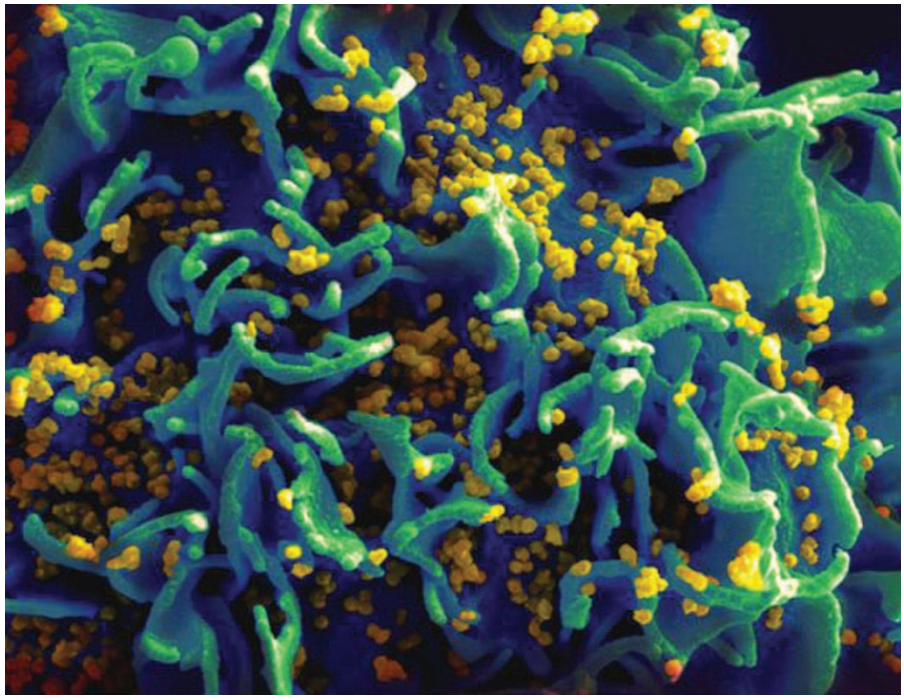
Underestimate of HIV reservoirs threatens purging approach

ATLANTA — At the annual Conference on Retroviruses and Opportunistic Infections (CROI) that took place almost exactly a year ago, researchers reported the first clinical evidence that the drug vorinostat could shock HIV out of its hiding places in patients, potentially allowing the once-dormant virus to be detected by the immune system and thereby cleared from the body. The release of the vorinostat findings hinted at a future in which HIV-infected individuals would not have to stay on lifelong drug therapy, as currently recommended. Amidst the buzz surrounding the results, there was just one word on everyone's lips: cure (see *Nat. Med.* **18**, 473, 2012).

A year on, however, few people were uttering that word here at this year's CROI meeting—at least not when it came to talking about patients in whom HIV had already taken hold. Activating latent HIV to clear the infection "could be a lot harder than we thought," Robert Siliciano, a molecular virologist at the Johns Hopkins University School of Medicine in Baltimore, told *Nature Medicine*. Siliciano kicked off the reality check at the very first plenary session of the meeting. There, he discussed unpublished work showing that the viral reservoir inside a given individual might be 40–50 times larger than researchers had originally calculated.

Scientists typically say that one in a million resting memory T cells in the body of an HIV-positive person on aggressive antiretroviral therapy contains HIV capable of replicating if the individual were to go off of drug therapy. This is known as the latent reservoir. They arrived at this number through a technique that uses a toxic compound to activate all T cells in a blood sample taken from a patient. But a closer examination of the virus particles suggests that the technique in fact wakes up only a fraction of the virus capable of replicating, thereby throwing off the math.

Siliciano's graduate student Ya-Chi Ho analyzed all the HIV gene sequences taken from patient blood samples and found that for every virus particle activated by the toxic compound, there were about 300 virus particles that remained dormant. She then discovered that 12% of those 'dormant' HIV particles actually contained a full suite of working genes needed for proper replication. These viral sequences should no longer be ignored, cautions Ho. "Just because the virus doesn't come out from this [T cell activation] assay doesn't mean that it's never inducible *in vivo* in the human body," she says.



