MEDICINE

For some cancer patients, viruses engineered to zero in on tumor cells work like a wonder drug. The task now is to build on this success

VIRUS THERAPY FOR CANCER

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Illustration by GUYC

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IN 1904 A WOMAN IN ITALY CONFRONTED two life-threatening events: first, diagnosis with cancer of the uterine cervix, then a dog bite. Doctors delivered the rabies vaccine for the bite, and subsequently her "enormously large" tumor disappeared ("*il tumore non esisteva più*"). The woman lived cancer-free until 1912. Soon thereafter several other Italian patients with cervical cancer also received the vaccine—a live rabies virus that had been weakened. As reported by Nicola De Pace in 1910, tumors in some patients shrank, presumably because the virus somehow killed the cancer. All eventually relapsed and died, however.

Even though the patients perished, the notion of treating cancer with viruses able to kill malignant cells—now termed oncolytic virotherapy—was born. And investigators had some success in laboratory animals. Yet for a long time only partial responses and rare cures in human trials ensured that the field stayed at the fringes of cancer research. Viral therapy for cancer faced several additional hurdles: uncertainty about its mechanisms and how to use viruses to achieve cures, a dearth of tools with which to engineer more effective viral strains and the habitual reluctance of physicians to infect patients with pathogens. Doctors elected to use poisons (chemotherapy) instead of microbes—mostly because they were more comfortable with those drugs and understood them better.

The story is very different today. Starting in the 1990s, researchers armed with a richer understanding of cancer and viruses and with tools for manipulating genes began to uncover the details of how viruses attack cancer cells. Investigators also started devising ways to genetically alter viruses to enhance their cancer-killing prowess and to prevent them from causing unwanted effects.

That work is beginning to pay off. One oncolytic virus was approved in China for head and neck cancer in 2005, and nearly a dozen are now in various stages of human testing in a wide variety of cancers. Recent results from the virus furthest along in testing give researchers hope that the U.S. Food and Drug Administration will approve one or more viruses as cancer therapies within a couple of years.

In particular, findings presented at the annual meeting of the American Society of Clinical Oncology in June 2013 showed that 11 percent of patients in a large trial of virotherapy against advanced metastatic melanoma (a skin cancer) had a "complete response"—showed no sign of the cancer—after treatment. The medicine, named T-VEC, consists of a version of the herpes simplex virus genetically altered to hit cancer with a double whammy—both to destroy cancer cells directly and to produce a protein (GM-CSF) meant to spur the immune system to also attack the cancer. In contrast to the side effects of many cancer therapies, the worst ones the virus caused in the study were flulike symptoms such as fatigue, chills and fever. Amgen, which makes the drug, released data on overall survival in November 2013 and the spring of 2014. Patients taking T-VEC gained four months over those taking GM-CSF alone.

The survival data may seem disappointing. Yet investigators are heartened that one in 10 patients had a complete response. The complete response rates achieved by T-VEC surpassed those of all recently approved drugs for metastatic melanoma, including a drug called vemurafenib, which was approved in 2011 to treat that cancer after a study reported in the *New England Journal of Medicine* determined that all signs of cancer disappeared in a much smaller ratio of patients—less than 1 percent.

Most encouraging, in the case of T-VEC, is a 2009 report showing that close to 90 percent of patients who responded to the therapy were alive more than three years later. A New Jersey woman named Sue Bohlin, for example, had no luck with standard treatments for her melanoma, and the cancer continued to spread, so she enrolled in a clinical trial of T-VEC. Three years after treatment with the drug, the now 61-year-old Bohlin remains cancer-free. "I'm one of the lucky ones," she says. "It's been a wonder drug for me."

