

# Micromanaging oncolytic viruses

By *Tim Fulmer, Senior Writer*

Oncolytic viruses have had limited success as cancer therapies, largely because of the need to balance potent antitumor activity with off-target toxicity. Two research teams have now applied a microRNA-based strategy that could restrict the replication of oncolytic viruses to tumors without the need for alterations to the viral genome that can lead to an overly attenuated strain.<sup>1,2</sup>

Oncolytic viruses are engineered to selectively target and replicate within tumor cells, triggering lysis and cell death. Selective targeting is usually achieved by mutating the viral genome to lower the likelihood that the virus will target or replicate within healthy cells. However, these mutations carry the risk of weakening the virus strain's ability to replicate within the tumor. Thus, attempts to balance potency and off-target pathogenicity have led to oncolytic viruses that are safe but lack efficacy in the clinic (see **Table 1, "Oncolytic virus therapies"**).<sup>3,4</sup>

Enter miRNAs, which are single-stranded, noncoding RNA molecules that downregulate gene expression by targeting specific mRNAs and preventing protein translation.

The key is that miRNA expression patterns are often tissue-specific. Thus, the two groups of researchers separately hypothesized that an oncolytic virus encoding sequences targeted by a tissue-specific miRNA would be prevented from replicating specifically in that tissue. This could be achieved by inserting the miRNA target sequence adjacent to genes required for viral replication.

The hope was that the virus would have limited off-target pathogenicity without the need for genetic attenuation.

In a paper in *Nature Medicine*, Stephen Russell and colleagues at the **Mayo Clinic College of Medicine** focused on coxsackievirus A21 (CVA21). The single-stranded RNA virus has previously shown activity in mouse models of cancer but has also caused fatal paralysis via inflammation and necrosis of skeletal muscle.

To address this toxicity, the researchers created a recombinant CVA21 strain that expressed sequences targeted by two muscle-specific miRNAs, miRNA-133 and miRNA-206.

In mouse models of multiple myeloma (MM), a single intratumoral dose of the recombinant CVA21 strain produced a decrease in tumor volume comparable to that seen using wild-type virus. Moreover, mice receiving the recombinant CVA21 strain had less muscle necrosis and significantly greater survival ( $p < 0.001$ ) than littermates that received wild-type CVA21, all of which developed hind-limb paralysis and required killing.

The authors concluded that incorporation of tissue-specific miRNA targets into oncolytic viruses "might increase their therapeutic index without perturbing their antitumor activity."

While the Mayo researchers focused on limiting muscle toxicity, a paper in the *Journal of Virology* describes a strategy to eliminate liver

toxicity associated with systemic delivery of oncolytic adenoviruses.

In that paper, researchers at the **University of Helsinki** and the **University of California, San Francisco**, constructed a recombinant adenovirus that contained a target sequence for liver-specific miRNA-122.

For a model, the researchers chose a liver cell line that expresses high levels of miRNA-122. Infection of those cells with a recombinant adenoviral strain containing three copies of the miRNA-122 target sequence inserted at a site adjacent to genes required for viral replication led to undetectable levels of adenoviral proteins.

"The papers potentially usher in a new era of oncolytic virus regulation," said John Bell, CSO of oncolytic virus company **Jennerex Biotherapeutics Inc.** "This approach may allow scientists to design viruses that maintain potency without having off-target infections." Bell is also a senior scientist at the **Ottawa Health Research Institute** and a professor of medicine at the **University of Ottawa**.

Jennerex's JX-594, a recombinant vaccinia virus (thymidine kinase deletion plus immunostimulatory granulocyte/macrophage colony-stimulating factor (GM-CSF)), is in a Phase II trial to treat unresectable hepatocellular carcinoma (HCC) and a Phase I trial to treat refractory solid tumors.

## Safe and stable

Despite the potential for fewer side effects, Jennerex CEO David Kirn said he was concerned that the miRNA targeting sequences are genetically unstable and could potentially mutate. That would cause the strain to revert back to a form that is toxic to normal tissue.

Bell agreed that escape mutants were a concern but added that "incorporation of multiple miRNAs throughout the viral genome might help mitigate the problem, and even when rare reversions arise, they could be eliminated by innate or adaptive immune responses."

Indeed, the *Nature Medicine* authors reported that CVA21 strains isolated from 6 of 11 infected mice were genetically identical to the original recombinant strain 45 days after virus administration. Virus isolated from three of the five remaining mice had at least 80% homology in the miRNA targeting regions compared with the same regions in the original recombinant strain.