Science Source

To a T: Nearly all HIV-infected T cells (pictured) may need to be purged to eliminate the reservoir.

Siliciano also teamed up with Martin Nowak, a mathematical biologist at Harvard University in Cambridge, Massachusetts, to measure how much of the reservoir would have to be purged to achieve a so-called 'functional cure' in which someone could safely stop taking antiretrovirals without fear of HIV rebounding. The analysis suggests at least 99.9% of all replication-competent HIV would have to be eliminated to significantly prolong or prevent rebound, beyond which the probability of HIV reactivation events becomes low enough to deem the strategy safe.

That's a tall order, especially if you consider research presented at CROI by Anthony Cillo, a graduate student in John Mellors's lab at the University of Pittsburgh in Pennsylvania. Cillo incubated HIV-infected memory T cells with either vorinostat, which inhibits the action of histone deacetylase (HDAC) enzymes, or an antibody-coated bead system that is also known to reverse viral latency. Importantly, he analyzed individual T cells to calculate exactly what percentage of the reservoir was activated, rather than just relying on overall viral RNA or DNA levels in the blood sample to judge the effect of the latency-activating compounds, as most researchers have done previously. In this way, Cillo showed that only 0.13% of HIV-containing T cells are activated by vorinostat, and just 1.5% with the antibody therapy. Add on top of that work published by Siliciano's group last year showing that even activated HIV-infected memory T cells treated with vorinostat in the presence of a patient's own killer T cells survived in a lab dish (*Immunity* **36**, 491–501, 2012).

With drugs like these, "you'd be hard-pressed to say that you've had a significant effect on the reservoir," says Cillo.

Given the totality of the data, it'll be an uphill battle to get to HIV eradication, notes Alison Hill, a graduate student with Nowak who led the mathematical modeling work. But on the upside, her model "doesn't say you have to get rid of every cell in the reservoir," she says. "We're not saying it's impossible."

No biggie

Even with all these obstacles, researchers are forging ahead with attempts to target viral latency in the clinic. "All of these things are, to me, not a big deal," says David Margolis, a clinical virologist at the University of North Carolina at Chapel Hill. "The big deal is that you have a rational, stepwise,

scientific approach to understand the mechanism of latent HIV activation and to make therapeutic progress." Margolis led the vorinostat trial reported at last year's CROI, which was subsequently published in *Nature* (**487**, 482–485, 2012), and he is currently leading a trial in which HIV-positive participants receive cycles of vorinostat therapy—three days on, four days off, for up to eight weeks—to see if he can activate more latent HIV than occurred with his earlier, single-dose protocol. (Sharon Lewin, an HIV specialist at Monash University in Australia, also recently wrapped up a 14-day trial of daily vorinostat treatment, the results of which were presented at this year's CROI.)

Additionally, the search is on to find better HDAC inhibitors to agitate HIV. To this end, scientists from Gilead, a drug company based in Foster City, California, assessed the latency induction potential of romidepsin, a drug that, like vorinostat, is approved for lymphoma treatment. As Gilead's George Wei reported here, romidepsin proved 500 times more potent than vorinostat in triggering HIV expression from T cells *in vitro*.

Another tactic could be combination therapy. At CROI, Daria Hazuda, head of infectious disease research at Merck Research Laboratories in West Point, Pennsylvania, reported the results of a high-throughput screen designed to identify additional compounds that work synergistically with vorinostat to reverse viral latency. In collaboration with Margolis's lab, Hazuda and her Merck team discovered a class of oncology drugs known as farnesyltransferase inhibitors that, in combination with vorinostat, proved far more potent at stimulating HIV production than vorinostat or its own. The farnesyltransferase inhibitors "have weak activity by themselves," Hazuda told *Nature Medicine*, "but when you put them together with an HDAC inhibitor, they turn on HIV gene expression to levels which are comparable to the most active activators in this system."