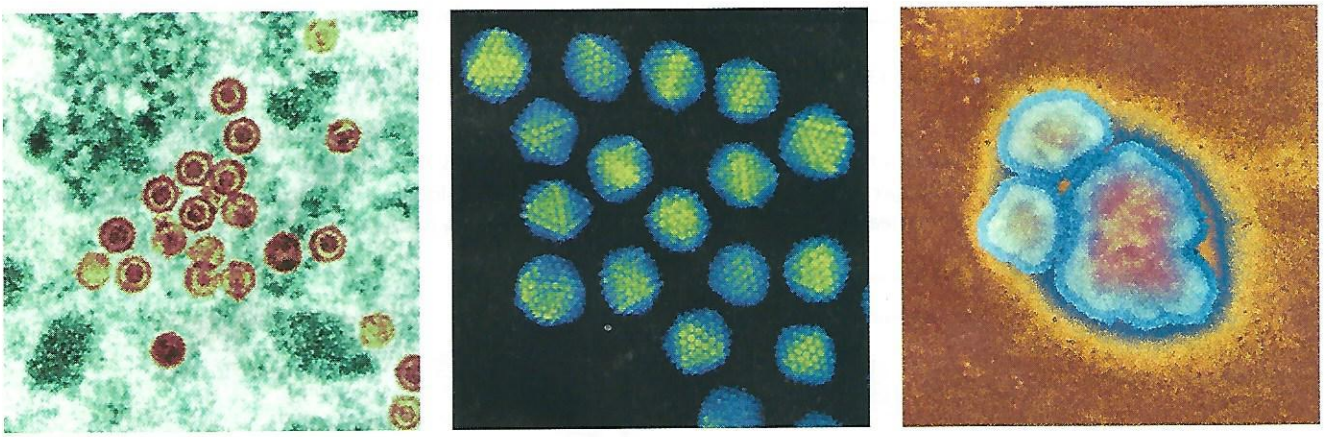
IN BRIEF

Specially engineered viruses could potentially infect and destroy human cancers without appreciably harming healthy tissues.

Once inside a tumor, such "oncolytic" viruses replicate extensively, yielding an army of virus clones able to seek out and infect more of the cancer cells.

Nearly a dozen viruses are being tested in humans as stand-alone therapies or in combination with existing treatments; several are in late-stage clinical evaluation.

Early on researchers attempted to suppress the immune system, to give the viruses time to act on the cancer cells before they were attacked as foreign. In an about-face, they are now engineering viruses to reawaken the immune system to fight the tumor.



PROGRAMMED CANCER KILLERS: Herpes simplex virus, adenovirus and measles (*left to right*) are three of about a dozen viruses that are being engineered to infect and kill cancer cells and, in some cases, boost the immune system's response to the disease.

The goal, of course, is to make Bohlin's experience the norm so that more than 11 percent of patients see their cancer disappear. Some of the viruses in clinical trials could well do that. Meanwhile researchers, including two of us (Stojdl and Mahoney), continue to explore ways to make virotherapy more effective for more people.

PROGRAMMABLE BIOLOGICAL MACHINES

VIRUSES OFFER a number of features that are appealing for cancer therapy, and scientists are trying to enhance several of them to improve their potency and safety. For one, certain viruses—either on their own or with some prodding—will selectively infect cancer cells while ignoring normal cells or will grow well only in cancer cells, leaving healthy cells relatively unscathed. Such selectivity is important for minimizing side effects, which are mainly caused by damage to normal tissues.

Once inside a cancer cell, viruses can be powerful killing machines. No virus can reproduce on its own, but if it finds the right conditions in a cell, it can hijack that cell's gene-copying and protein-making machinery to make new copies of itself. If all goes well in the case of cancer treatment, a virus will generate an army of clones that charge out of the infected tumor cell to seek and infect neighboring or even distant cancer cells. At times, the escaping viruses literally blow apart an infected cell as they exit—a process known as cell lysis—hence the name "oncolytic" virotherapy. In other cases, the viruses kill more stealthily, subtly programming a tumor cell to initiate a self-destruct sequence, called cell suicide, or apoptosis. In essence, viruses delivered as a drug convert infected cells into factories within the body that churn out more and more drug, then close for business.

Another advantageous component of virotherapy is its multipronged approach to attacking a cancer. Many cancer drugs interfere with only one aspect of cell functioning, a common drawback because malignant cells often eventually find ways of compensating for the effect. Also, cancers are really an ecosystem of cells that all descend from one deranged ancestral cell but now possess different genetic and other aberrations—so a drug that works on some cells may not work on others. These are two reasons why cancers become resistant to treatment, allowing tumors to rebound and kill patients. For such reasons, physicians often attack cancer from multiple angles with more than one kind of treatment, much as doctors treat patients with HIV today. Virotherapy, by itself, is more akin to combination than single therapy because viruses disrupt many processes in the cell at once so that the cell is less likely to become resistant.

