A growing assortment of viruses that replicate in and kill cancer cells, but not normal tissue, may be new weapons in the war on cancer

Training Viruses to Attack Cancers

Viruses have spawned more than their share of misery over human history. Cancer has an equally grim record. Now several companies and about a dozen research teams are working on recruiting one kind of scourge against another. They are developing viruses that are either naturally harmless to normal tissue or have been genetically altered to make them so and are turning them loose on cancer cells. The hope is that the viruses will do what doctors, with their scalpels, chemotherapy, and radiation beams, all too often cannot: eradicate cancer without damaging normal tissue.

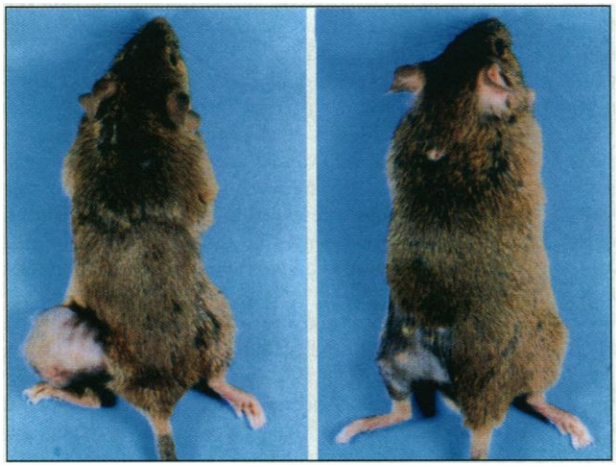
Here the viruses play a different role than they do in experimental therapies that rely on viruses to ferry therapeutic genes into cancer cells, where the new genes might correct the genetic errors underlying the uncontrolled cell growth. These gene-carrying viruses have been disarmed so that they can't multiply and spread. But the secret of the new anticancer viruses is precisely their ability to replicate and spread, killing the cells—albeit only within the cancer. "It's like a chain reaction that spreads until it gets to the tumor boundary," explains Jeffrey Ostrove, a virologist with NeuroVir Inc. in Vancouver, British Columbia.

So far, researchers have come up with a half-dozen of these tumor-killing viruses, the latest of which is a reovirus described on page 1332 by Patrick Lee, a virologist at the University of Calgary in Alberta, and his colleagues. The reovirus—a type of virus that doesn't cause problems in humans—is not yet in clinical trials, but two other viruses are, and early results from one indicate that it can shrink tumors, particularly when used in conjunction with other therapies. "A lot of people are very excited because of the lack of side effects and the hope of specificity to cancer cells," says Steven Linke, a molecular biologist at the National Cancer Institute in Bethesda, Maryland.

Much more work will be needed to see whether this preliminary promise will hold up. But viruses that simply kill cancer cells—so-called oncolytic viruses—are just the first wave of this new type of cancer therapy. Also in the works are oncolytic viruses that not only kill cancer cells but also carry genes that make the cells more susceptible to radiation or chemotherapy, thereby delivering a double blow to the tumor. As such, they represent "a whole new avenue of potential treatments," says Robert Martuza, a neurosurgeon at Georgetown University in Washington, D.C.

Ras appeal

Lee and his colleagues didn't start out looking for new ways to treat cancer; they were using human reoviruses to study how viruses in general work. Reoviruses grow fast, are easy to work with, and are apparently harmless to people, although they can kill newborn mice. Researchers already knew that, in order to infect cells, reoviruses have to latch onto molecules of sialic acid on the cell surface. But Lee and his colleagues realized that something more is also needed, because the virus replicates only in a subset of the cells that carry sialic acid.



Cancer buster. Tumor injected with a reovirus (*right*) shrinks while untreated tumor (*left*) continues to grow.

CREDIT: P. W. K. LEE ET AL.

