Dismantling cancer's immune defense

By Michael J. Haas, Senior Writer

A team of North American researchers has shown that a class of validated cancer drugs—histone deacetylase inhibitors—could help boost the efficacy of oncolytic viruses, which have been around since the early 1990s but have rarely progressed to late-stage clinical trials. The exact mechanism of the synergy between the viruses and the inhibitors remains to be elucidated, but companies polled by *SciBX* said the combination showed promise—especially because both classes of therapeutics are well tolerated in cancer patients.

Oncolytic virus company **Jennerex Biotherapeutics Inc.**, which participated in the research, plans to in-license the findings and is already considering clinical trial designs.

As reported in the *Proceedings of the National Academy of Sciences*, histone deacetylase (HDAC) inhibitors suppressed the innate antiviral response of a variety of tumor cell types—leaving those cells vulnerable to infection by an oncolytic virus and enhancing subsequent virus-induced apoptosis.¹

The research team was led by John Hiscott, professor of microbiology, immunology and medicine at **McGill University** and director of the molecular oncology group at **Jewish General Hospital** in Montreal, and John Bell, professor of medicine at **University of Ottawa** and senior scientist at its **Ottawa Health Research Institute (OHRI)**, as well as CSO of Jennerex.

Dynamic duo

Oncolytic viruses are engineered to only infect and replicate in cancer cells and thus lower tumor burden while leaving normal cells unharmed. HDAC inhibitors induce gene transcription by limiting histone binding to DNA and thus make chromatin, the protein-DNA complex that makes up chromosomes, transcriptionally active.

“It was unexpected that these compounds would synergize so effectively—or at all, really, given the global role HDACs play in chromatin remodeling,” Hiscott told *SciBX*. “The observation that HDAC inhibitors augmented the response of cancer to oncolytic viruses was pretty dramatic.”

Over the last decade at least 10 companies have developed oncolytic viruses to treat cancer, but thus far only two have made it beyond Phase II trials. **Onyx Pharmaceuticals Inc.** had its ONYX-015 in a Phase III trial to treat head and neck cancer but discontinued the study in early 2003 as part of a company restructuring. In 2005, H101, an oncolytic adenovirus from **Shanghai Sunway Biotech Co. Ltd.**, received

approval in China for use in combination with chemotherapy to treat late-stage refractory nasopharyngeal cancer.

In 2003, an OHRI team, also led by Bell, reported that about three-fourths of all tumor cell types have a defect in their innate immunity—the initial response of any cell to infection that in turn triggers a systemic immune response. The defect was characterized by a decrease in interferon (IFN) expression,² and it made tumors more vulnerable to viral infection than normal tissues.

Many oncolytic viruses seek to exploit this fact. However, the degree of IFN deficiency varies among tumor types, and some tumors can still mount an innate immune response that is sufficient to inhibit oncolytic virus infection and replication.

“We wanted to find something that would level this response in all tumors and make them all equally responsive to oncolytic virus therapy,” Bell said.

In parallel, there was growing evidence that HDAC activity may play a role in activation and transcription of IFN-related genes in response to viral infection. A paper by David Levy, professor of molecular pathology and microbiology at the **New York University School of Medicine**, and colleagues described the need for HDAC activity to induce IFN-stimulated gene expression and antiviral responses.³

“All indications point to interferon genes, gene products and signaling pathways as key,” Hiscott said.

Therefore, for their current *PNAS* study, Bell and Hiscott hypothesized that HDAC inhibitors might increase the apoptotic activity of an oncolytic virus by abrogating any IFN-activated immune defense that a tumor could mount.