Of the mice that had a mutated miRNA insert, 2 were free of disease symptoms at the end of the 70-day study. Only 1 of the 11 isolated strains had significantly lower retention of the miRNA targeting sequence.

Kalle Saksela, corresponding author on the *Journal of Virology* paper, told *SciBX* that his lab "observed no adenoviral escape mutants during the two-week *in vitro* infection experiments, suggesting that the reversion rate is rather slow and that the number of reversion mutants, when they arise, is probably small enough to be cleared by the immune system without serious side effects."

Saksela is chairman of the Department of Virology at the University of Helsinki.

Paul Hallenbeck, president and CEO of **Neotropix Inc.**, said another potential challenge facing the miRNA-based strategy is lowering toxicity in multiple types of healthy tissues.

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**Table 1. Oncolytic virus therapies.** At least 13 oncolytic virus therapies are in development for various cancers.

Company	Product	Description	Indication	Status
Shanghai Sunway Biotech Co. Ltd.	Oncorine	Modified adenovirus with deletion of an E1B-55kd segment	Head and neck cancer	Marketed (China)
BioVex Inc.	OncoVEX GM-CSF	Modified herpes simplex virus (HSV) encoding granulocyte/macrophage colony-stimulating factor (GM-CSF)	Various cancers	Phase II
Jennerex Biotherapeutics Inc.	JX-594	Recombinant vaccinia virus (thymidine kinase deletion plus GM-CSF)	Various cancers	Phase II
MediGene AG (Xetra:MDG) <sup>A</sup>	NV1020	Engineered HSV	Liver metastasis	Phase II
Oncolytics Biotech Inc. (TSX:ONC; NASDAQ:ONCY)	Reolysin	Formulation of human reovirus type 3	Various cancers	Phase II
Jennerex	JX-929	Recombinant vaccinia virus carrying cytosine deaminase	Cancer	Phase I
MediGene <sup>A</sup>	G207	HSV modified to produce a toxic metabolite in tumor cells	Brain cancer	Phase I
Neotropix Inc.	NTX-010	Naturally occurring oncolytic picornavirus	Cancer	Phase I
Viralytics Ltd. (ASX:VLA)	Cavatek	Coxsackievirus A21 (CVA21)	Melanoma; solid tumors	Phase I
M's Science Corp.	HF10	Mutant of HSV-1	Head and neck cancer	Pilot (Japan)
Genelux Corp.	GL-ONC1	Genetically stable modified vaccinia oncolytic virus	Breast cancer	Preclinical
Introgen Therapeutics Inc. (NASDAQ:INGN)	INGN 007	Replicating adenovirus vector overexpressing adenosine diphosphate	Cancer	Preclinical
Jennerex	JX-963	Recombinant vaccinia virus	Cancer	Preclinical

<sup>A</sup>In June, MediGene said it plans to either spin off or discontinue its oncolytic HSV program.

“For instance, in the *Journal of Virology* paper, the strategy resulted only in reducing viral replication in liver cells. However, while the liver is indeed a major organ of toxicity, the strategy is likely not to have changed the fact that adenoviruses replicate and cause toxicity in many other normal tissues,” Hollenbeck said.

Saksela responded that “our main goal was to prevent the acute hepatotoxicity that has been associated with the systemic delivery of oncolytic adenoviral strains and adenoviral vectors in primates and humans. The risk of toxicity to other tissues will clearly be a function of the levels of virus that are administered. At the levels we are delivering, the main concern is clearly liver toxicity.”

Neotropix’s NTX-010, a tumor-targeting single-stranded RNA virus, is in a Phase I trial in patients with small cell lung cancer (SCLC). Early next year, the company hopes to begin a Phase II SCLC trial and a Phase I/II trial in neuroblastoma and other solid pediatric cancers.

As to the *Nature Medicine* findings, Hallenbeck similarly thinks the modified CVA21 strain may have to address safety issues that extend beyond the muscles. “CVA21 not only causes muscle toxicity but also causes respiratory infections that may not be altered by the strategy in the *Nature Medicine* paper,” he said.

In the only reported instance of CVA21 in patients—a Phase I safety trial of intratumoral wild-type CVA21 to treat late stage melanoma—Australian biotech **Viralytics Ltd.** did not report any serious

off-target tissue toxicities.

Aladar Szalay, president and CEO of **Genelux Corp.**, said he wanted to see more efficacy data. In particular, he’s looking for data on the host immune response to miRNA-based oncolytic viruses.

“A key aspect of any oncolytic virus strategy is its ability to trigger an acute inflammation response at the site of the tumor,” he said.