In 1993, Lee's team found a clue to what that might be by showing that the virus does better in cells that also have surface receptors for a molecular signal called epidermal growth factor (EGF). Three years later, Lee's group showed that it wasn't the EGF receptor per se that is important but the signaling pathway the receptor activates when it binds the growth factor. And this year, they reported that what enables the virus to thrive is one particular component of that pathway, the protein made by the *ras* gene. To replicate, the virus needs the Ras protein because it blocks the activity of another protein in the cell, called PKR, that would otherwise prevent the synthesis of viral proteins.

That discovery pointed Lee toward the current work, because *ras* is one of the oncogenes that can, when inappropriately activated, spark cancer cell growth. Lee realized that the virus would probably replicate readily in tumors that have an overactive *ras* gene, which include some colon, pancreatic, and lung cancers. To test this idea, he and his colleagues transplanted cells from a human brain cancer called glioblastoma into immune-deficient mice that would not reject the cells. The cancer cells had high levels of Ras protein because of mutations in proteins that control the oncogene's activity. After the cancer had taken hold, the researchers shot the tumor full of virus. "The virus was extremely potent," says Lee. The tumors shrank or disappeared in 65% to 80% of the mice tested.

Lee notes that unpublished results from his team show that the virus also kills cultured cells derived from breast, prostate, and pancreatic cancers, but none of noncancerous cell lines, which have low *ras* activity, tested. "They've shown pretty convincingly that this virus has specificity for *ras*-mutated cells," says Frank McCormick, a molecular biologist at the University of California, San Francisco.

McCormick, who earlier led the team that developed another oncolytic virus at ONYX Pharmaceuticals, a biotech firm in Richmond, California, worries about the safety of reovirus in humans, however. He notes that some of the treated mice in Lee's experiments died, presumably as a result of the infection. But other experts dismiss the

concern. Reoviruses "are completely nonpathogenic in people," says virologist Wolfgang Joklik of Duke University in Durham, North Carolina. "I wouldn't worry about them." Lee is working with the Canadian government to get permission to try the reovirus in people with breast or head and neck cancers that haven't responded to conventional therapies.

In the clinic

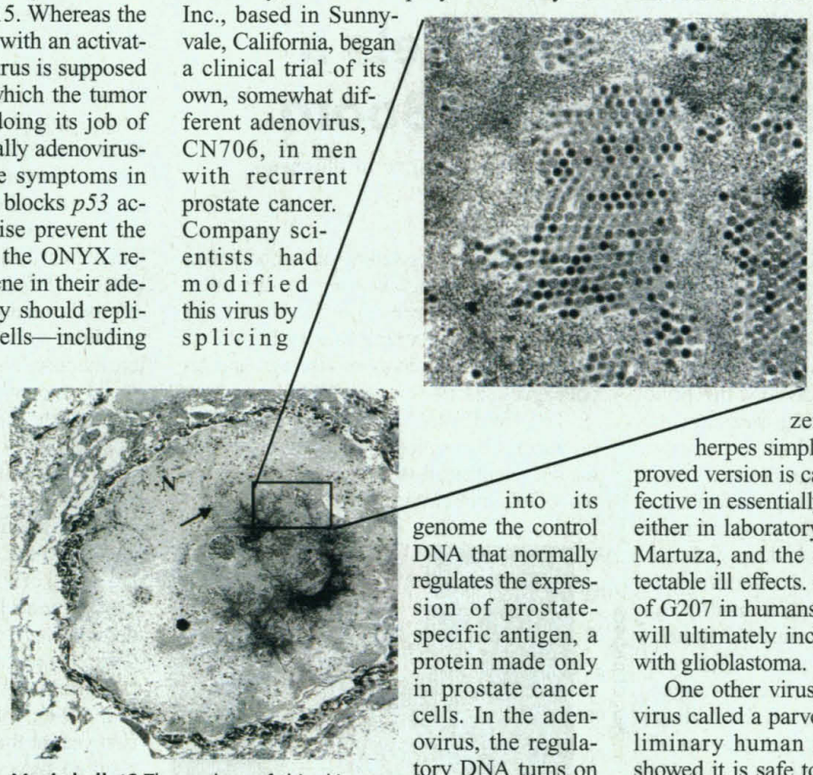
Other oncolytic viruses are already in the clinic. The farthest along is the one developed by ONYX, a genetically modified adenovirus called ONYX-015. Whereas the reovirus targets cancer cells with an activated *ras*, the modified adenovirus is supposed to work in cancer cells in which the tumor suppressor gene *p53* isn't doing its job of preventing cell growth. Usually adenoviruses, which can cause flulike symptoms in people, make a protein that blocks *p53* activity, which would otherwise prevent the virus from replicating. But the ONYX researchers deactivated that gene in their adenovirus, which consequently should replicate in and kill only those cells—including many cancer cells—whose *p53* is out of commission for other reasons (*Science*, 18 October 1996, pp. 342 and 373).