They tested their hypothesis in a range of *in vitro*, *in vivo* and *ex vivo* studies using *vesicular stomatitis virus* (VSV)—which replicates poorly in normal cells but efficiently in IFN-defective cells—combined with one of two small molecule HDAC inhibitors: SNDX-275 from **Syndax Pharmaceuticals Inc.** or Zolinza vorinostat from **Merck & Co. Inc.** SNDX-275 (formerly MS-275), which until 2007 had been marketed for nonclinical research purposes, is in multiple Phase II trials for various cancers. Zolinza is marketed to treat cutaneous T cell lymphoma (CTCL).

First, the team found that the combination of VSV and an HDAC inhibitor increased viral replication and virus-induced apoptosis of human prostate cancer cells compared with the effects of VSV alone. Additional studies confirmed that the HDAC inhibitors suppressed the IFN immune response induced by VSV in the prostate cancer cells.

Next, in human cancer tissue samples—prostate, ovary and sarcoma—the team showed that VSV infected and ultimately killed cancer cells only when administered in combination with an HDAC inhibitor. They also found that HDAC inhibition did not enhance the virus’ ability to infect and replicate in normal human prostate, colon, muscle and lung tissues. Instead, those tissues remained refractory to VSV infection.

Last, in mouse models of melanoma, prostate, colon and metastatic breast cancer, VSV plus SNDX-275 either slowed or halted tumor growth without affecting normal tissues.

In *PNAS*, the researchers wrote that they obtained similar results from preliminary studies combining an HDAC inhibitor with either *vaccinia virus* or *Semliki Forest virus*.

“The key finding is that HDAC inhibitors made tumors—but not normal tissues—more sensitive to oncolytic viruses,” Hiscott said.

“The beauty of this approach is that there are very few off-target effects from the virus,” and the combination therapy is administered for only a few weeks—thereby avoiding the side effects associated with longer-term use of some HDAC inhibitors, such as vomiting, anorexia and weight loss, Bell said.

“These findings show that oncolytic viruses have favorable and exciting interactions with standard therapeutics for cancer,” said David Kirn, president and CEO of Jennerex and coauthor on the *PNAS* paper.

The company’s JX-594 is a recombinant *vaccinia virus* genetically modified to be selective for cancer and to generate a systemic, anti-tumor immune response. The virus is in a Phase II trial to treat unresectable hepatocellular carcinoma (HCC) and a Phase I trial to treat solid tumors in malignant melanoma, lung cancer, renal cell carcinoma (RCC) and squamous cell cancer of the head and neck (SCCHN) that are refractory to standard therapy.

“Patients given our lead candidate, JX-594, experience flu-like symptoms that last only a few days and are usually self-limiting,” Kirn said.

Combination safe

Other companies contacted by *SciBX* agreed that the *PNAS* paper made a case for combining an oncolytic virus and an HDAC inhibitor to treat cancer—especially if the combination’s safety profile carries over to humans.

Axel Mescheder, executive board member for R&D at **MediGene AG**, said he “appreciated the *PNAS* paper because it confirms what we have thought about the potential for use of oncolytic viruses in combination” with other anticancer therapeutics.

“Since the first reports on the efficacy of oncolytic viruses about 10 years ago, it has been clear that they can be useful on their own but may have additive or synergistic effects with other therapies such as chemo- and radiotherapy,” he said.

“We are convinced that oncolytic virus therapy is an interesting and positive approach, and the combination with HDAC inhibitors is a very smart one that has a future in clinical trials,” Mescheder added.

He thinks the combination of virus and HDAC inhibitor could be safer and less toxic than combination chemotherapy. But he did say that enhancing the ability of an oncolytic virus to replicate and spread might increase the levels of systemically available virus. Thus, dosing might have to be closely monitored to minimize the risk of infecting normal cells.

He said further studies should include combinations of different viruses and HDAC inhibitors in a range of animal models and tumor types. The goal, he said, would be to investigate “the potential for additive toxicity or other adverse events that are difficult to predict, such as cytokine release.”

