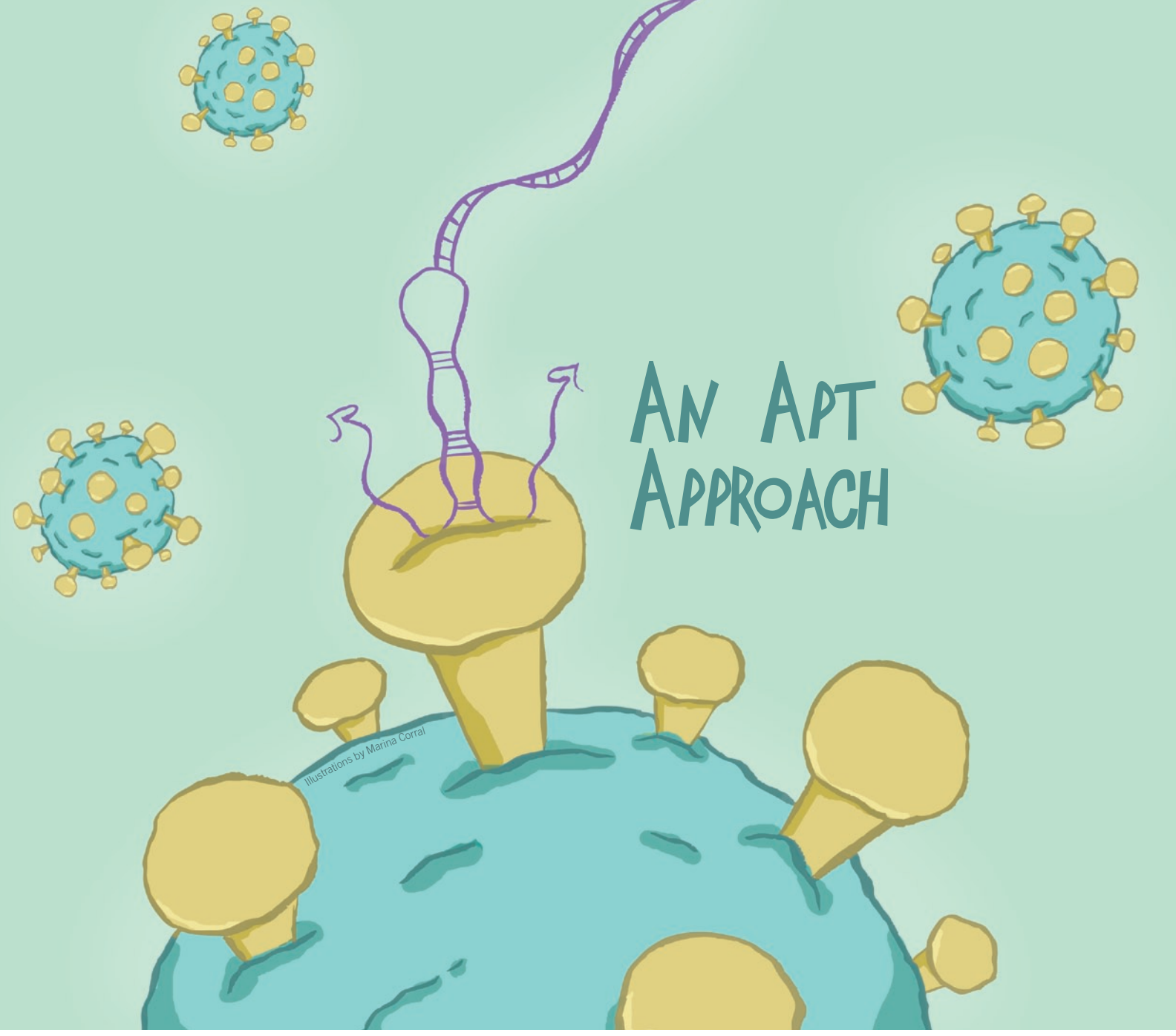


A new microbicide built from two types of RNA has shown promise against HIV in lab experiments, but moving it toward becoming a marketable product is proving difficult. **Alan Dove** investigates what's interfering with this novel therapeutic RNA strategy.

In December, newspaper headlines announced more disappointing news in the fight against AIDS. A international team of researchers reported that yet another microbicide failed to block transmission of HIV. In the largest international clinical trial to date, involving close to 10,000 women in four African countries, the PRO 2000 vaginal microbicide did no better than a placebo gel.

The news was the latest chapter in the dismal history of anti-HIV microbicides, a class of products that public health agencies have been clamoring for almost since the HIV epidemic began. It's been a tall order, and, with the latest failure, many researchers have given up on the idea of ever developing such a product.

