Flushing out HIV

By Lev Osherovich, Senior Writer

Highly active antiretroviral therapy, or HAART, keeps HIV in check for many patients. But it doesn't fully eliminate the virus, which can lie dormant in CD4+ T helper cells, making the therapeutic cocktail a life-long necessity. A team at **The Johns Hopkins University School of Medicine** now has identified a compound that reactivates latent HIV, potentially providing a way to completely eliminate the virus.[1] However, toxicity issues mean further translational work will be necessary before the finding can be advanced into the clinic.

As part of its life cycle, HIV integrates into the genome of infected CD4+ cells. Most infected cells weaken and die as the virus hijacks their metabolic machinery. However, a small fraction of infected cells manage to subdue viral replication and instead enter a resting state, sequestered in the lymph nodes.

It is these dormant memory T cells that become the perpetual reservoir of the virus. These memory cells can occasionally be reactivated, and if a patient has stopped taking HAART, the resurgent cells can resume production of viral particles.

Thus, patients must take HAART for the rest of their lives.

"The latent virus, in the form of viral DNA, integrates into the host genome," said Robert Siliciano, professor of medicine at Johns Hopkins and lead author on the *Journal of Clinical Investigation* paper. "In that form it can't be seen by the immune system or by drugs, which target active steps in replication."

Siliciano estimates that about one in a million T cells in a patient on HAART is latently infected. In these dormant cells, the latent virus is transcriptionally silent and "there's essentially no way to activate it besides activating the host cells," he said.

David Margolis, professor of medicine at **The University of North Carolina at Chapel Hill**, also has been addressing HIV latency. He noted that the expense and side effects of long-term HAART use, as well as the eventual risk of drug-resistant strains, make it desirable for researchers to find a way to fully eliminate HIV infection.

Margolis, together with researchers at **Merck & Co. Inc.** and **Johnson & Johnson**'s Tibotec unit, is part of an academic-industry consortium that aims to cure HIV infection.[2]

"In light of the failures of vaccine trials and the realization that millions of people will require chronic therapy," eradication is an attractive goal, he said. The challenge is to figure out how to reactivate the virus "without doing too much damage to the host."

Releasing HIV

One way to reactivate latent HIV would be to directly stimulate the dormant T cells in which it is embedded. But widespread T cell activation could cause undesirable immunological complications, so the challenge is to selectively induce HIV activation without generally inducing T cell proliferation.

In their study, Siliciano's team looked for compounds that did just this. They developed a method of culturing latently infected cells *in vitro* without activating them and then screened for small molecules that reactivated the virus without inducing cell proliferation.

First, the team transfected primary human CD4+ T cells with an antiapoptotic gene that extended their survival in cell culture in a quiescent state resembling resting memory T cells.

The team then infected the cells with a crippled version of HIV

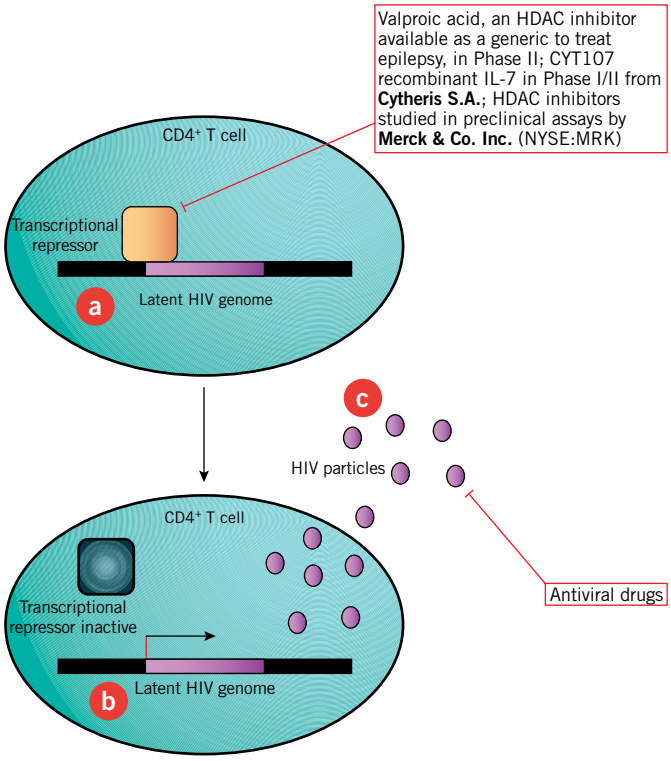

Figure 1. Flushing out HIV. Yang *et al.* report a screening method to identify compounds that help clear latent HIV infections. The idea is to turn on expression of HIV that has integrated in the genomes of CD4+ T helper cells. Ordinarily, HIV expression is blocked by transcriptional repressors [**a**]. Inhibition of these repressors leads to production of HIV transcripts and viral particles [**b**], which can then be killed by conventional antivirals [**c**].

