Interfering with HIV

By Lev Osherovich, Senior Writer

A team of Korean and American researchers has shown in a humanized mouse model that small interfering RNA–based therapeutics could be harnessed to treat HIV.[1] However, because administering these therapeutics would require repeated i.v. dosing, the approach may only be applicable to multidrug-resistant patients. Thus, companies will need to be convinced that the approach is worth developing for this relatively small sector of the HIV market.

The study, published in *Cell*, was led by Sang-Kyung Lee, assistant professor at **Hanyang University**, and Premlata Shankar, who was an assistant professor of pediatrics at **Harvard Medical School** when the work was conducted. Shankar is now a professor of biomedical sciences at **Texas Tech University**.

The mouse-based study showed that siRNAs linked to a T cell–specific antibody can be delivered systemically to T cells *in vivo*. This was previously considered challenging because these primary targets of HIV don't readily internalize extracellular RNAs. The work thus lays out a general approach to targeting leukocytes with siRNA that could be useful for treating not only HIV but also other blood-borne viruses and lymphatic cancers.

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> ***—Judy Lieberman, Harvard Medical School***

"I think this is a very elegant study that shows biological efficacy for siRNAs and a method that might be useful for other diseases," said Judy Lieberman, professor of pediatrics at Harvard Medical School. Lieberman was not an author on the paper, but she pioneered several of the techniques used in the study.[2]

"There's nothing dramatically new about the methodology, though getting it to work for HIV is a tour de force," she said.

Lieberman noted that the cocktail of siRNAs used in the study showed some efficacy in blocking initial infection. This suggests that siRNA could potentially be used as a prophylactic against chronic infection after initial exposure but before a reservoir of virally infected quiescent cells takes hold. However, this might be a hard sell because marketed combination drugs have shown efficacy in the postexposure prophylaxis setting.[3]

Moving target

siRNAs can be used to knock down mRNAs encoded by disease-associated viral or host genes. But there are only a handful of clinical trials of siRNA-based therapies, most of which involve local delivery of therapeutics to RNA-friendly tissues such as the retina.[4]

"For a clinical therapy for HIV, the challenge is to get the siRNA to the right cells," which are the T cells, said Priti Kumar, an instructor at Harvard Medical School's Immune Disease Institute and the study's lead author. "The other question is how to get the T cells to take up" the siRNA.

The team overcame both challenges with a delivery vehicle consisting of a T cell–targeting antibody fragment and an RNA-binding oligopeptide. The antibody fragment recognized CD7, a T cell–specific surface receptor that is internalized by the T cells upon antibody binding.[5] The RNA-binding oligopeptide consisted of nine arginine residues that covalently linked the siRNA moieties to the antibody.[6]

This targeting complex was loaded with a cocktail of siRNAs targeting two HIV genes encoding the viral replication proteins Vif and Tat, as well as targeting the gene encoding host cell receptor CC chemokine receptor 5, which is required for viral entry.

As designed, binding of the antibody-siRNA complex to CD7 would trigger endocytosis, delivering the siRNA to the interior of the T cells. During subsequent endosomal processing of the internalized antibody, the siRNA would escape into the cytoplasm and disrupt viral and host mRNAs.

Lieberman noted that similar antibody-based vectors have been used to deliver siRNAs to brain cells and tumors in preclinical studies. Therapeutics based on this approach are in preclinical development by **Alnylam Pharmaceuticals Inc.**, which has licensed the siRNA delivery technology developed by Lieberman.

To test their siRNA cocktail, the team used a mouse strain with transplanted human T cells that had been infected with HIV.

They found that injections of the siRNAs knocked down both virus and host gene expression in T cells for about a week, improved T cell survival and lowered plasma viral load compared with what was seen in untreated controls.

The team then worked out a dosing regimen to treat chronic persistent HIV infection. Repeated doses of the siRNA cocktail every 4–5 days suppressed viral titer and preserved high T cell levels compared with what was seen in mock-treated controls for the duration of the 40-day experiment.

"The treatment reduced viremia in infected mice to practically zero," said Kumar. She also noted that the siRNA cocktail was effective even in mice with HIV-positive T cells from drug-resistant humans.

John Rossi, professor of medicine at **City of Hope National Medical Center**, thinks the key advance in the paper is the use of a humanized HIV mouse model to prove the potential of targeting T cells *in vivo* for HIV therapy. Rossi is a cofounder of siRNA company **Dicerna Pharmaceuticals Inc.**

Antibody-based siRNA delivery to cultured T cells has been shown before, said Rossi. "What's important is that they can put it together in a living animal to prevent and treat HIV."