Earlier this fall, Anthony Hall and his colleagues at the University of Otago in Dunedin, New Zealand, questioned this picture, reporting in *Nature Medicine* that the virus does infect cancer cells that have a normal *p53* gene and even seems to need the gene to destroy the infected cells. But McCormick thinks that these cell lines have other genetic changes that inhibit *p53* activity. Linke adds that the bottom line is how well the viruses kill tumors: "If these viruses can selectively kill tumor cells without adversely affecting normal cells, perhaps the genetic status of the tumor cells is not such an important issue."

Preliminary results indicate that ONYX-015 can meet those criteria. The first clinical trials of the virus took place in 1996 and demonstrated that it is safe to use. Since then, two more groups, each consisting of 30 patients whose head and neck cancers had not responded to previous therapies, have been undergoing treatment with the adenovirus.

The results so far show that the virus alone shrank tumors by at least 50% in slightly more than a third of the patients studied. But "even more dramatic has been the efficacy of the virus in combination with chemotherapy," says McCormick. Within a month, tumors completely disappeared in two of the first 10 patients treated with both therapies, and in seven more, the tumor shrank by more than 50%, with no significant side effects. Overall, ONYX-015 is proving to be "definitely much more effective than indicated from our mouse data," says McCormick. ONYX has also started testing this virus against pancreatic, colon, and ovarian cancers and plans to use it in people with brain tumors soon.

In July, another company called Calydon Inc., based in Sunnyvale, California, began a clinical trial of its own, somewhat different adenovirus, CN706, in men with recurrent prostate cancer. Company scientists had modified this virus by splicing into its genome the control DNA that normally regulates the expression of prostate-specific antigen, a protein made only in prostate cancer cells. In the adenovirus, the regulatory DNA turns on a viral gene that spurs viral replication, but only in response to the right combination of hormones and transcription factors. Because this array of molecular messages is found only in prostate cancer, "it's a very neat way of targeting the specificity [of the virus]," says Jonathan Simons, an oncologist at Johns Hopkins University in Baltimore, Maryland, who is assessing the safety of CN706 for Calydon.



Magic bullet? The nucleus of this skin cancer cell is bursting with replicating adenovirus particles.

CREDIT: ONYX PHARMACEUTICALS

Results of this trial are not yet available, but Simons says he is excited about the potential of using these viruses to treat prostate and other cancers that grow rather slowly. Because conventional therapies are designed to attack rapidly dividing cells, they don't work well on prostate cancer. "But [adenoviruses] kill independent of the cell cycle," he points out.

A third oncolytic virus that has moved into clinical trials is a herpesvirus produced by Georgetown's Martuza. Herpesviruses can cause encephalitis and other problems in humans, so Martuza and his colleagues needed to disable the virus so it could no longer reproduce in normal cells. In the first stage of this work, completed in 1991, they inactivated genes that produce enzymes the virus needs to replicate. As a result, the virus could multiply only in actively dividing cells, such as cancer cells, that make enough of these enzymes themselves. The researchers then demonstrated in lab culture that the modified virus destroys glioblastoma cells but not normal cells (*Science*, 10 May 1991, p. 854).

