The fourth front against cancer

The first clinical trials to test engineered viruses that attack tumour cells have yielded promising results for future cancer therapies

Philip Hunter

Exactly a century ago, US pathologist Francis Peyton Rous demonstrated that cancer can be transmitted among chickens by a virus. Although few believed him at the time, the virus was eventually named after him—Rous sarcoma virus—and Rous was awarded the Nobel Prize for Medicine, 55 years later in 1966. In 1964, Anthony Epstein, Bert Achong and Yvonne Barr isolated the first human cancer virus from lymphoma cells. These were important breakthroughs for cancer research and led to the discovery of more cancer-causing viruses—so-called oncoviruses. Today, seven viruses are thought to be responsible for more than 11% of all diagnosed cancers worldwide (Parkin, 2006). This knowledge has led to the development of vaccines against some oncoviruses, notably hepatitis B and, in 2009, against the two types of human papillomavirus (HPV-16 and HPV-18), after the German virologist Harald zur Hausen had demonstrated that it causes most cases of cervical cancer.

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Brad Thompson, president and CEO of Oncolytics Biotech Inc. (Calgary, Canada), is one of several scientists who think that viruses might also be able to kill cancer cells. Several candidate viruses are being—or are about to be—tested in phase 3 trials to compare the new viral treatments with established treatments, including

radiotherapy, chemotherapy and surgery. “With multiple companies’ viral programs in phase 3 testing using study designs agreed by the US FDA [Food and Drug Administration] under the Special Protocol Assessment process, we are on the cusp of seeing if these therapies can successfully play a broader role in the treatment of cancer,” Thompson said.

The latest trials are built on a growing body of evidence from *in vitro* experiments—backed up by several early phase 1 and phase 2 clinical trials—showing that some so-called oncolytic viruses can be highly effective against cancer in the form of both solid tumours and metastasis. The deliberate injection of viruses to treat disease obviously raises important safety and regulatory questions, but some researchers in the field have been so encouraged by recent results that they think that viruses could become a new weapon against cancer.

Three viruses have the greatest chance of being approved for clinical use within a few years, and two of them are now in phase 3 trials. These are a herpes virus called OncoVEX GM-CSF (BioVex; Woburn, Massachusetts, USA); a reovirus called Reolysin, developed by Oncolytics; and a vaccinia virus called JX-594 (Jennerex; San Francisco, California, USA). Another treatment developed by the Institute of Cancer Research (ICR) in London, UK, uses an adenovirus against solid tumours. Although this research is at an earlier stage, it is generating interest because it involves a different mechanism of action from the others and has shown early promise against a range of common cancers, including breast and bowel.

These viruses exert different mechanisms of action against tumour cells, which can broadly be assigned to three categories that are not mutually exclusive. The virus can be used to simply destroy cancer cells directly through lysis, just as it would destroy healthy cells when causing an infectious disease. This enables the second avenue of attack: the cell lysis spills antigens specific to the tumour into the blood-stream and elicits a systemic immune response against the tumour cells, including those that have not been infected by the virus, such as metastasized cells. Alternatively, the virus can be used as a vector to express an enzyme in tumour cells. This is followed by the administration of a prodrug that combines with the enzyme to generate cytotoxic compounds that destroy the target cell and surrounding cells.

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Caroline Springer and colleagues at the ICR in London developed this third method using a modified adenovirus in a system called gene-directed enzyme-prodrug therapy (GDEPT). The adenovirus is engineered to replicate only in the presence of human telomerase reverse transcriptase (hTERT). The enzyme is not expressed in most normal cells, but activated in many tumour cells, which enables them to replicate uncontrollably and, in effect, become immortal. “We achieve selectivity as we have used the hTERT promoter to drive replication of our viral

vector and hence expression of CPG2, meaning that the virus only replicates where human telomerase is expressed, that is in around 90% of human tumours," said Springer.

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The adenoviruses also contain the gene for the bacterial enzyme carboxypeptidase G2 (CPG2), which converts prodrugs containing nitrogen mustard into cytotoxic compounds. Nitrogen mustards are DNA alkylating agents, similar to the mustard gas used during the Second World War. When combined with CPG2, they form crosslinks in the DNA of the tumour cell that block DNA replication and result in apoptotic cell death.

The researchers at the ICR developed the therapy by trying a variety of prodrugs in combination with the CPG2 enzyme (Hedley *et al*, 2007). Once they had settled on nitrogen-mustard compounds, the greatest difficulty was to find a

biotechnology company that was prepared to produce the virus and meet the rigorous safety requirements. "The delay has been caused by the difficulty of scaling up viruses to the health and safety requirements for a trial, which are more complicated than for small molecules," Springer explained. "As a result, what has tended to happen is that a few biotech companies have set up in the field but then dropped out." Eventually, the ICR rescued the project by setting up its own manufacturing facility for the adenovirus in light of encouraging early results. A phase 1 trial is due to start in 2012, involving patients with head and neck cancer.

The attraction of this system is that the combination of virus and prodrug is apparently able to destroy the entire tumour, even if the CPG2 enzyme is expressed in only a small proportion of cells. Springer explained that this is due to the bystander effect. "In our case, the CPG2 bacterial enzyme produced by the viral vector is quite stable, so we are able to administer a prodrug that is cleaved by the enzyme, [producing] a cytotoxic drug that can kill neighbouring cells, regardless of whether CPG2 was expressed in them." In fact, only 2% of the cells in the injected tumour need to express CPG2 in order for all of them to

be eradicated; the bystander effect wipes out the other 98%.

