Oncorus, Inc.

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Viral vanguard:

Designing cancer-killing viruses to chase metastatic tumors

By Shraddha Chakradhar

Two years ago, Alice Brown and her team of scientists at PsiOxus Therapeutics began an experiment in which she seeded mice with tumor cells to model metastatic cancer in these rodents. The commonly used model has caveats—it doesn't perfectly replicate the human version of cancer progression—but the process did result in mice with lungs filled with multiple tumors. Hoping to save the mice from the cancers, the group injected into the tails of some of the mice a new cancer-killing virus that the company had developed. Brown, who is director of biology at PsiOxus, hoped that the virus would be able to survive in the bloodstream long enough to reach the tumors. More than three weeks after the injections, her team re-examined the lung tissue from the treated animals and saw that previously diseased tissue was now mostly clear. The animals receiving the experimental therapy lived more than twice as long as the control animals, whose extensive tumors were untreated. Brown's vision had been realized: the therapy worked, and it provided encouraging evidence that an infusion of cancer-killing viruses might be able to treat metastatic disease far from the site of injection.

Brown's preclinical work in mice was just published[1] as *Nature Medicine* went to press, but after the promising preclinical results, pharma giant Bristol-Myers Squibb (BMS) in New York City licensed a modified version of the compound, and is hoping to move it forward into clinical trials. It is the latest development in a quest that has stretched back more than 60 years. Early attempts to use viruses to treat cancer included targeting Hodgkin's lymphoma with the hepatitis B virus, and using a subtype of adenovirus to kill cervical cancer cells. In these endeavors, scientists understood that once a virus gained entry into a tumor cell, it seemed to replicate unchecked. Ultimately, the tumorous cells would burst open.

In some of these early trials, the virus would make some patients sick. In 1949, 22 people with lymphoma were given unmodified hepatitis B—which can replicate in normal cells—as part of a trial, but 13 of them developed viral hepatitis, and one person died during the trial[2]. The challenge, then, was to have viruses replicate only in cancer cells and not in normal cells in the body.

With the arrival of genetic-engineering technologies in the 1990s, scientists were able to design viruses that would replicate more frequently in cancerous cells than in normal cells. Virus particles are diluted once they enter the bloodstream, which means that a large enough concentration of viruses has to be injected to ensure that they can reach tumor cells. But researchers and companies still struggled to make enough engineered virus particles with which to dose patients. Scientists often ran out of their supply of specially modified virus particles before they reached a dose at which any efficacy could be observed, explains Brian Lichty, cofounder of Turnstone Biologics, an Ottawa-based company that is developing oncolytic viruses. Lichty notes that advances in cell-culturing, particularly purification methods, in the 1990s and 2000s allowed for large-scale production of engineered viruses. Where scientists could previously manufacture a single dose containing several million viral particles, Lichty says that large-scale manufacturing can now produce a dose with more than 100 million particles.

With the advent of improved genetic-engineering and manufacturing technology for oncolytic viruses, the next milestone for many scientists is targeting multiple tumors in one patient with a single injection.

Few oncolytic viruses have successfully been brought to market. An unmodified viral therapy called Rigvir was approved for use in melanoma by Latvia's State Agency of Medicines in 2004, but it is not yet approved for sale in the European Union. Shanghai Sunway Biotech in China developed a drug called Oncorine (H101), which reached the market in 2005, but it is approved for use only in China. More recently, in 2015, US and European regulatory authorities approved Amgen's Imlygic (talimogene laherparepvec, or T-VEC), a modified version of herpes simplex virus (HSV)-type 1. Imlygic has so far been approved for use only in melanoma cases in which the lesions cannot be surgically removed, sometimes because they have spread too extensively throughout the body. The viral therapy also poses some challenges in the way that it is administered: it's injected directly into tumors, and the procedure can require special

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training of physicians and nurses. Furthermore, injecting tumors directly can be uncomfortable for patients.

Another, more urgent limitation of Imlygic is its limited effect on tumors that are not near the skin. Although the therapy is engineered to trigger the body's immune response against melanoma tumors, evidence of the therapy's ability to act on noninjected tumors below the surface of the skin is only modest. In the phase 3 trial that helped Imlygic to earn approval, 64% of the injected tumors on and just below the skin shrank to at least half of their original size. By comparison, only 15% of tumors around the internal organs (and thereby too deep to be treated, in this case)—in individuals who received an injection of Imlygic directly near the surface of the skin—achieved the same response[3].

