of approaches makes viruses more targeted.

A further strategy for targeting virotherapy makes the most of one of cancer's hallmarks: the ability of tumor cells to divide again and again in an uncontrolled manner. Healthy cells make proteins that serve as natural brakes on cell division—notably, the retinoblastoma (Rb) and p53 proteins. As cells turn cancerous, however, the genes that code for one or the other of these proteins become mutated or otherwise inactivated. Certain viruses, including adenovirus, interfere with the braking mechanisms of a normal cell by making proteins that stick to and inactivate Rb or p53. They do this because they can replicate only in cells that are preparing to divide.

Several research groups and biotechnology companies have engineered adenoviruses that fail to make the Rb or p53 blockers. Normal cells, which make these blockers, will stall the replication of these viruses by putting the brake on cell division. But these viruses will replicate in cells in which the Rb or p53 proteins are already disabled—cancer cells—and kill them. Curiel is planning clinical trials of the approach for ovarian cancer.

Researchers are also arming therapeutic viruses with genes that make the cells they infect uniquely susceptible to chemotherapy. The technique involves splicing into the viruses genes that encode enzymes that turn nontoxic precursors, or “prodrugs,” into noxious chemotherapies. In one example, which was reported in 2002, André Lieber of the University of Washington and his co-workers designed adenoviruses to carry genes encoding the enzymes capable of converting innocuous prodrugs into the anticancer compounds camptothecin and 5-fluorouracil. The scientists engineered the viruses so that they could make the enzymes only in actively dividing cells, such as cancer cells. When they injected the viruses and the prodrugs into mice bearing implanted human colon or cervical cancer cells, they found that the viruses reproduced and spread in the tumors.

Such “smart” virotherapies are the vanguard of the future.

But physicians will also need to track the activity of virotherapies in a patient's body to best assess how well the strategies are working and refine them further. Virotherapists are now teaming with radiologists to establish novel imaging technologies to easily measure how effectively a given virotherapy is replicating.

The imaging strategies involve inserting a gene that governs the production of a tracer molecule into a virus or virus-infected cell. The tracer can be either a fluorescent protein that can be observed directly or one that binds readily to the radionuclides used in standard radiological imaging techniques. The fluorescent protein might work best for cancers that are accessible by an endoscope, such as cancers of the larynx. Physicians could peer into the endoscope and see exactly where the viruses—and therefore, cancer cells—are by looking for fluorescence. So far the approach has worked best with viruses that do not kill cells, however. Nevertheless, we are convinced that such sophisticated imaging technologies will enable scientists to draw more meaningful conclusions from future clinical trials of virotherapy.

In 1995 gene therapy pioneer W. French Anderson of the University of Southern California School of Medicine predicted in this magazine that “by 2000 . . . early versions of injectable vectors that target specific cells will be in clinical trials.” These trials indeed began on schedule, as well as some he could not have envisioned then. We envision a substantial role for viruses—that is, *therapeutic* viruses—in 21st-century medicine. SA

MORE TO EXPLORE

Gene Therapy: Designer Promoters for Tumour Targeting.

D. M. Nettelbeck, V. Jérôme and R. Müller in *Trends in Genetics*, Vol. 16, pages 174–181; 2000.

Replicative Adenoviruses for Cancer Therapy. R. Alemany, C. Balagué and D. T. Curiel in *Nature Biotechnology*, Vol. 18, pages 723–727; 2000.

Vector Targeting for Therapeutic Gene Delivery. Edited by D. T. Curiel and J. T. Douglas. John Wiley & Sons, 2002.

Cytolytic Viruses as Potential Anti-Cancer Agents. C.J.A. Ring in *Journal of General Virology*, Vol. 83, pages 491–502; 2002.

Gene therapy clinical trials database of *Journal of Gene Medicine*: www.wiley.com/legacy/wileychi/genmed/clinical/

American Society of Gene Therapy: www.asgt.org