But scientists may simply have gone about developing such medicines in the wrong way, according to Judy Lieberman, an immunologist at Harvard Medical School in Boston. PRO 2000, like most previous anti-HIV microbicides, was a polymer with nonspecific antiviral activity. Yet the real future of microbicides, Lieberman contends, lies in an entirely different direction: using specially designed RNA molecules to stop HIV's own RNA from being translated into new virus particles.

Lieberman's lab has designed short interfering RNA molecules—called siRNAs—that bind HIV's protein-coding RNAs and flag them for destruction. This process, known as RNA interference (RNAi), has worked beautifully in the laboratory to silence certain genes in cell culture, but getting the technique to work in the clinic has been harder. The siRNAs are unstable in the bloodstream and have a tough time getting inside cells.

That's why Lieberman is turning to another class of synthetic RNAs called aptamers that bind proteins on the cell surface. Developed independently by two American groups in the early 1990s, aptamers are artificial RNA

molecules that fold into a specific three-dimensional shape to fit together with a target protein. By attaching siRNAs to the aptamers, researchers hope to get their small RNA–based drugs where they want them and have them stick there.

So far, though, biotech companies have been skittish to embrace this approach. "It's impossible to get any commercial interest in doing this," says Lieberman. She acknowledges that the combination of two untested technologies, RNAi and microbicides, and a hard target, HIV, might seem like a foolhardy research venture, but her preclinical results already look more promising than those of earlier microbicides.

Indeed, it would be a bad idea to bet against Lieberman. Throughout her 30-year scientific career, she has had a knack for being in the right place at the right time—starting when she was a physicist at Rockefeller University and the Institute for Advanced Study in Princeton, New Jersey, where she participated in the unified theory of weak and electromagnetic forces, through to setting up her own lab, which was the first to show that RNAi could be used to protect model animals from disease. "All these fields that took off, I was part of," she says, "and it has been really exciting."

> "We've seen several examples where the aptamer has worked a lot better than the antibody."
> —Paloma Giangrande

A complex trajectory

Contributing to the Standard Model of particle physics—which describes particle interactions—is an uncommon accomplishment for a biomedical researcher, but Lieberman followed an uncommon career path. After receiving her doctorate in theoretical physics and completing two short postdoc positions in the field, she decided that physics research was not for her. In 1975, a friend suggested she consider medical school, and Lieberman liked the idea. "So I decided that I would do that," she recalls. "I would go to med school and become a clinician and never do research again."

That didn't go quite as planned. During her medical internship at Tufts Medical Center in Boston, she saw some of the first people with a mysterious new disease called Acquired Immune Deficiency Syndrome, and Lieberman soon found herself immersed in AIDS research. She did a research rotation with Herman Eisen at the Massachusetts Institute of Technology in Cambridge, just as his lab was cloning the first T cell receptors. Lieberman knew that transfusing T cells could cure mice of virally induced cancer, so she decided to try the same approach with HIV.

The clinical trials with the T cells didn't pan out, so in the 2000s Lieberman turned to another newly discovered form of therapy: RNAi. In 2003, Lieberman reported that injecting siRNAs prevented livery injury in mice with hepatitis—the first evidence that the technology could treat disease in a living organism (*Nat. Med.* **9**, 347–351, 2003).

But similar techniques have failed clinically. Researchers have tried using antibodies, viral vectors, liposomes and other modifications to deliver therapeutic siRNAs in animal models of a range of disorders, including cancer and infectious diseases, but the results have been inconsistent. Although these techniques seemed to deliver siRNAs to some types of cells, they didn't work for others. "Just because you can get things to receptors that are on cell surfaces doesn't mean that you're going to get productive siRNA knockdown," says Matthew Levy, a biochemist at Albert Einstein College of Medicine in New York.

Now, many say the brightest hopes on the RNAi horizon are aptamers. These single-stranded RNA structures are created entirely in a test tube, so the search for aptamers avoids some of the limitations of finding the right antibodies—immune proteins that also bind structures on the surfaces of cells—to attach to siRNAs. For example, generating antibodies against some highly conserved mammalian proteins is tricky, because the immune system often can't tell the difference between the foreign protein and the body's own version. Generating aptamers against these same targets, however, is relatively straightforward, and no natural equivalent exists to confound therapeutic applications.

What's more, aptamers often have higher affinities for proteins than do the corresponding antibodies. "They turn out to be really great for targeting, and they turn out to be good alternatives to antibodies in general," Levy says.

To produce aptamers, researchers first create a library of short, random sequences and then expose them to the target bits of protein to identify the RNA sequences that bind the protein best. Repeated rounds of the process—known as *in vitro* selection or systematic evolution of ligands by exponential enrichment (SELEX)—can yield aptamers with improved affinities for their targets.