To further ensure that the virus is safe, the team has since knocked out a virulence gene that enables the herpesvirus to cause encephalitis. Because of the multiple changes in the virus's genome, "the chance that it can revert to the wild-type is virtually zero," says Martuza. The herpes simplex G207, as the new, improved version is called, "so far has been effective in essentially all solid tumors," tested either in laboratory dishes or rodents, says Martuza, and the animals suffered no detectable ill effects. Tests to assess the safety of G207 in humans began last February and will ultimately include two dozen patients with glioblastoma.

One other virus, a small, nonpathogenic virus called a parvovirus, went through preliminary human trials 8 years ago that showed it is safe to use. But its developers, Jean Rommelaere and his colleagues at the INSERM lab of the German Cancer Research Center in Heidelberg, Germany, wanted to improve the virus's tumor-killing potential before proceeding with further tests. "In the race between tumor proliferation and viral amplification, sometimes, the tumor is the winner," Rommelaere explains. So his team has spent several years developing ways to give the virus an added advantage, such as by adding genes that will recruit immune system cells to aid in tumor killing. In new cell-culture and animal studies, the virus's anticancer effect is now "more pronounced," he adds.

Other researchers are also trying to bolster the tumor-killing potential of the oncolytic viruses. At the University of Alabama, Birmingham, James Markert and his colleagues find that adding genes for the cytokines interleukin-2 or interleukin-5 boosts the immune system's attack on the tumor.

Others are trying to modify the viruses so that they will make the cells more susceptible to traditional cancer treatments as well as kill them directly.

For example, at Massachusetts General Hospital in Boston, neurosurgeon Antonio Chiocca and his colleagues have added a rat gene for a protein called cytochrome P-450 to the genome of a herpesvirus. Cytochrome P-450 converts cyclophosphamide, a drug used for cancer chemotherapy, to its active form. Consequently, as this virus spreads through a tumor, it not only kills the cells directly but also makes them susceptible to cyclophosphamide. Chiocca's team is now evaluating this virus in animal studies. And there's talk of putting in genes for compounds that will make a tumor more sensitive to radiation. "That's the beauty of this viral technology: With one agent you can deliver an oncolytic effect, a pro–drug activating effect, even a radiation-sensitizing effect," Chiocca says.

Of course, early excitement about a potential cancer therapy often gives way to disappointment, or at least realism. "Caution must be exercised, since the long-term side effects are not really known," says Linke. But the concept of making tumors get sick and fade away has undeniable appeal, says Simons. "This is the kind of thinking we need in new cancer pharmacologies.

–ELIZABETH PENNISI

PALEONTOLOGY

Popular Interest Fuels a Dinosaur Research Boom

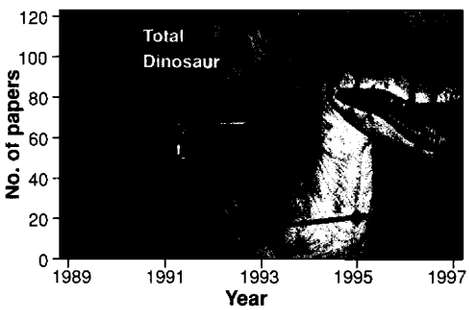
Paleontologists are learning to capitalize on the popularity of dinosaurs, and new discoveries, labs, and exhibits are the result

In 1994, paleontologist Cathleen May was running out of time and money. The University of California, Berkeley, graduate student had discovered an *Apatosaurus* skeleton in Curecanti National Recreation Area, near Gunnison, Colorado, but the bones were in danger of eroding away and her grant from the National Park Service was too small to excavate them. Many a similar skeleton has been left in the field for lack of funds, but May found an unexpected savior: Hollywood. She hooked up with an L.A. animation firm keen on creating a virtual dino dig for kids, gave interviews, let the company film the site, and wound up with $24,000 over 2 years. That was enough to finish the dig. The 20-meter-long *Apatosaurus*, one of the oldest known, is now headed for the Museum of Western Colorado in Grand Junction.

