

## Innovations

### Smarter viruses

#### Onyx Pharmaceuticals, Inc.

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Common anti-cancer drugs remain crude, killing any cell that divides rapidly. Onyx Pharmaceuticals, Inc. (Richmond, CA, USA) hopes to change that situation by targeting only cells with the sorts of genetic defects characteristic of cancer cells.

Onyx's weapon, ONYX-015, is a modified adenovirus (cold virus) that lacks the E1B-55K gene. Without E1B-55K, ONYX-015 can no longer inactivate the cellular p53 protein, and so cannot replicate in normal cells. But, in tumor cells that lack p53, ONYX-015 does just fine. It can replicate, and kill, the cancer cells.

ONYX-015 is exhibit A in an emerging pack of replication-selective viruses, which are finding favor with many gene therapists. "It is a very, very important strategy to follow," says Michael Blaese, head of human therapeutics at ValiGen (US), Inc. (Newtown, PA, USA) and former chief of the clinical gene therapy branch of the National Human Genome Research Institute (Bethesda, MD, USA). "The traditional dead viruses used for gene therapy simply will not work very well because they cannot get around that well."

But exhibit A is having its problems. Since its triumphant unveiling in 1996, ONYX-015 has been plagued by doubts about whether the virus' ability to replicate correlates with the status of the p53 pathway. Despite a recent paper from Onyx's founder, the ultimate verdict may have to wait for the results of ongoing clinical trials.

#### Company first, idea second

Before Onyx there was Chiron, and before Chiron there was Cetus.

Both Onyx's scientific founder Frank McCormick and its current CEO Hollings Renton started out at Cetus Corporation before moving to Chiron Corporation (Emeryville, CA, USA) when Chiron acquired Cetus. In 1992, McCormick says he "was approached by some venture capitalists, who convinced me that this would be a good time to start my own company. I was very happy in the job that I already had, but they made me an offer I could not refuse."

McCormick took his research interest in ras signaling with him. At the new company "the science was not very different," he says. "But building something from the ground up was very exciting."

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#### Onyx's therapy discriminates based on the genetic changes that define a cancer cell.

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Soon after Onyx began, McCormick attended a conference where an adenovirus protein called E1A was a major topic of conversation. E1A was proposed to act in the same manner as E1B-55K, but its target seemed to be the tumor suppressor Rb rather than p53. So far the major evidence for this role was that E1A and Rb bound to each other. But, asked McCormick, "what was the formal proof that Rb was the major target?" Viruses with a mutant E1A that could no longer bind Rb should be replication-defective, but that replication failure could also be caused by another, unknown consequence of the mutation.

"I was trying to think of a better way to prove that Rb is the major target," says McCormick. "I realized there are cell lines that lack Rb, so adenovirus would not need E1A any more. Then I realized, if that were true, the adenovirus without E1A should only be able to replicate in tumor cells (that lack Rb). The same is true for E1B-55K and p53. As well

as being a really good experiment this could lead to a therapeutic."

"I could not wait to get to work the next morning and bounce (the idea) off my colleagues," he says. Within a few months he had a team of virologists working on the problem, using a virus from which Arnie Berk (University of California, Los Angeles, CA, USA) had deleted E1B-55K. "The advantage of being founder of the company," says McCormick, "is I could marshal the resources to quickly test this out."

#### Success turns to doubt

Three years later McCormick had a paper in *Science*. Richard Klausner, director of the National Cancer Institute (Bethesda, MD, USA), hailed the discovery of such a cancer-specific virus as "a long-held fantasy."

But almost immediately there were signs of discord. Berk drew attention to old data indicating that adenoviruses mutant for E1B-55K could replicate in some cells containing functional p53, and not in some cells that lacked p53. This lack of correlation led Berk to suggest that "the story is much more complicated."

The Onyx researchers admitted as much in their next publication the following year. Killing by ONYX-015 was still restricted to cancer cells, but not all of the susceptible cancer cells were defective in p53.

A flood of similar observations followed from other laboratories in 1998 and 1999. "I was very worried," says McCormick. "The lack of correlation with p53 status was a concern." But McCormick felt the answer was waiting. "We were pretty convinced that the cells were finding other ways to knock out the (p53) pathway," he says. "Until we could identify these we were on thin ice."

In the meantime, the criticisms kept piling up. "The worst part about it was that some of my colleagues were really quite abusive," says McCormick. "It was not a very objective discussion. It was quite hostile." David Ornelles of Wake

Forest University (Winston-Salem, NC, USA) stumbled into the dispute when he started using the viruses for his unrelated research. "I think," he says, "that there was an element of human ego that elevated this above and beyond what the science justified."