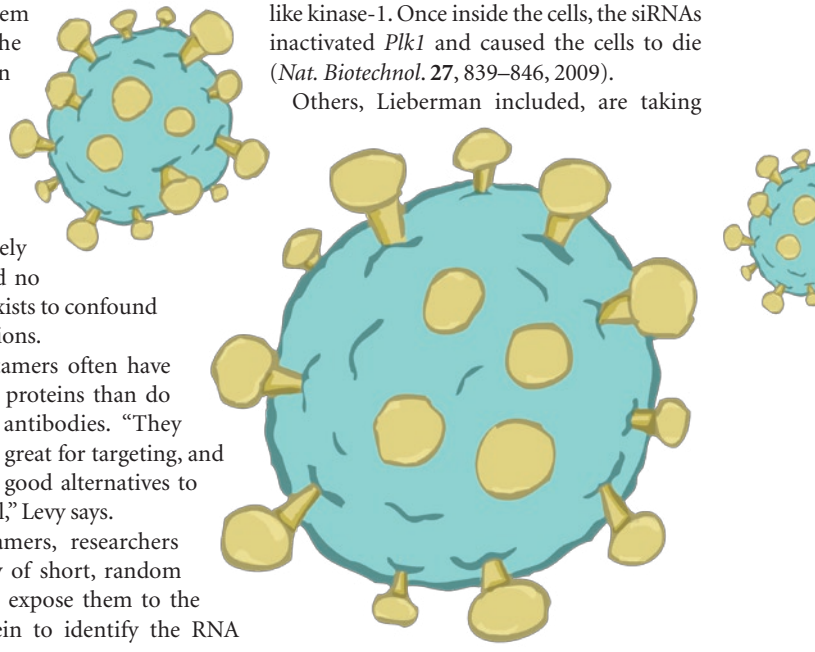
Unmodified aptamers, however, are unstable in the bloodstream, with half-lives usually measured in minutes. This led some to question their clinical utility, but researchers have recently discovered chemical modifications that can stabilize aptamers, and many scientists are now bullish about the technology's therapeutic prospects. "We've seen several examples where the aptamer has worked a lot better than the antibody" at binding specific targets, says Paloma Giangrande, an RNA researcher at the University of Iowa in Iowa City. "[Aptamers] are easily synthesized versus antibodies, and their shelf life is also longer and more stable."

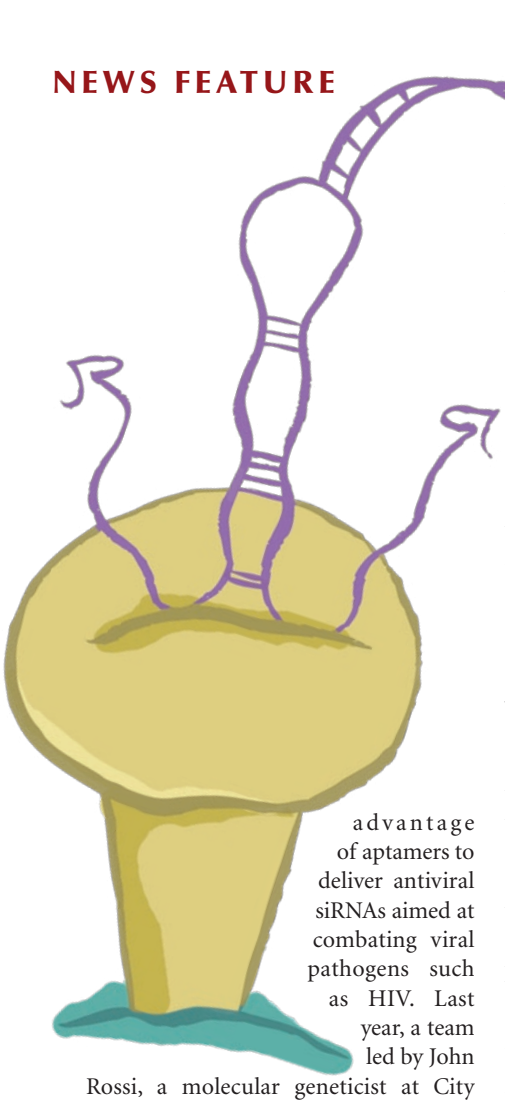
It takes two

With siRNAs and aptamers maturing technologically, it seemed natural to combine the two techniques. Because the building blocks for both are RNA molecules, linked aptamer-siRNA complexes are relatively easy to manufacture, and, at least in principle, the combination can shut down virtually any gene in any cell.

For example, Giangrande and her colleagues developed an all-RNA aptamer-siRNA drug to kill prostate tumors in mice and in human cell cultures. Her team combined an aptamer aimed at the prostate-specific membrane antigen—a surface protein expressed on normal and cancerous prostate cells—with an siRNA matching a prostate cancer–specific prosurvival gene called *Plk1*, encoding polo-like kinase-1. Once inside the cells, the siRNAs inactivated *Plk1* and caused the cells to die (*Nat. Biotechnol.* **27**, 839–846, 2009).