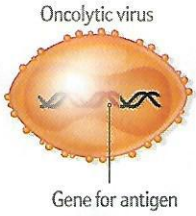
Beyond directly destroying tumor cells, when a virus infects a cell, it elicits several "bystander" mechanisms that can kill cancer cells that have resisted infection, including so-called vascular collapse [*see box on next page*]. Whereas oncolytic viruses are predominantly selective for tumor cells, some strains also infect tumor blood vessels. This secondary infection, in turn, attracts immune cells that damage the blood vessels, choking off blood flow to the tumor. Another important mechanism involves the rapid recruitment of immune cells to the tumor to fight off the initial infection. This immune response has long been viewed as a major impediment to successful virotherapy; after all, a prompt, strong attack should, in theory, erase virus-infected cells before the microorganisms have a chance to reach many cells. In fact, early efforts focused on keeping the immune system at bay to give the virus time to infiltrate the tumor.

Yet more recent work has shown that these immune cells sometimes get redirected toward the cancer itself and are, in many cases, critical for therapeutic success. Although we do not know the full details of how, when and why this switchover occurs, we do know that the process of infecting and killing tumor cells generates cellular debris that induces the production of small immune-stimulating molecules called cytokines and also activates the immune system's dendritic cells. Dendritic cells normally survey the body for any entities not native to the body and alert the immune system's T cells to mount a response against the apparent invader. In this case, the dendritic cells are thought to treat tumor components as "foreign" and to awaken the immune system to the fact that there is a tumor growing.

In addition to all these potential benefits, viruses can be programmed to behave in ways that natural viruses would not: they can be genetically altered to, for instance, decrease their ability to reproduce in healthy cells and increase their selective replication in cancer cells. The virus's genome can also be revised to give the viruses other cancer-fighting traits, such as the T-VEC virus's ability to pump up the body's immune attack against a tumor.

How Oncolytic Viruses Destroy Tumors

Not all viruses attack cancer cells, but some are especially good at targeting tumors and ignoring healthy tissues. Researchers are learning how to modify these viruses (*inset at left*) to awaken a stronger immune response against the tumor (*below*). Ideally, this approach would be paired with new treatments (*not shown*) that block a tumor's ability to suppress the immune system.



Researchers can insert genes for tumor antigens—molecules that elicit immune responses—into viruses. Infected tumor cells then produce the antigens, enhancing immune activity against such cells.

Direct Killing (Lysis) of Cancer Cells

Once inside a cancer cell, the virus forces it to make many more viruses. This new viral army charges out of the infected cell, killing it, and seeks out new cancer cells to infect. Or the viruses may simply reprogram infected tumor cells to self-destruct in a process known as apoptosis.

Vascular Collapse

Viruses also infect the cells that line the blood vessels around the tumor. As those cells die, they begin attracting the attention of white blood cells called neutrophils, which help to trigger the production of blood clots, leading to the eventual collapse of the blood vessel and the choking off of the tumor's nutrient supply.

Adaptive Immune Response

When an infected cancer cell bursts, it releases antigens—including the engineered antigens—which are engulfed by dendritic cells of the immune system. These antigens in turn are presented to the body's T cells, which go hunting for other cancer cells that share the same antigen.

Innate Immune Response

The death of virus-infected cells spurs the release of immune-stimulating molecules—including danger signals and cytokines—which prompt natural killer cells to destroy other infected or noninfected tumor cells.

Illustration by Tami Tolpa

SUPERVIRUSES

RESEARCHERS ARE EXPLOITING all this knowledge to enhance virotherapy in several ways, some of which are being tested in clinical trials now under way. One approach aims to engineer viruses to home in on certain molecules known as receptors that occur in greater quantities on cancer cells than on normal cells. Attachment to these receptors helps viruses to enter cells. This engineering should therefore help ensure that much more virus is taken up by cancer cells than by their healthy cousins.

A second, more advanced approach aims to enhance the tendency of viruses to replicate best in cancer cells. Because malignant cells replicate constantly, they generate a great deal of raw material. Viruses need these raw materials as well, and so they will often proliferate, or grow, better in a malignant cell than in other cells they manage to enter. Knowing of this proclivity, scientists have engineered viruses that are hyperresponsive to the raw materials present in excessive amounts in tumor cells. For example, they can genetically alter a virus so that it cannot direct the production of thymidine, a building block of DNA. Without this ability, the virus is forced to find an outside source of thymidine, and tumor cells have plenty. Normal cells do not offer enough thymidine for the virus to replicate. This approach is in early and midstage clinical testing.

John Bell's group at the Ottawa Hospital Research Institute (in which Stojdl was a postdoctoral researcher) and Glen Barber's group at the University of Miami have identified another reason that viruses can thrive in cancer cells: as cells undergo genetic and other changes that push them toward malignancy, they often lose some of their defenses against microbial attack, such as the ability to produce an antiviral molecule called interferon. These groups and others have taken advantage of this weakness to design viruses, such as an engineered version of vesicular stomatitis virus (VSV), that will not grow in any cell except tumors with defects in their antiviral defenses. One of these VSVs is being evaluated in patients with liver cancer.