Hollywood isn't such an unlikely sponsor these days. After 65 million years of extinction, dinosaurs have conquered school yards, bookstores, and the video rental market. And the insatiable public appetite for the beasts is boosting research. Students are crowding into dinosaur paleontology classes, corporations and philanthropists are pledging support and donating specimens, and money is flowing into the field from movie and book spin-offs. Such nontraditional funding has its dangers, and despite all the activity, few researchers are flush with funds. But some say such sources are the key to survival for dinosaur paleontology.

Many researchers agree that popular enthusiasm and funding have combined with new discoveries to reanimate the field. A new analysis suggests that the number of dinosaur papers is on the rise, and new positions are appearing at a time when other areas of paleontology are barely holding steady. Spectacular fossil discoveries follow one upon another. This issue of *Science* reports the latest find: an African specimen with a fish-eating, crocodilelike skull, which paleontologist Paul Sereno of the University of Chicago and his colleagues describe on page 1298.

The field wasn't always so active. Dinosaurs have long been popular with the public, but scientifically they were a sleeper from the 1930s through the 1970s. Despite big dinosaur exhibits, most major museums had no Ph.D. dinosaur paleontologist. "Dinosaurs were considered gee-whiz things, good to show to the public but not particularly important from an evolutionary point of view," recalls Edwin Colbert, retired curator of dinosaurs at the American Museum of Natural History (AMNH) in New York City.



Hungry for more. Dinosaur money has helped fatten the pages of the *Journal of Vertebrate Paleontology*, allowing more papers to be published in all fields, including dinosaurs.

But in the 1970s, the field was rocked by the controversial idea that dinosaurs were warm-blooded and active like birds. The notion that the last dinosaurs were wiped out by an asteroid impact stirred even more interest. From then on, research seemed to take off: Since 1969, the number of dinosaur genera described has more than doubled, to about 350, notes Peter Dodson of the University of Pennsylvania School of Veterinary Medicine in Philadelphia. Papers have surged too. Back in the late 1980s, only about one in 10 papers in the *Journal of Vertebrate Paleontology* was on dinosaurs, according to a new analysis by Richard Cifelli, a mammal paleontologist at the University of Oklahoma, Norman, and former *JVP* editor. But by 1997, almost 25% of the articles in *JVP* were on dinosaurs (see figure).

Many paleontologists say that public interest bordering on mania has pumped specimens, students, and even jobs into the field. The movie *Jurassic Park*, for example, grossed nearly $900 million—vastly more money than all government agencies combined have ever spent on vertebrate paleontology. Although scientists don't share directly in these profits, the enthusiasm those figures reflect "doesn't hurt," admits Sereno, who has been featured in a half-dozen television documentaries and was listed as one of *People* magazine's 50 Most Beautiful People in 1997.

In 1986, paleontologist Timothy Rowe's first year at the University of Texas, Austin, his dino survey class had one of the largest enrollments in department history. "In some ways it's been my meal ticket here," he says. The next year, when he added labs to the course, he was able to fund six graduate students in paleontology as teaching assistants. Because many public universities distribute funding by the number of undergraduates taught, "a course that brings in 500 students really turns heads," says mammal expert Cifelli, who also teaches a dinosaur survey course.

And although many of the larger museums still haven't hired dinosaur specialists, popular interest has fueled the birth of regional museums that rely on dinosaurs as the main attraction, such as the Museum of the Rockies in Bozeman, Montana, and the Royal Tyrrell Museum of Paleontology in Drumheller, Alberta. "Where there used to be five museums where you could see dinosaur collections, now there's literally hundreds," says Sereno. Each one creates op-

SOURCE: R. CIFELLI/UNIVERSITY OF OKLAHOMA, NORMAN; PHOTO: MICHAEL SCHEMELLING STRINGER/AP