Compounds that could reactivate latent HIV include histone deacetylase (HDAC) inhibitors and IL-7. The former are thought to inhibit transcriptional repressors that prevent HIV gene expression, and the latter promotes CD4+ T cell proliferation, which induces expression of latent HIV. Selected compounds tested to treat HIV are listed.

that did not readily replicate but underwent normal integration and transcriptional silencing. The engineered HIV strain also contained a *green fluorescent protein* (*GFP*) transgene, allowing the researchers to visually detect viral activation.

Siliciano's team found that after two months in cell culture, a small fraction of the T cells stopped making GFP, indicating that the virus had become quiescent.

The researchers then treated these latently infected cells with a library of 4,400 drugs and biologically active compounds, looking for reactivation of GFP expression. They found 17 compounds that induced high expression of GFP compared with that in untreated controls, indicating that the virus had awakened.

One compound—5-hydroxynaphthalene-1,4-dione (5HN), a quinone found in black walnut trees—was especially potent at activating HIV expression without directly turning on T cells. In cell culture, 5HN did not promote T cell activation as measured by cell growth and cytokine production.

Siliciano's next goal is to better understand the mechanism of action of 5HN, which may involve activation of NF-κB, and to scale-up the cell culture methods for further screening.

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>
> ***—Rafick-Pierre Sékaly, Vaccine and Gene Therapy Institute at the Florida Center for Innovation***

High hurdles

Although the precise mechanism is not yet clear, 5HN and other compounds identified by Siliciano's team appear to trigger expression of latent HIV genes (*see* **Figure 1, "Flushing out HIV"**). However, deploying these compounds in the clinic leads to concerns about toxicity and specificity.

Margolis said the *JCI* study "is an advance toward activating virus expression," which is complementary to his own team's efforts to eliminate latent virus by antagonizing repression of viral genes. Margolis and collaborators at Merck recently conducted cell culture studies of the company's histone deacetylase (HDAC) inhibitors as antagonists of HIV latency.[3]

"It's unclear what the pathway of activation by 5HN is," said Margolis. "Though exposure to the compound doesn't overtly induce activation of the cells, it's certain that some changes go on."

Margolis and colleagues at Chapel Hill are running Phase II trials of the nonselective HDAC inhibitor valproic acid in combination with conventional antiretrovirals to treat HIV. Valproic acid is available as a generic for psychiatric indications and epilepsy.

These approaches are "very novel but certainly not the only pathway" to reactivating latent HIV, said Rafick-Pierre Sékaly, co-director and scientific director of the **Vaccine and Gene Therapy Institute at the Florida Center for Innovation**.

Sékaly also cautioned that 5HN and HDAC inhibitors could have undesirable effects besides activating latent HIV.

"These molecules are hitting some very broad pathways involved in chromosome remodeling," said Sékaly. "Specifically targeting the infected cells is a challenge."

Earlier this year, Sékaly's team reported on the role of IL-7 in maintaining the HIV reservoir.[4]

Sékaly noted that the lack of a good animal model for HIV latency and the difficulty in scaling-up cultures of latently infected cells have hindered progress in the field.

Michel Morre, president and CEO of **Cytheris S.A.**, thinks the Siliciano team's work could potentially identify targets for achieving HIV eradication but said the compounds in the study aren't suitable for the clinic.

"I would not consider those molecules as drug candidates," said Morre. "Some of these compounds are extremely toxic." He added that he had concerns "about activating the expression of other genes, including oncogenes."

Morre said that biologics like Cytheris' CYT107 could be a safer alternative. The recombinant IL-7, which stimulates T cell proliferation, is in Phase I/IIa studies as an adjuvant to HAART to treat HIV.

"Our data show that we can use CYT107 to wake up latent HIV, but we haven't yet looked at the effect on the reservoir," he said.

Morre said Cytheris is planning a trial of CYT107 in combination with HAART to eradicate HIV infection.

Siliciano acknowledged that 5HN and other hits from his screen are "probably too toxic to use in humans" but said they could be the basis for next-generation molecules.

Meanwhile, Merck is sitting on the fence until additional mechanistic studies are done.

"It is still too early to know whether the approach of activating the expression of HIV in latently infected cells in the presence of intensified HAART could be part of a therapeutic regimen aimed at clearing the virus from an infected patient," said Amy Espeseth, director of vaccines and biologics in external basic research at Merck Research Labs.

According to Siliciano, no patents have been sought on the molecules or the screening platform.

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REFERENCES

1. Yang, H.-C. *et al. J. Clin. Invest.*; published online Oct. 1, 2009; doi:10.1172/JCI39199
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2. Richman, D.D. *et al. Science* **323**, 1304–1307 (2009)
3. Archin, N.M. *et al. AIDS* **23**, 1799–1806 (2009)
4. Chomont, N. *et al. Nat. Med*. **15**, 893–900 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

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