To us and many of our colleagues, the greatest gains are going to come if we can enhance the ability of viruses to elicit immune responses against tumors. In the T-VEC trials, investigators found that the virus did not reach every metastatic cancer cell that had spread away from the primary tumor. Even so, 11 percent of patients experienced a complete response—no sign of cancer anywhere in the body—presumably because the engineered virus stimulated the immune system to seek out and destroy cells that the virus did not reach. In support of this possibility, the researchers found activated T cells at sites of metastases.

In another immunity-related strategy, pioneered by our colleagues at McMaster University in Ontario and the Mayo Clinic in Rochester, Minn., Stojdl is engineering into therapeutic viruses genes that encode molecules called tumor antigens that can elicit an immune response when present on tumor cells (for example, melanoma-associated antigen, or MAGE). In treated animals, the antigens are displayed to the immune system, prompting it to home in on and kill cancer cells at the same time that the oncolytic virus both kills cancer cells directly and changes the tumor microenvironment in a way that awakens other antitumor immune responses. Human studies are expected to start this year.

The idea of revving up the immune system is promising. But we have learned an important lesson from decades of immunotherapy research: tumors have evolved many ways to evade immune attack, and co-treating patients with other agents that relieve the immune suppression within the tumor may also be needed. It does not matter how much we boost the immune system if the tumor is highly adept at tamping down the response.

With colleagues at the University of Calgary, one of us (Mahoney) is trying to shut down the immune-suppressing cells that are known to lurk within tumors at the same time as patients receive oncolytic viruses. With those cells under wraps, the immune system activated by the virus should be able to escape suppression and thus fight cancers more effectively. By targeting the suppressor cells, we are taking advantage of decades of work by other researchers who have been designing molecules able to target and shut down immunosuppression; such drugs, including monoclonal antibodies that latch onto a molecule called PD-1, are among the most promising next-generation cancer therapies. Almost certainly such combination strategies, as well as deploying viruses together with traditional approaches, will be the future of oncolytic virus therapy because of their potential to help patients who do not respond to stand-alone virus treatment.

As we consider combination treatments, however, we must be careful. Although virotherapy has so far proved to be safe in clinical trials—there have been very few serious adverse events reported in patients, which contrasts sharply with most other experimental cancer medicines—we cannot be sure how our viruses will behave when combined with other, complementary immunotherapy strategies or when we increase the dose. "Oncolytic virotherapy has been very safe so far," says our colleague Stephen Russell, a professor of medicine at the Mayo Clinic. "But as we work toward increasing its potency and broadening its utility—particularly in the context of modulating host immunity—we run the risk of introducing toxicity, and we need to be aware of that," he cautions.

Harnessing the power of viruses to treat cancer has been a long work in progress. As the result of decades of research into molecular genetics, cancer biology, tumor immunology, immunotherapy, virology and gene therapy, investigators finally have the collective tool set and knowledge they need to exploit these interactions between viruses and the body for cancer therapy. That oncolytic virus therapy can work has been proved. The question now is how to make it work for more patients and to finally realize the promise of De Pace's 100-year-old dream of putting viruses to good use by saving the lives of people with cancer. SA

MORE TO EXPLORE

Novel Oncolytic Viruses: Riding High on the Next Wave? Marianne M. Stanford et al. in *Cytokine & Growth Factor Reviews*, Vol. 21, Nos. 2–3, pages 177–183; April–June 2010.

Thunder and Lightning: Immunotherapy and Oncolytic Viruses Collide. Alan Melcher et al. in *Molecular Therapy*, Vol. 19, No. 6, pages 1008–1016; June 2011.

The Emerging Role of Viruses in the Treatment of Solid Tumours. M. G. Bourke et al. in *Cancer Treatment Reviews*, Vol. 37, No. 8, pages 618–632; December 2011.

Virotherapy—Cancer Targeted Pharmacology. Alison Tedcastle et al. in *Drug Discovery Today*, Vol. 17, Nos. 5–6, pages 215–220; March 2012.

A list of clinical trials involving oncolytic virotherapies: http://clinicaltrials.gov/ct2/results?term=oncolytic+virus&Search=Search

FROM OUR ARCHIVES

Cancer's Off Switch. Jedd D. Wolchok; May 2014.