Now, companies such as PsiOxus are taking a new approach by choosing viruses that, unlike HSV-1, are less readily neutralized by the immune system. Being able to deliver these therapies intravenously, rather than by single-site injection, is key, experts explain. That is why Brown's experiments in mice are under such close observation. "If you have metastatic disease, you might not even be able to know where in the body the metastatic disease is," Brown says. Now that a couple of oncolytic viruses have gained market approval, the prospect of using this approach for cancers that have spread is more real, she adds. "It's really an exciting time."

Disabled defenses

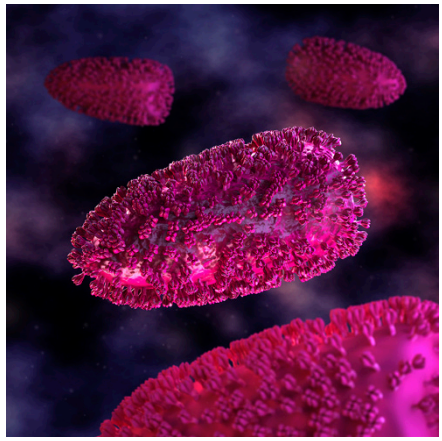
The use of viruses to target cancer cells relies on the fact that most tumors have a weakened immune defense against these pathogens. Healthy cells release cytokines known as interferons that signal the need to mount a response against viruses, but scientists have observed a diminished ability of tumor cells to fend off viruses[4]. In one of the first studies to demonstrate this difference, researchers infected multiple normal and tumor cell lines with vesicular stomatitis virus (VSV)—a virus that mainly infects farm animals—and checked the cells for viral particles 24 hours after infection. The normal cells had at least tenfold fewer virus particles than the tumor cells did. To confirm that cancer cells weren't able to mount their own antiviral response with the help of interferon signaling, the researchers repeated the experiment, but first primed the cell lines with interferon-α. "Some tumor cell lines are very able to detect the virus and respond to the virus," says Lichty, a coauthor of the study. "But if the cells don't respond to interferon, they're less likely to respond to the virus."

Many of the companies developing therapies have made modifications to further limit viruses so that they function only in tumor cells. For example, the viruses in Imlygic were engineered to lack copies of a gene called *ICP34.5*, a process that decreased the virus's ability to replicate in normal cells.

The quest to deliver oncolytic viruses systemically—a key requisite for tackling metastatic cancer—will involve developing viral therapies that are not easily recognized and eliminated by the immune system. Owing to the long, shared history that humans have with viruses, many viruses are quickly recognized by the immune system. When this happens, a virus injected intravenously as an oncolytic agent will be rapidly neutralized by antibodies, and won't be able to travel around the body to infect metastatic tumors. "Many of us have seen HSV in our immune system, and so it's going to be neutralized completely," Brown says. In the US, for example, more than 90% of adults, by some estimates[5], have been exposed to HSV-1, which would render a systemic injection of oncolytic HSV-1 ineffective in much of the population. Brown adds that other types of viruses can be similarly sequestered if the virus happens to bind to receptors on cells in the blood. But choosing the right kind of virus and modifying it to avoid elimination could enable viruses to reach otherwise inaccessible metastases.

Turnstone's solution to this problem was to explore all known viruses. Lichty says that many companies might have focused on developing oncolytic treatments by modifying viruses known to cause infections in people. But Turnstone liked the idea of finding a virus that didn't cause disease in humans. From an initial batch of a couple hundred viruses, the company narrowed the list to several dozen. In the process, they identified a small subset of rhabdoviruses, a group of single-stranded RNA viruses that can cause rabies and some forms of encephalitis. The rhabdovirus species that the company settled on, *Maraba vesiculovirus*, has no known pathogenic effect in humans. In normal cells, rhabdoviruses are readily killed following interferon signaling, Lichty explains, and this quality would potentially mean that they could get "very nice results" as far as selectively attacking tumor cells[6].

MedImmune, which is headquartered in Gaithersburg, Maryland, similarly sought out a virus that has not been known to circulate among people or normally cause disease in humans. Its candidate, MEDI5395, consists of a modified Newcastle disease virus, a single-stranded RNA virus that is known to be pathogenic in bird populations. Although the virus can be transmitted to humans through contact with poultry, this is rare. Unlike certain strains of bird flu, which can be fatal to humans, Newcastle disease virus only sometimes causes mild flu-like symptoms in humans, and is considered nonthreatening to humans.