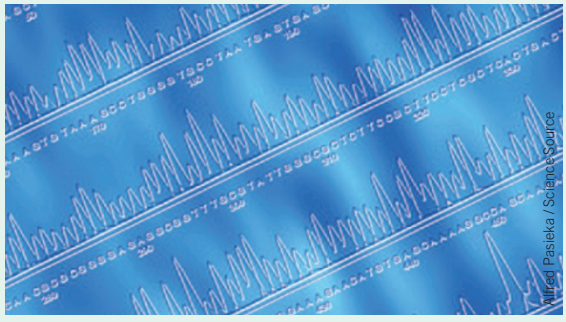
Although the progress might be incremental, Margolis is buoyed by findings like these. "This is just the beginning, but we'll never get to the finish line if we don't start," he says.

Elie Dolgin

Regulatory agency struggles under the weight of genomic data

With the precipitously falling price of genome sequencing, generating reams of data is easy these days—analyzing all those As, Ts, Cs and Gs is the hard part. Yet, it's not just physicians and scientists who are faced with this analytical bottleneck posed by high-throughput sequencing (HTS). Regulators are now receiving huge batches of sequence data that support new drug applications—and they are struggling to figure out what to do with them.

High-throughput sequencing technology produces millions of sequences all at once in an automated and parallel fashion. Because of its high-throughput nature, it outperforms the time efficiency of the older Sanger sequencing by a factor of 1,000 and allows researchers to obtain an entire genome from an individual in a matter of days. The technology has paved the way for indentifying targets for cancer drugs, such as mutations in the protein kinase BRAF in melanoma (*N. Engl. J. Med.* **364**, 2507–2516, 2011) or in HER2 in breast cancer (*Cancer Discov.* **3**, 224–237, 2013).



Peak performance: FDA gets a grasp on DNA data.

"The science of HTS is moving fast, [and] applications will continue to grow that require certain infrastructure," says Carolyn Wilson, the associate director for research at the Center for Biologics Evaluation and Research (CBER), a division of the FDA in Rockville, Maryland. The FDA now plans to build that infrastructure through a newly established 'genomics working group', launched earlier this year and tasked with developing approaches for handling and storing genomic data both internally and with relevant external partners.

On 27 February, Wilson, who chairs the group, presented an update to the agency's Science Board where she emphasized the need to develop the infrastructure and bioinformatic tools to meet today's demands. "We have been working to bring different components of the agency together to store this data better and to develop a strategic plan for evaluating HTS data quality and interpreting it for regulatory decision making," she told *Nature Medicine*. To this end, the working group has also partnered with other branches of the US government, including the National Center for Biotechnology Information and the National Institute of Standards and Technology, to establish quality standards.

Delineating data benchmarks is important, notes George Weinstock, associate director of the Genome Institute at Washington University in St. Louis. He cites a lack of off-the-shelf reliable software to analyze genomic data. As such, most academic labs use various homegrown tools. These different programs often disagree in the results they produce from the same data sets. "Standards for both data quality as well as data analysis may be required," he says.

According to Wilson, the FDA has been receiving an increasing amount of HTS data from industry and academia, but she would not comment on whether the agency has used such data to support approval of specific drugs, citing confidentiality. She did note, however, that the FDA anticipates it will use more genomic data for in-house research in the future. For example, the CBER plans to use HTS to detect emerging infectious agents in the nation's blood and tissue supplies and also to assess the safety and quality of products such as vaccines. Additionally, the FDA is planning to use HTS to evaluate how drug-resistant viruses develop after antiviral drug treatments.

Although it's unlikely that the FDA will make proprietary drug approval data available in the near future, some researchers still dream of access to genomic information handled by the agency. "The key is for the FDA to put out [these data] into the market as raw data so that it can be reanalyzed by the public," says Michael Becich, chairman of the department of biomedical informatics at the University of Pittsburgh School of Medicine in Pennsylvania. "It would be very useful if the FDA considered this in their framework."

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