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>
> Anthony Fauci, NIAID

TARGETS & MECHANISMS

RETHINKING THE RESERVOIR

By Selina Koch, Staff Writer

It's time to rethink using "shock and kill" in HIV - a strategy that has made no serious progress in the last 16 years towards its goal of getting patients permanently off antiretroviral therapy (ART) - according to a group at The Scripps Research Institute. Rather than trying to flush out the last traces of infection from all the viral reservoirs, the researchers believe a better approach might be to put the latent virus to sleep permanently.

Using cells from HIV patients treated with ART, a team led by Susana Valente showed that by inhibiting the transcription factor HIV tat, it could suppress viral replication and prevent viral rebound after withdrawal of ART.

"We are targeting suppression of the virus to such a point that no virus will be expressed in cells," said Valente, an associate professor in the Department of Immunology and Microbial Sciences at Scripps.

Despite the success of ART in suppressing HIV to the point where no virus is detectable in the blood, the virus inevitably rebounds within just a few weeks of withdrawing treatment. And while many infected patients have lived for decades on the therapy, researchers are still trying to find a way to eliminate the virus completely because lifelong treatment is costly, makes patient adherence more difficult, and results in accumulating toxicities, said Anthony Fauci, director of NIH's National Institute of Allergy and Infectious Diseases (NIAID).

"The trouble is 'shock and kill' has been totally unsuccessful thus far, no matter what method we've tried," Fauci told BioCentury. "I think suppressing the viral reservoir and not allowing it to reactivate is a fresh new approach that should be pursued."

Fauci said the concept behind "shock and kill" has been to pair a chemical agent that can induce viral reactivation in quiescent cells with ART to prevent the newly generated viruses from infecting other cells, "and hope that the cells that are spitting out the virus get sick and die or are eliminated by an immune response."

But two key issues have prevented "shock and kill" from working, he said.

First, the existing methods of stimulating viral transcription only reactivate a small fraction of the reservoir, leaving most latently infected T cells untouched.

Second, HIV patients often have compromised immune systems that fail to attack T cells in which the virus has been reactivated. Instead, he said, "the cells spit out some virus and then go back into their latent state."

Andrew Rice, a professor in the Department of Molecular Virology & Microbiology at Baylor College of Medicine, told BioCentury that because of the low efficiency rates of reactivation and elimination, the strategy could only work with multiple rounds of "shocking" to empty all of the reservoir, and that treatments would likely need to be paired with an immunotherapy to boost immune-mediated clearance of the infected cells.

The reason ART can't eradicate the virus is that HIV inserts itself into the genome of the host cell and remains there for as long as the cell is alive.

While ARTs are very good at inhibiting certain viral functions, including entry into cells and reverse transcription, Valente said, "once the virus is in the host gene there are no drugs that have any ability to eliminate it; the viral genome becomes like a cellular gene."

She and her colleagues reasoned it might make more sense to drive the latent virus underground permanently by blocking the ability of tat to ramp up DNA replication — a virtually essential step in reactivating the dormant virus.

In a study published in July in the American Society for Microbiology's journal *mBio*, the team used a derivative of the steroidal alkaloid cortistatin to inhibit tat, and showed the compound could suppress both viral replication and rebound after ART removal. (See Distillery, page 18)

"Cortistatin offers a really nice combination of targeting a viral protein that targets viral RNA, is not related to a human protein, and is one that the virus depends on," said Valente.

TAT-TLE TALE

Valente told BioCentury that the team targeted tat because the protein is a key transcriptional regulator that acts at an early stage in the process, and without it, Pol II-mediated transcription is "incredibly inefficient."

She said tat acts after integration of the viral genome into host DNA to jump-start viral transcription by recruiting a protein complex to the viral promoter that activates Pol II and releases inhibition from negative elongation factors. Tat also brings in histone acetylases to allow unwinding of the chromatin, increasing elongation efficiency. According to Valente, the tat-driven process is a feedback loop that "amplifies transcription by several logs."

To suppress the transcription factor, the group used didehydro-cortistatin A (dCA) — a cortistatin analog the researchers had previously shown binds tat and inhibits tat-induced transcription from the viral promoter.

In a cell-based model of HIV infection, dCA suppressed viral replication below detectable levels by day 82. When treatment was discontinued on day 103, viral replication did not restart during four months of subsequent monitoring.

In addition, the team tested the effect of dCA on viral rebound after ART withdrawal, using T cells harboring latent virus from HIV patients on ART.

First, the researchers incubated the cells *in vitro* with an antiretroviral cocktail containing Sustiva efavirenz, Retrovir zidovudine and Isentress raltegravir for 22 days, and then withdrew the treatment. Within six days, the virus was reactivated.

When they incubated cells with dCA in addition to the cocktail, the subsequent viral rebound was reduced by 93.5% and 93.1% in cells from the two patients respectively, compared with the rebound seen in the absence of dCA.