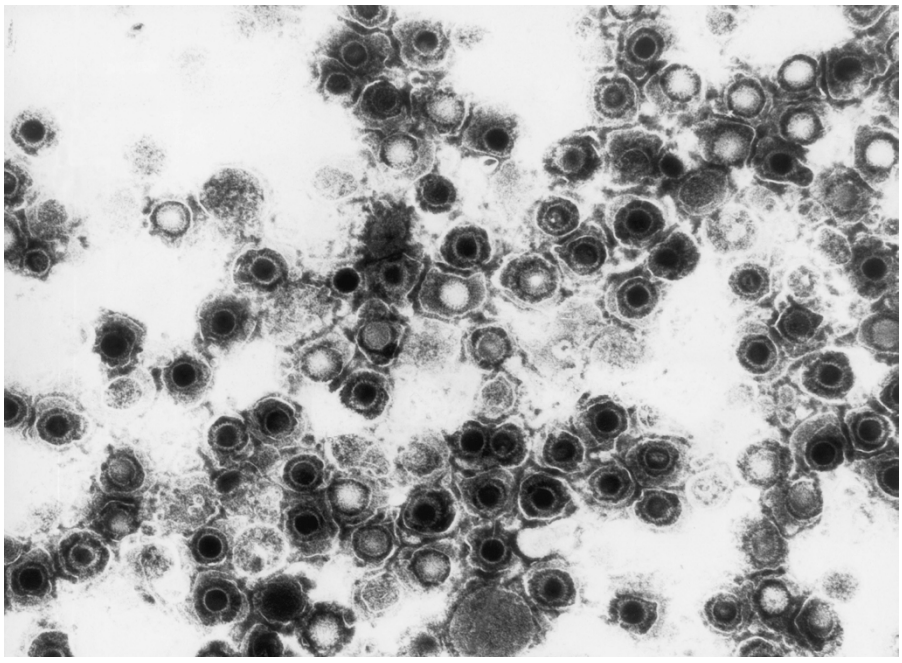
Turnstone Biologics

Cancer killer: Artist's rendering of *Maraba* virus.

In the case of PsiOxus, Brown and her colleagues have been conducting experiments using a virus designed as a genetic mix of two different strains of adenovirus, a group best known for causing the common cold and other mild respiratory illnesses. The resulting chimeric virus, called enadenotucirev, is unknown to the immune system at first injection and seems to evade the immune system for longer than regular adenoviruses. In a study comparing how much enadenotucirev is neutralized in blood relative to the AD5 strain of adenovirus, researchers found that the quantity of enadenotucirev was reduced by only tenfold after five days. By contrast, AD5 was reduced by nearly 1,000-fold[7]. Brown says that although the immune system will eventually recognize this virus, her team designed the virus to have a very short life cycle, allowing it to replicate and infect many cancer cells before it is caught by the immune system. Results from a phase 1 trial in patients with metastatic epithelial cancer revealed that the virus causes flu-like symptoms, but Brown says that PsiOxus doesn't know whether it's the virus itself or its oncolytic activity that is responsible for these side effects. John Beadle, CEO of PsiOxus, says that many of the early failures in the oncolytic-virus field could be due to scientists guessing at the right viral candidate. "We actually don't know what makes the best oncolytic virus."

Delivery dilemma

Beyond finding the right virus to treat metastatic cancer, companies are also considering how best to administer it so that it can reach the tumors that have spread through



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Tumor therapy: Herpes simplex virus, which forms the basis of Amgen's Imlygic.

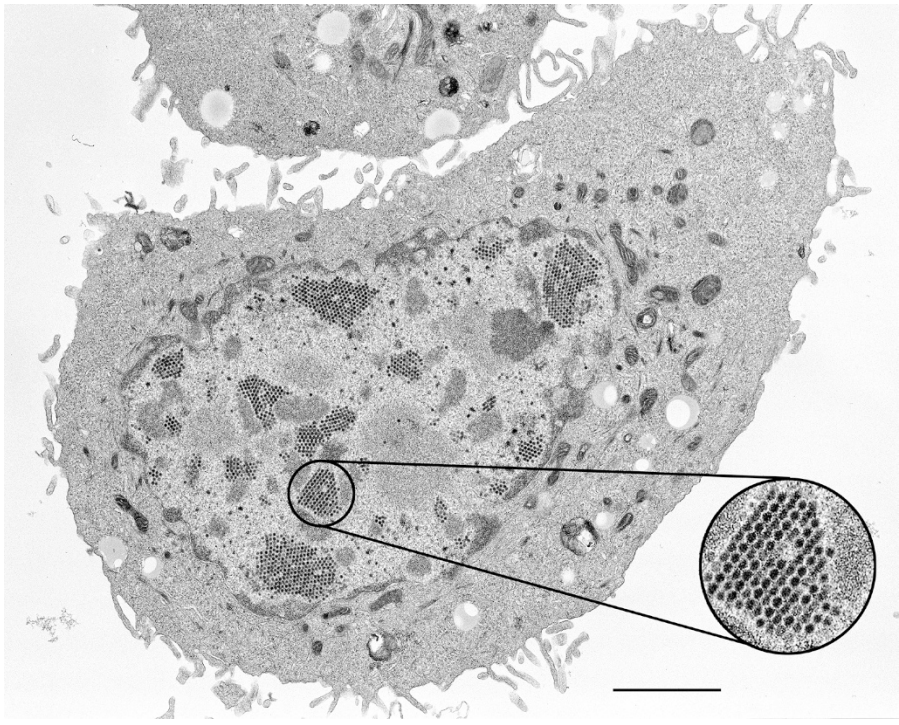
the body. They think that the best approach is to inject the virus directly into the bloodstream through intravenous (IV) infusion.

