Viral Vectors Still Pack Surprises

Viruses may be lowly parasites, but their power to invade cells has won them a big part in gene therapy. Stripped of disease-causing elements, they work as natural syringes to inject DNA into human cells. Such "viral vectors" now dominate gene therapy: Nearly three-quarters of all protocols use them. Even so, researchers view their parasitic past with suspicion and worry about unforeseen problems in the clinic. The tamest viruses have produced surprises, as researchers using adeno-associated virus (AAV) learned recently.

In September, federal overseers asked Stanford University's Mark Kay to put "on hold" a clinical trial using an AAV vector to treat hemophilia B, an inherited blood disorder. The reason: Signs of AAV in the patient's semen raised a concern that gene therapy might have changed the man's inheritable DNA.

It's not unusual to detect traces of a vector after gene therapy, Kay says. But in this case, the signal persisted "at a low level" for weeks before it cleared, he says. Kay alerted the Recombinant DNA Advisory Committee (RAC), an oversight group at the National Institutes of Health (NIH), and the Food and Drug Administration (FDA). The FDA asked for a pause; the case will be discussed in the RAC on 5 December.

The RAC forbids any gene therapy that changes the "germ line"—eggs or sperm—either inadvertently or for genetic enhancement, because germ line mutations could be passed on to future generations. Kay already takes steps to prevent inadvertent alterations. His team informs patients that there is a small risk of germ line changes and, before therapy, offers to bank the sperm of male patients and asks them to use barrier contraception until their semen is clear of vector signal.

Kay doubts that germ line changes occurred in this hemophilia patient. Instead, he thinks the AAV signal probably came from typical "shedding" of vector seen in body fluids. But he hopes the RAC discussion will lead to a consensus on risk. "We're changing germ lines all the time in cancer therapy" with DNA-mutating chemotherapy—and that doesn't bother people, Kay notes. But he understands that gene therapy is "new territory." He favors guidelines that would allow these safety trials to continue if the probability of germ line alteration remains low.

Widely regarded as ultrasafe, AAV ran into another hurdle earlier this year. Although wild-type AAV infects many people, it doesn't seem to cause illness. But researchers got a scare last winter when mice that had been injected with an AAV vector developed liver tumors. This discovery prompted a short pause in two clinical trials using an AAV vector and an inquiry by U.S. health agencies in March. A joint review by FDA and RAC concluded that the AAV vector probably did not cause the mouse cancers. Clinical trials using AAV have resumed.

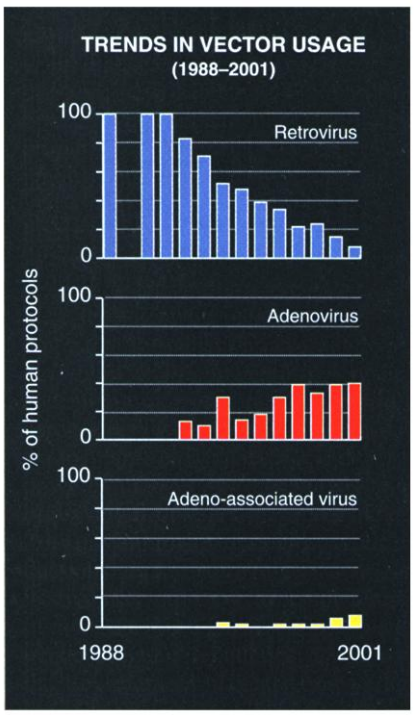
The cancer scare arose when molecular biologist Mark Sands of Washington University in St. Louis, Missouri, was reviewing data on mice in a gene therapy test. Sands is developing an AAV vector to treat people with inherited enzyme deficiencies, concentrating on a fatal disorder called mucopolysaccharidosis type VII, in which the body fails to process waste in lysosomes. Sands created knockout mice with this disorder and successfully treated them with AAV-vector gene therapy. But during a routine pathology review last year, he discovered that three of five mice sacrificed late in life—at 18 months, the human equivalent of 55-year-olds—had massive liver or blood vessel tumors. "It scared me. I had never seen tumors like this," says Sands, although he had used identical mice in many experiments—and this particular group of 59 had seemed tumor-free until the end of the study. On reexamination, three additional animals, the youngest sacrificed at 8 months, were found to have had tumors.

Sands was concerned that the AAV vector might have inserted new genes into the mouse DNA in a way that triggered cancerous growth. After reviewing the data, experts at a joint FDA-RAC meeting in March ruled out "insertional mutagenesis" as a cause of cancer. Sands agrees. But that does not rule out other possible vector-induced changes, Sands notes.

What actually caused the cancers remains unclear. Some panel members suggested that the knockout mice may have been prone to liver cancer. R. Jude Samulski of the University of North Carolina, Chapel Hill, a vector expert who took part in the RAC review, suggests that when these mice are cured of their inherited enzyme disorder, another genetic flaw may cause cancer in old age. But Sands hasn't seen evidence that the mice are prone to cancer. And it troubles him that other researchers have not allowed mice to live as long as he did for safety testing.

Although the scientific puzzle remains unsolved, Mark Kay and Terence Flotte, a gene therapist at the University of Florida, Gainesville, are confident that AAV vector can be used safely in gene therapy. The NIH and FDA, meanwhile, have asked Sands to do another mouse study to see if he can repeat the results. The research will require "hundreds" of animals, he says, and "years" to complete.

–ELIOT MARSHALL



Shifting focus. This sample illustrates the relative decline of retrovirus vectors (active only in dividing cells) and the rise of adenovirus and adeno-associated virus vectors (active in dividing and nondividing cells).

cholesterol and a synthetic lipid—also gave promising results in a phase II trial in patients with tumors that could not be surgically removed, a team from Valentis Inc. of Burlingame, California, reported at the ASGT annual meeting in June. The treatment kept cancer from spreading for more than 4 months when combined with traditional chemotherapy—38% longer than patients receiving chemotherapy alone.

Up-and-coming vectors

The number of clinical trials using nonviral vectors for gene therapy is growing (see table on p. 641), but many diseases can't be treated using the nonviral gene delivery methods that are farthest along. That's because most methods have delivered only low levels of active genes for short periods of time. Researchers are currently hammering out other approaches in the lab. They're trying to improve upon current vectors by finding ways to penetrate a higher percentage of cells in target tissues and make imported genes last longer once inside the cells.

Short-lived gene expression is fine for vaccines, cancer therapies, and angiogenesis. Indeed, Isner called it "a major-league safety advantage" for vascular gene therapy, because only temporary gene expression is needed to grow new vessels, and because in-