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Merck & Co. Inc. markets the HIV integrase inhibitor Isentress and the non-nucleoside reverse transcriptase inhibitor (NNRTI) Sustiva. GlaxoSmithKline plc markets the nucleoside reverse transcriptase inhibitor (NRTI) Retrovir.

Valente's group then tried to chemically induce viral replication in the quiescent patient cells using the PKC activator prostratin, which is commonly used to kick-start viral transcription in "shock and kill" experiments. However, cells treated with the ART cocktail plus dCA showed 99.9% less prostratin-mediated reactivation than cells that had received only the HIV drugs. That suggested viral transcription had been fully suppressed and had become refractory to viral rebound even in the face of a strong inducing stimulus.

Valente thinks the persistent viral suppression that occurs after dCA is removed is likely mediated by epigenetic mechanisms.

"What we think is happening is that the HIV promoter accumulates very strong repressive epigenetic marks in the presence of this drug that are somewhat different than what you would get under normal latency and so we don't see viral rebound," she said.

While the group didn't find changes in methylation at the viral promoter in the study, she said the researchers are investigating other epigenetic modifications such as citrullination to get a handle on the molecular mechanisms by which dCA persistently suppresses viral transcription.

ALL OR SOME

Although Fauci noted that it's early days, he told BioCentury that given "shock and kill" is not working out, "the opposite approach is worth pursuing," and the study is based on good logic.

"There's no doubt that is a good *in vitro* study," said Fauci. "The real question is whether this will be translatable *in vivo*."

Paul Wender, a professor of chemistry at Stanford University, told BioCentury that although the "shock and kill" strategy

"clearly has the head start" and will remain an active area of research, he thinks "viral silencing is an approach that merits attention."

Wender, whose lab developed the synthetic method for prostratin, said his lab and others have since developed reactivating agents that are more potent than prostratin and he would like to see if dCA could suppress viral rebound in the face of those.

He also wants to see how effective the compound is *in vivo*, and said that in order for the approach to work, dCA would have to prevent 100% of the reservoir from reactivating after ART is discontinued.

"They're not yet in the position where they can say this is permanent or that it eliminates reactivation," said Wender. "Nearly permanent silencing just isn't going to be good enough" because the infection could return.

Wender also noted that the half-life of $CD4^+$ T cells is about 44 months. At that rate, he said, and assuming even a modest reservoir size of one million cells, it would take about 70 years to eliminate the reservoir in most patients on ART.

Valente told BioCentury that her lab is synthesizing dCA analogs to optimize the compound's effects and safety but that the team will take dCA forward into proof-of-concept experiments in animals. She said her group has already begun studies in humanized mice and hopes to start experiments in non-human primates next year.

In addition, she noted that a recently publicized case of a French teenage patient suggests that it might not be necessary to completely eliminate or suppress the reservoir to achieve a functional cure.

The girl, who is now 18, was infected from birth and was treated immediately and aggressively with ART until she was six years old but was then lost to follow-up and has been off ART for the past 12 years without significant viral rebound. "She still has the virus in her," said Valente, "but she has no detectable viremia."

What's of particular interest in that case, said Valente, is that the girl tried stopping ART twice during her first six years and each time the virus rebounded, indicating that she was not an "elite controller". Elite controllers represent a small subset of patients who are genetically predisposed to be able to control HIV infection without drugs. Despite the initial inability to control her infection, Valente said six years of ART made the girl competent to suppress her infection on her own, presumably because her reservoir had shrunk over time as the latently infected cells died.

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Rice agreed that reaching every infected cell might not be necessary, and noted that theoretical estimates suggest about 90% of cells would need to be eliminated or suppressed. He also noted that the French girl started treatment earlier than most patients and may have had a smaller reservoir, suggesting it could take longer to repeat the result in other patients.

"Maybe a viral suppressor like this HIV tat inhibitor could drive a lot of the reservoir into some kind of deep latency so that you wouldn't have to reduce reservoir size to such an extent," Rice said. If so, "the tat inhibitor approach has the potential to contribute to a functional cure for HIV infection."

COMPANIES AND INSTITUTIONS MENTIONED

American Society for Microbiology, Washington, D.C.
Baylor College of Medicine, Houston, Texas
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Merck & Co. Inc. (NYSE:MRK), Kenilworth, N.J.
National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Md.
National Institutes of Health (NIH), Bethesda, Md.
The Scripps Research Institute, La Jolla, Calif.
Stanford University, Stanford, Calif.

TARGETS AND COMPOUNDS

HIV tat - HIV tat protein
PKC - Protein kinase C
Pol II - RNA polymerase II

REFERENCES

Mousseau, G., et al. "The tat inhibitor didehydro-cortistatin A prevents HIV-1 reactivation from latency." *mBio* **(2015)**

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