McCormick responded with a paper in the October 2000 issue of *Nature Medicine*. He concluded that ONYX-015 could replicate in a colorectal cancer cell line that had an intact p53 gene because the cell line did not express functional p14<sup>ARF</sup>. This protein is an inhibitor of an inhibitor of p53. Viral and other proliferative signals normally induce p14<sup>ARF</sup> expression, which frees up p53. Adenovirus presumably needs E1B-55K to counteract this newly liberated p53. But in the cancer cells, the lack of p14<sup>ARF</sup> meant that p53 was not liberated and E1B-55K was no longer needed. (This is despite the fact that an intersecting pathway – from irradiation to p53 to transcriptional induction of p53 targets – was completely intact in the same cell line.)

McCormick showed that a number of other p53+ cell lines that support ONYX-015 replication also had p14<sup>ARF</sup> defects, and reintroduction of wild-type p14<sup>ARF</sup> suppressed ONYX-015 replication. Onyx's senior vice president of research and development Leonard Post thinks this will answer many of the critics. "I do not know that we can say that (p14<sup>ARF</sup>) explains all of it," he says. "It explains a lot of it."

McCormick is even more confident. "I presume," he says, "that the controversy about p53 status is now behind us."

#### **But on it goes**

Antony Braithwaite (University of Otago, Dunedin, New Zealand) is not so sure.

McCormick began his adenovirus odyssey trying to confirm the function of an adenovirus protein. He now uses the presumed function of E1B-55K, which is supported by the results of

transfection experiments, as one of his defenses. The status of the p53 pathway must be important, he says, because "the virus would not have a protein (E1B-55K) to inactivate p53 if it did not have to."

But Braithwaite is proposing that E1B-55K has an entirely different, if still p53-related function. He thinks that a complex of p53, E1B-55K, and another protein called E4orf6 regulates the cell cycle so that virus replication and cell death are correctly coordinated. "The complex is regulating the cell replication machinery to maximize (virus) production, then it is utilizing a point of sensitivity in the cell cycle to induce cell death," says Braithwaite. "That is the theory. I have not got all the data to prove that yet."

What Braithwaite claims to have shown is that p53 is necessary for maximal viral replication and rapid cell death. Both replication and death can occur without p53, he says, but they are less extensive and slower. In a *Cancer Research* paper in May 2000, Braithwaite reported that the failure of the rapid cell death pathway is correlated with the presence of E1B-55K mutants that cannot bind p53.

Braithwaite cannot yet explain how the Onyx virus selectively kills transformed cells. And it is difficult to compare his work to that of McCormick, as Braithwaite's readout is cell death rather than the more usual replication measure of plaque-forming units.

McCormick, meanwhile, says he "agree(s) with (Braithwaite's) proposition that the E1B-55K/E4orf6 complex may have unknown and important roles in virus replication. However, these functions are independent of p53. After all, as dozens of papers have described, p53 is actively degraded during adenovirus infection."

#### **The proof is in the phase III trial**

Braithwaite thinks a compromise may be possible. "We appear to be at opposite poles from Onyx and others," he says. But "the differences

may be quantitative rather than qualitative."

The final verdict may come from Onyx's phase III trial for head and neck cancer. The phase II trial, combining ONYX-015 with chemotherapy, "gave us a great deal of confidence," says CEO Renton. Nineteen of thirty patients with advanced tumors showed 50% or greater response to therapy.

Clinical trials and the debate about mechanism coincided. "It did not slow us down," says Renton. "As long as (ONYX-015) had a good strong therapeutic index we wanted to go ahead." The trials showed no correlation between treatment success and p53 status, although p14<sup>ARF</sup> function was not measured. In the phase III trials "the idea is to take all comers and retrospectively analyze who does best," says McCormick.

Side effects have been minimal so far, perhaps because the selectively replicating virus can be used at lower doses than traditional non-replicating gene therapy vectors. One "legitimate concern," according to McCormick, was apoptosis of normal cells in response to even a non-productive infection. In culture ONYX-015 induces a significant amount of cell death, but whatever cell death occurs in vivo does not appear to be a problem.

Also ongoing are phase II trials for multiple cancer types, and a phase I trial using intravenous delivery (other trials involve direct injection into tumors). Onyx is designing new viruses – viruses that will make prodrug-converting enzymes, and viruses targeted to receptors on tumor cells – in the hope that these viruses will have an even greater selective advantage. "The kind of results we are getting are as exciting as anything else in cancer therapy," says McCormick, "and we are just starting."

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