MediGene is developing two oncolytic viruses to treat cancer. NV1020, an engineered herpes simplex virus (HSV), recently completed

a Phase II trial to treat liver metastases from colorectal cancer. G207, an HSV modified to produce a toxic metabolite in tumor cells, recently completed a Phase I study in glioblastoma multiforme (GBM).

In a Phase II trial, NV1020 had side effects similar to Jennerex’s JX-594. “In most patients, NV1020 was well tolerated, with flu-like symptoms as the dominant side effect,” Mescheder said.

The company hopes to partner or spin out its oncolytic virus program, he said.

Peter Ordentlich, cofounder and director of scientific affairs at Syndax, agreed with Mescheder that the combination of agents was a feasible strategy for treating cancer. “HDAC inhibitors could prolong the effect

of the virus and increase its systemic exposure,” thereby overcoming two of the hurdles often associated with oncolytic viruses, he said.

Ordentlich noted another interesting feature of the combination reported in *PNAS*: in the colon cancer model, the research team showed that halting SNDX-275 therapy slowed virus activity and that reinitiating therapy also reinitiated virus activity.

“This could be used like a dialing mechanism to turn the virus on and off” and could possibly prolong the time it takes the tumor to develop resistance, he said.

Ordentlich added that dialing the virus activity down or up could prevent excessive tumor lysis—which he described as a treatment-induced explosion of a tumor that releases large amounts of toxic compounds into a patient’s system. The effect is often a dose-limiting toxic effect for a cancer therapy, he said.

Still, like Mescheder, Ordentlich was concerned that an HDAC inhibitor might increase the effect of the virus on normal cells. Thus he said more work is needed to understand how HDAC inhibitors broaden the effect of a virus and to identify the optimum species of virus to use in the combination.

Indeed, Ordentlich said Syndax probably would not pursue combinations of SNDX-275 with an oncolytic virus until one or the other agent is approved for use. “But,” he added, “we would not be averse to conducting more preclinical work in this area with our HDAC inhibitor” and another company’s oncolytic virus.

Syndax has SNDX-275 (entinostat) in four Phase II trials: two to treat non-small cell lung cancer (NSCLC) and two to treat metastatic breast cancer.

The compound is also in clinical trials sponsored by the **National Cancer Institute** to treat NSCLC, chronic myeloid leukemia (CML), acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).

Syndax in-licensed worldwide rights to the compound from **Bayer AG**’s Bayer Schering AG unit in 2007, at which time the biotech halted commercial sale of the compound.

Microanalyzing combinations

Having established the activity of the HDAC and virus combination, Bell and Hiscott are now trying to elucidate the mechanism behind the synergistic effect to determine what makes a particular combination efficacious.

Hiscott’s group is conducting microarray analyses of human tumor samples, which Bell said “could determine the best combination of

“The key finding is that HDAC inhibitors made tumors—but not normal tissues—more sensitive to oncolytic viruses.”
—John Hiscott, McGill University

virus and inhibitor to use, according to the genetic profile of the tumor.”

Hiscott added that he and Bell are also interested in studying both the HDAC-regulated events that normally occur on chromatin and the genes affected by the action of different HDAC inhibitors.

“Microarray analysis might indicate which genes are affected by HDAC inhibitors. It is also known that some HDAC inhibitors are more selective than others—so there is a question about which HDACs to target,” Hiscott said. “We want to explore the best combinations of virus and inhibitor in animal models and human tissues.”

The team is studying combinations of HDAC inhibitors and either *vaccinia virus* or *Semliki Forest virus* in animal models of cancer.

Bell said that the University of Ottawa and McGill University have submitted a patent application for the concept of treating cancer with a combination of oncolytic virus and HDAC inhibitor.

Jennerex intends to in-license the findings reported in *PNAS*. Kirn said the company is considering clinical trial designs that would combine its lead oncolytic virus, JX-594, with an HDAC inhibitor to

treat cancer, but it has not decided which HDAC inhibitor to use.

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COMPANIES AND INSTITUTIONS MENTIONED

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