A major objective of the forthcoming phase 1 study is therefore to establish beyond doubt that the virus is behaving as the researchers believe and that it behaves in the same way in a large number of patients. "That's the problem with such trials; defining exactly what the end point is. You have to assess whether expression—of CPG2 in our case—really has taken place. We need to be really certain what [causes] the oncolytic effect," Springer explained.

This proof of concept has already been achieved for a few other viruses, including the OncoVEX GM-CSF herpes virus, which is now in phase 3 trials in patients with malignant melanoma, the most aggressive form of skin cancer. According to BioVex, the company that developed the virus, OncoVEX GM-CSF has already improved the long-term survival of a significant number of patients from the 2008 phase 2 trial; 8 out of 50 patients with advanced metastatic melanoma made a complete recovery and remain in good health after treatment, with no recurrence of the disease.

Similarly to all emerging treatments that are still to receive full clinical approval,

viral cancer therapy can only be used on patients with advanced disease for whom existing therapies have failed, so this represents a promising success rate. Nevertheless, according to Alan Melcher, Professor of Clinical Oncology and Biotherapy at the University of Leeds in the UK, there can only be talk of a complete cure after successful phase 3 trials that directly compare the viral therapy with the current gold-standard treatment for a particular tumour. "We can't call these cures yet," said Melcher, whose lab specializes in oncolytic viruses. "Only when we get phase 3 trial data will we get a full idea how effective these can be."

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This aside, OncoVEX GM-CSF still gives grounds for optimism, not only because of the improved long-term survival rates, but also because of evidence that the therapy confers resistance against recurrence through a vaccination effect. This involves the release of tumour-specific antigens into the blood after viral lysis of the tumour cells. According to BioVex, these antigens, such as tumour necrosis factor, recruit T cells that then operate against all related tumours in the body, including those that did not receive a direct injection of the herpes virus. The result is an apparent long-standing immune response against the cancer, although BioVex agrees that phase 3 trials are needed to provide conclusive evidence. Nevertheless, the biotechnology company Amgen is clearly betting on the success of the virus: it just bought BioVex at a cost of US\$425 million. "The fact that a big pharma company has come in and invested that sort of money must mean they believe there is something in it," Melcher commented.

Another virus with good results from phase 1 and 2 trials is Reolysin, developed by Oncolytics Biotech Inc. It is based on a reovirus (respiratory enteric orphan virus)—a family of viruses that commonly infect the respiratory and/or digestive tracts, usually without causing any noticeable symptoms. According to Oncolytics, Reolysin replicates almost

exclusively in cells with an active RAS pathway, as these cells cannot activate the antiviral responses that normally inhibit the expression of crucial virus proteins. As RAS has a crucial role in cell differentiation and replication, it is activated in many tumour cells, which provides a convenient cue for attack by Reolysin, according to Patrick Lee from Dalhousie University's Department of Microbiology and Immunology (Halifax, Canada). "Based on results from *in vitro* and *in vivo* animal studies, reovirus has been shown to be effective against essentially all types of cancer including breast, brain, lymphoma, melanoma, prostate, pancreatic, bladder, ovarian and colon cancer," he said.

Early research by Lee and colleagues has also shown that the reovirus has the potential to treat some intractable tumours that have so far resisted other therapies. One of their studies has shown that oncolytic reovirus induces the regression of human breast cancer samples—xenografted into mice—by attacking the underlying cancer stem cells (Marcato *et al*, 2009). According to Lee, their work has demonstrated that cancer stem cells are as susceptible to reovirus treatment as other cells within the tumour, although it remains to be established whether this would lead to long-lasting cures in patients.

The third virus, the efficacy of which has already been demonstrated in phase 1 and 2 trials, is the DNA vaccinia virus, best known for its role as a vaccine that helped to eradicate smallpox. Jennerex therapeutics has developed an engineered version called JX-594 and is testing it in two phase 2 trials in patients with liver cancer. According to Caroline Breitbach, Director for Translational Research at Jennerex, the virus has already proven to be effective in earlier trials: "Objective tumour responses have been demonstrated in a variety of cancers including liver, colon, kidney, lung and melanoma."

Similarly to Reolysin, JX-594 works, at least in part, by targeting the activated RAS pathway of tumour cells. Yet, Jennerex point to an additional advantage of vaccinia: it also targets and destroys the blood vessels supplying the tumour, which can accelerate the rate of shrinkage by starving the tumour of nutrients. There is a slight downside, though: vaccinia causes mild flu-like symptoms in many patients, as did the smallpox vaccine. Although these side effects rarely

cause complications in healthy people, they could become more serious in patients with advanced cancer.

Importantly, Jennerex has demonstrated the efficacy of both intravenous delivery of the virus and direct injection into tumours. This makes it easier to target all tumour cells, especially in cases in which the cancer has metastasized to different places. It also provides an extra treatment option: in some cases direct injection into tumours might work best and in others, especially where metastasis has already occurred, intravenous injection would be preferable.

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For Reolysin, JX-594 and OncoVEX GM-CSF, the outcome of the current or forthcoming phase 3 trials is crucial if they are to realize their promise and gain clinical approval. Nevertheless, it seems likely that improved understanding of the molecular details of cancer, combined with an increasingly advanced ability to modify viruses to create potent oncolytic varieties, could create a fourth therapy option in addition to surgery, chemotherapy and radiotherapy. In the longer term, it might even be possible to dispense with the viruses and exploit their key molecules and pathways without the risks, however small, of injecting patients with a live infectious agent.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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