At City of Hope, Rossi is planning a Phase I trial of an siRNA-based

gene therapy for HIV. His method uses a lentivirus-based transgene that expresses small hairpin RNAs and hairpin ribozymes to target the same viral and host factors as the *Cell* study.

The technique, developed in partnership with **Benitec Ltd.**, involves extracting T cells from HIV patients, transfecting them with the therapeutic transgene *in vitro* and putting them back into the patient's circulation.

"There's an unmet medical need in salvage patients who are resistant to multiple drugs. Here, they are targeting multiple cellular and viral targets—you can multiplex targets in one treatment."

—Milind Desphande, Achillion Pharmaceuticals Inc.

Resistance is useless

Kumar thinks targeting multiple viral and host genes with siRNA minimizes the chances of viral escape through mutation. Resistance to small molecule HIV drugs arises through the accumulation of point mutations in the viral genome, setting a limit on how long any given combination of drugs can be used by each patient. In contrast, siRNA therapeutics are processed by the cellular machinery into a multitude of small RNA strands that bind to too many target sites for resistance to take hold.

"We chose targets in the HIV genome that are conserved and are very important" for viral survival and thus are not likely to mutate quickly enough to evade destruction by siRNA, Kumar said.

Milind Desphande, CSO and EVP of research at infectious disease company **Achillion Pharmaceuticals Inc.**, told *SciBX* that salvage therapy would be the natural market for siRNA HIV therapeutics.

"There's an unmet medical need in salvage patients who are resistant to multiple drugs," said Desphande. "Here, they are targeting multiple cellular and viral targets—you can multiplex targets in one treatment."

Achillion's lead compound is elvucitabine, a nucleoside analog viral polymerase inhibitor that the company said has higher potency and longer plasma half-life than comparable marketed molecules. Elvucitabine recently completed Phase II trials and the company hopes to partner the molecule by year end.

Desphande did caution that even if the finds in the *Cell* study translate to humans, there would be significant challenges to marketing an siRNA-based HIV therapeutic. A key issue would be the need for weekly i.v. dosing.

A case in point is Fuzeon enfuvirtide, an injectable HIV viral fusion inhibitor from **Trimeris Inc.** and **Roche** that was approved in 2003. The drug has faced many problems, including initial manufacturing difficulties and being an injectable in a field dominated by oral drugs.[7] In contrast, orally administered fusion and entry blockers like Selzentry maraviroc from **Pfizer Inc.** and Isentress raltegravir from **Merck & Co. Inc.** have met with greater success.

Moreover, the number of multidrug-resistant salvage patients in the U.S. is relatively small—about 90,000 out of a total HIV-positive population of 1.1 million, according to Desphande.

Perhaps because of these issues, few companies have shown enthusiasm for siRNA-based HIV therapeutics, according to Lieberman. "It's very challenging to find commercial partners in this indication," she said.

Instead, the immediate commercial significance of the *Cell* paper may be in validating the general approach of targeting cellular receptors to deliver siRNA. If the right antibody fragments can be designed, variants on the technique could be used to deliver siRNA cocktails against other blood-borne viruses and lymphatic cancers.

"This is an important paper that very nicely demonstrates the ability of targeted delivery of siRNA to have a profound impact on disease," said Antonin de Fougerolles, VP of research, immunology, metabolic and viral disease at Alnylam.

According to de Fougerolles, the promise of the paper "lies in the ability to selectively deliver an RNAi therapeutic that can be designed against any gene in the human or viral genome to cell types which are critical to human disease pathology."

Alnylam's ALN-RSV01 is an siRNA therapeutic in Phase II trials to treat respiratory syncytial virus (RSV) infection. The compound is partnered with **Kyowa Hakko Kogyo Ltd.**

Kumar told *SciBX* that her next steps will be to adapt the CD7 antibody fragment for use in humans and to improve the efficiency of drug delivery by packaging the antibody-siRNA complex into liposomes or nanoparticles.

Harvard Medical School has filed patents for the methodology and composition of matter described in this study. The technology is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY), Cambridge, Mass.
Benitec Ltd. (ASX:BLT), Hawthorne East, Australia
City of Hope National Medical Center, Duarte, Calif.
Dicerna Pharmaceuticals Inc., Boston, Mass.
Hanyang University, Seoul, South Korea
Harvard Medical School, Boston, Mass.
Kyowa Hakko Kogyo Co. Ltd. (Tokyo:4151), Tokyo, Japan
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Roche (SWX:ROG), Basel, Switzerland
Texas Tech University, El Paso, Texas
Trimeris Inc. (NASDAQ:TRMS), Morrisville, N.C.