“Indeed, oncolysis in combination with a host innate immune response is generally much more effective than oncolysis alone for tumor regression and shrinkage.”

Thus, said Szalay, who is also professor of medicine at **University of Würzburg**, “I would like to see proof that the miRNA-modified Coxsackievirus strain in the *Nature Medicine* paper replicates sufficiently within tumors to cause release of tumor antigens and tumor debris into the microenvironment and the subsequent triggering of a robust immune response.”

Genelux’s GL-ONC1 (GLV-1h68), a genetically attenuated vaccinia virus that induces oncolysis and also expresses a luciferase-GFP fusion protein for noninvasive tumor imaging, is in preclinical testing. The strain has shown activity in murine xenograft breast cancer models following i.v. delivery.<sup>5</sup>

**Next steps**

Saksela said he is interested in combining the miRNA with other tumor-targeting approaches to create an optimal oncolytic adenovirus.

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**“The beauty of the miRNA strategy is that it can be combined with a variety of tumor-targeting strategies and is potentially applicable to any RNA or DNA virus.”**

**—Kalle Saksela, University of Helsinki**

# Fragment-based reality check

By Michael J. Haas, Senior Writer

Researchers at the **Burnham Institute for Medical Research** have described a drug discovery technique that combines fragment-based synthetic methods and virtual screening to identify inhibitors of protein-protein interactions faster and more efficiently than either method alone.<sup>1</sup> In a proof-of-principle study in human breast cancer cells, reported in the *Journal of Medicinal Chemistry*, the team identified a selective, low-micromolar inhibitor of X-linked inhibitor of apoptosis.

Team leader Maurizio Pellecchia, professor of infectious diseases at Burnham, told *SciBX* that an essential feature of the approach is using NMR to confirm the binding behavior of hits. He said this is particularly useful in the initial rounds of screening, in which the potency of the

protein-fragment interaction is less important than the geometry of that interaction.

“Even a fragment with a weak binding interaction that still fits the binding pocket is of interest” and NMR can measure those weak interactions—which may be in the millimolar range—better than other spectroscopic methods, he said.

Traditionally, understanding protein-protein interactions—and designing drug compounds to inhibit them—has depended on the detailed structural information obtained by X-ray crystallography on protein-ligand or protein-drug cocrystals. But growing such crystals is difficult and time consuming. Thus, despite great strides made to optimize the process (see **Box 1**, “**Chips and drips**”), alternative approaches have been developed over time.

Fragment-based discovery involves screening synthetic libraries of small, drug-like molecular fragments for members that show weak interactions with the target protein. Hits are combined into larger structures by chemical synthesis to produce leads with micromolar binding affinity.

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“For example, the liver-specific miRNA silencing sequence could be combined with additional mutational modifications that limit adenoviral entry and/or replication to the tumor,” he said. “The beauty of the miRNA strategy is that it can be combined with a variety of tumor-targeting strategies and is potentially applicable to any RNA or DNA virus,” he said.

Saksela said his group has used the same miRNA-based approach to restrict poliovirus from replicating in the mouse CNS, thereby producing an attenuated strain that could potentially be used in a vaccine.<sup>6</sup>

Additional next steps for Saksela and colleagues include better characterization of miRNA expression in different tissues.

“For the most part, the published literature on tissue-specific miRNAs is rich enough to provide potential candidate sequences for miRNA targeting. However, tissue specificity by itself may not be enough to ensure the strategy’s success. We suspect that the expression level of a given miRNA in its tissue will also impact the effectiveness of the approach,” he said.

Over the longer term, said Saksela, researchers eventually may identify miRNAs that are downregulated or entirely absent in certain tumors but expressed in most normal cells. “An oncolytic virus targeted by such an miRNA would be prevented from replicating in all cells except the tumor and thus might represent the ideal tumor-targeted therapy,” he said.

Indeed, Bell and colleagues at the University of Ottawa are doing just that: his group is taking advantage of let-7 miRNA, which is abundantly expressed in most mammalian cells but downregulated in some cancers. They found that an oncolytic vesicular stomatitis virus (VSV) expressing let-7 targeting sequences had lower replication in normal cells while retaining oncolytic activity in cell culture and murine cancer models.<sup>7</sup>

Stephen Russell, corresponding author on the *Nature Medicine* paper and professor of medicine at the Mayo Clinic, told *SciBX* a patent application has been filed for the miRNA targeting strategy and is available for licensing. He also said he hopes to secure funding to begin testing the strategy in the clinic.

The *Journal of Virology* findings are not patented, according to Saksela.

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## COMPANIES AND INSTITUTIONS MENTIONED

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