Others, Lieberman included, are taking





advantage of aptamers to deliver antiviral siRNAs aimed at combating viral pathogens such as HIV. Last year, a team led by John Rossi, a molecular geneticist at City of Hope Comprehensive Cancer Center in Duarte, California, and Ramesh Akkina, a microbiologist at Colorado State University in Fort Collins, pioneered this tactic with aptamer-siRNA that targeted a surface protein on the outside of HIV cells called gp120 (*Nucleic Acids Res.* **37**, 3094–3109, 2009). Akkina likens the system to a molecular guided missile, with the aptamer directing the molecule to the infected cell and the siRNA delivering the gene-silencing payload that kills the virus.

Since proving the concept in cultured cells, Akkina and Rossi have moved the work into mice. Their preliminary unpublished data show that the aptamer-siRNA duos can lower the HIV viral load in a mouse model of the disease. Akkina concedes that reducing the viral load is not the same as eliminating the virus, but for people with HIV it may be enough to keep the virus in check. "If you can keep the viral load down, then you basically are doing an effective immune reconstitution so that the individual can lead a pretty much normal life," he says.

Like Rossi and Akkina, most researchers in this small burgeoning field have focused on silencing viral genes inside cells that are already infected. But Lieberman is taking a prophylactic approach. To protect the cells in greatest danger, she uses aptamers directed toward the cell surface receptor that HIV uses to gain entry into T cells. The aptamer-siRNA molecules bind the receptor and get taken into the cell just like the virus.

These aptamers carry two types of siRNAs: one to silence essential HIV genes, the other to quell host genes the virus needs. In this way, when invading virus particles arrive at a treated cell, they find the drawbridge up and crocodiles in the moat. "I think we were the only group that realized that you might want to target both viral genes and host genes that were important in HIV," says Lieberman.

Tracey Schaal

Running interference: Judy Lieberman

Silence is golden

Lieberman's latest work builds on an earlier strategy the lab developed to prevent herpes virus infection, in which her team also used siRNAs against viral and host genes but attached them to cholesterol molecules rather than to aptamers. The cholesterol carried the siRNAs into cells in the vaginal epithelia of mice, protecting them against subsequent challenge with a vaginally delivered dose of the herpes virus (*Cell Host Microbe* **5**, 84–94, 2009). "It had no toxicity, and we got durable protection," Lieberman says.

But the cholesterol delivery approach didn't deliver siRNAs to the immune cells targeted by HIV—possibly because those cells are generally more selective in terms of the molecules they take up—so Lieberman turned to aptamers. In unpublished work, her team has found that aptamer-siRNAs target T cells in cultured vaginal tissue and render them highly resistant to HIV. Lieberman hopes to start mouse and monkey trials of the HIV microbicide soon.

If the strategy works as well in humans as it has in preclinical experiments, it could form the basis for a new microbicide that a woman could apply hours or even days before having sex, thereby conferring substantial protection against sexually transmitted disease. That's a promising sales pitch for public health agencies, but investors are likely to remain leery. Aside from the miserable track record of microbicides, the first aptamer-based drug on the market was also a flop.

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> —Judy Lieberman

In 2004, the US Food and Drug Administration approved Macugen as an aptamer–based treatment for age-related macular degeneration, and, for a brief period, the drug (which does not include siRNA) enjoyed considerable success. But, two years later, an antibody–based therapy for the disease, called Lucentis, arrived and quickly took over the market; in its first three months Lucentis raked in $153 million in sales, whereas Macugen sales slumped to $9 million for the same period. Unsure of why Macugen performed worse in clinical trials, many investors have since steered clear of aptamers altogether.

Even so, a few companies are still betting on aptamer drugs. Cambridge, Massachusetts–based Archemix is the technology's lead proponent, with an aptamer to treat a blood-clotting disease called thrombotic microangiopathy currently in phase 2 trials. Archemix's results may look promising, concedes George Farmer, a biotechnology analyst at Canaccord Adams in New York, but he says that most investors will probably still wait for more definitive proof that small RNA molecules really work in the clinic.

"All this stuff is still kind of a great idea, but I don't really see anything materializing just yet," Farmer says. "It works great in a Petri dish, but I don't know if it's going to work so great systemically."

Lieberman remains upbeat, though, both about her microbicide strategy and the future of HIV treatment with aptamer-siRNAs. "HIV is not that infectious an agent," Lieberman says. "It takes several hundred encounters to become infected, and if you are able to treat at the population level, you can block infection. So, from that point of view, I'm optimistic."

Alan Dove is a freelance science writer based in Springfield, Massachusetts.