Of those viruses being tested through IV delivery, several have shown promising results. Both Turnstone and PsiOxus are investigating their respective candidates in clinical trials in which patients with metastatic disease are given the virus via IV infusion. Preliminary results from clinical trials of enadenotucirev indicate that the virus is able to travel through the body to infect metastatic tumors, according to Beadle. Beadle adds that his research team has observed an infiltration of virus particles in organ tissue samples far from injection sites, which suggests that the virus might be traveling through the bloodstream without being neutralized. As *Nature Medicine* went to press, results from Turnstone's investigator-led trial of its lead *Maraba* virus candidate MG1-MAGEA3 had not yet been released. But Lichty says that patients in the trial seem to tolerate the virus well.

Stephen Russell, a clinician–researcher at the Mayo Clinic in Rochester, Minnesota, says that achieving systemic administration of oncolytic viruses is "absolutely the goal" of the oncolytic-virus field. Russell, who has also developed an oncolytic VSV, says that overcoming virus neutralization is a barrier. "Viruses are fairly rapidly sequestered by phagocytic cells in the liver and spleen," Russell adds, saying that IV dosing also has to account for some of the virus to be lost to these phagocytic cells. "IV dosing is a very achievable goal," he says, and the need is especially great for those tumors that don't respond to conventional therapy.

Certain types of metastases might not be treatable through IV infusions. E. Antonio Chiocca, a neurosurgeon and researcher at Harvard Medical School, explains that it is especially important to consider metastases in the brain, because "the brain is one of the places where metastatic cancers go and hide." His group is launching a trial, sponsored by the US National Institutes of Health, in individuals with a type of brain cancer called glioblastoma, in which an oncolytic herpes simplex virus will be injected directly into brain tumors.

But direct injection of viruses into a tumor could still have a place within the oncolytic-virus field. Cancers of the peritoneal cavity—such as bladder and ovarian cancers—are difficult to treat through IV infusion. For these cancers, injecting a therapy directly into the cavity might elicit a tumor response. At least one company—Genelux in San Diego—is testing an oncolytic virus against metastatic ovarian cancer by injecting it directly into the body's peritoneum. Their lead candidate, a *Vaccinia* virus-based therapy called GL-ONC1, has thus far shown to be safe and effective in targeting multiple tumors around the peritoneal cavity, according to Thomas Zindrick, CEO of Genelux. To achieve a widespread response, researchers engineered Imlygic to include the granulocyte-macrophage colony-stimulating factor, or *GM-CSF*, gene. The gene encodes a protein that stimulates the growth of hematopoietic stem cells into dendritic cells. These antigen-presenting cells in turn alert the body's immune system and summon T cells to the site of the oncolytic virus and tumor, thereby inducing a more widespread immune response against tumors[8]. However, given the small effect on nonsurface tumors in the phase 3 trial, how effective Imlygic



Prof David Ferguson, University of Oxford

Potent parasite: PsiOxus' enadenotucirev (clusters of fine dots) within lung tumors.

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A selection of companies developing oncolytic viruses

Candidate name	Company	Virus type	Indications	Delivery mode	Phase in development
Reolysin	Oncolytics Biotech	Reovirus	Breast cancer	Intravenous (i.v.) infusion	Phase 2, going into phase 3
ONCR-001	Oncorus	Herpes simplex virus	Glioblastoma	Intratumoral (i.t.) injection	Preclinical
MEDI5395	MedImmune	Newcastle disease virus	Range of solid tumors	i.t. and i.v.	Preclinical
Imlygic/T-VEC	Amgen	Herpes simplex virus	Head and neck cancer, liver, advanced melanoma	i.t.	Phase 1, 2, and 3
MG1-MAGEA3	Turnstone Biologics	Maraba virus	Non-small-cell lung cancer (NSCLC), breast and esophageal cancers	i.v.	Phase 1/2
GL-ONC1	Genelux	Vaccinia	Ovarian cancer, solid tumors	Intraperitoneal injection and i.v.	Phase 1/2
Enadenotucirev	PsiOxus Therapeutics	Mix of two adenoviruses	Head and neck cancer, NSCLC	i.v.	Phase 1/2
CG0070	Cold Genesys	Adenovirus	Bladder cancer	Intravesical (directly into the bladder)	Phase 2
Pexa-Vec (JX-594)	Sillajen	Vaccinia	Hepatocellular carcinoma	i.v.	Phase 3
JX-929	Sillajen	Vaccinia	Melanoma	i.v. and i.t.	Phase 1
Cavatak	Viralytics	Coxsackievirus	Melanoma, prostate, lung and bladder cancers	i.v. and i.t.	Phase 1 and 2
MV-NIS	Vyriad	Measles virus	NSCLC, myeloma, bladder and ovarian cancers	i.t, i.v., intravesical and intraperitoneal, respectively	Various trials, with most advanced being in phase 2
VSV-IFNb-NIS	Vyriad	Vesicular stomatitis virus	Solid cancers and hemato-logical cancers	i.t and i.v, respectively	Preclinical going into phase 1

is at treating metastases in organs is still unknown. Robert Coffin, who is known as the inventor of Imlygic, says that the therapy is a "starting point," and that more potent versions are needed to effectively treat metastases throughout the body. He adds, however, that the only way, as far as he can see, to ensure that oncolytic viral therapy fully delivers on its promise of maximum anticancer potency is to inject tumors directly.

Immunotherapy added

Much of the excitement surrounding oncolytic viruses is related to their potential to be combined with immunotherapies. One of Amgen's trials combines Imlygic with Keytruda (pembrolizumab), an immunotherapeutic antibody that targets a protein known as programmed cell death 1, or PD-1. PsiOxus's partnership with BMS includes a trial that administers enadenotucirev in combination with BMS's Opdivo (nivolumab), a checkpoint-inhibitor antibody that also targets PD-1. MedImmune told *Nature Medicine* that the company is exploring the possibility of combining its oncolytic virus with immunotherapy. It adds that the potential of such viruses for use with immunotherapy might have recently increased interest in oncolytic therapy.

To capitalize on the opportunity to partner with immunotherapies, some companies are further enhancing their viruses to better induce immune responses. Turnstone's modifications to its Maraba virus candidate to more effectively target metastatic disease have blurred the line between oncolytic viral therapy and a vaccine. "We encode in the virus tumor antigens, and so we refer to it as an oncolytic vaccine," Lichty says of MG1-MAGEA3, which has been engineered to express melanoma-associated antigen A3 (MAGEA3). "I think of it like passing a baton," Lichty says. "The virus does its part for a period of time before the immune system comes in." The company plans to create different versions of the therapy that vary according to which antigens the virus will be engineered to express.

PsiOxus has developed a platform in which genes for immune-activating elements such as cytokines are inserted into its oncolytic virus. When the engineered virus enters a cell, the expression of the gene produces cytokines and other immune-activating elements, including antibodies, T cell–engaging ligands, or a combination of the three, to incite an immune response against the host tumor cell. "It's still oncolytic, it's the same virus," Beadle says, "But now, the virus forces the tumor to change itself or to produce something that will change the tumor." The company is currently running a trial to see how well its virus can reach metastases in cases of bladder, non-small-cell lung, kidney, and colon cancers. Results are expected in the second half of next year.

The positive results from her preclinical work make a compelling case for IV dosing of oncolytic viruses, says Brown. "The message here is that if you deliver one dose of virus [intravenously], it can infect and kill at many unconnected tumor sites," she says. She adds that directly injecting a tumor, where possible, would not yield such results. There are still serious hurdles to overcome in the field, including questions about whether patients receiving oncolytic therapies will eventually develop antibodies that neutralize follow-up viral doses. But in the meantime for Brown and others developing oncolytic-virus therapies, the ability to combine specialized viruses with immunotherapies such as checkpoint inhibitors offers a chance not only to treat multiple tumors throughout the body, but also to make oncolytic viruses more mainstream. "People are now really coming to understand oncolytic viruses," Zindrick says. "It's a fantastic time to be in the field."

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