Hidden Killers
A larger-than-imagined reservoir of HIV is evading current antiretroviral therapies.

There was a time when a positive test for human immunodeficiency virus (HIV) meant certain death. Today, the future is much brighter for people with HIV, thanks to advances in drug treatments. But even the most powerful therapies can’t flush out the dormant virus that lurks in a patient’s body—a reservoir that, according to HHMI Investigator Robert Siliciano at the Johns Hopkins University, may be up to 60 times larger than previously imagined.

HIV wipes out the immune system by converting the very cells that are meant to help kill it into virus-producing factories. During an infection, HIV injects its genetic material into activated CD4+ T cells. The HIV DNA integrates into a T cell’s genome, causing it to create more virus. Most of the infected T cells in this viral production mode die, but some survive and go into a resting state in which virus production is shut off. About 100 to 1,000 resting CD4+ T cells per million harbor dormant HIV sequences called proviruses. These sleeper viruses are invisible to circulating immune cells—and to current antiretroviral therapies.

Until recently, scientists were unable to get an accurate fix on the size of the provirus reservoir. Moreover, they didn’t know how many of these stealthy invaders could wake up and mount an attack against the immune system. Siliciano and Ya-Chi Ho, an HHMI international student research fellow, published a technique in the October 24, 2013, issue of Cell that has revealed both the size and the composition of this reservoir. The good news is that about 88 percent of these dormant viruses have genetic defects that make them unable to reactivate. Unfortunately, the remaining 12 percent are fully functional and have the potential to reawaken and attack at any time. The findings suggest that previous calculations vastly underestimated the magnitude of the provirus population—by about 60-fold.

To effectively target HIV, future therapies need to consider these dormant viruses. “It doesn’t mean that it’s hopeless, but it does mean we need to focus on getting an even clearer idea of the scope of the problem,” says Siliciano. Currently, he and his colleagues are working on simple clinical assays to assess reservoir size. – Nicole Krege

IN BRIEF
isolated newly made RNA from human cells and compared the sequences to the DNA in the same cells. They discovered that RDDs are not formed when RNA polymerase creates an RNA copy of the DNA template. Instead, the changes often occur right after the synthesis, when the new RNA folds back on its DNA template, forming a DNA-RNA hybrid called an R-loop. These structures are thought to play a role in gene regulation. The group published its findings on March 13, 2014, in Cell Reports.

Now that Cheung knows when RDD formation occurs, she’s looking for the processes that form these mismatches. “The knowledge that RDDs are not as rare as previously thought tells us that genetic studies need to give RNA variation some attention,” she says. Figuring out the mechanisms behind RDD formation will further the understanding of how RNA processing contributes to genetic diversity.

REPROGRAMMING CANCER
Glioblastomas are the most common brain tumors. They are also the most lethal, in part because of a small population of stem cells that live inside each tumor. Although the stem cells comprise only a small portion of the tumor—just a few percent—they are not trivial. They cause aggressive tumor growth as well as resistance to radiation and chemotherapy. Recent findings by HHMI scientists point to a way to disarm these cells by focusing on what makes them so different from the rest of the tumor.

HHMI Early Career Scientists Bradley Bernstein, at Massachusetts General Hospital, and Aviv Regev, at Massachusetts Institute of Technology, collaborated to examine the circuits that regulate genes in both stem cells and non-stem cells from glioblastoma tumors. The researchers found four transcription factors—proteins that turn genes on and off—that were present only in the stem cells. When they expressed a cocktail of four transcription factors in the non-stem cells from the glioblastomas, those cells turned into stem cells. The experiments, published April 24, 2014, in Cell, show that transcription factors can override a glioblastoma cell’s programming and drive it into a more aggressive state.

Bernstein and Regev hope to use this information to target these aggressive stem cells with small molecule inhibitors.

NEURONAL RECOVERY
In the body, neurons use molecule-filled sacs, called vesicles, to communicate. When it’s time to send a message, a neuron’s vesicles fuse with its membrane, releasing their contents into the synaptic space between the cells. If there is a lot to communicate, the neurons burn through their vesicles quickly. Rapid replenishment of vesicles is of the utmost importance—the neurons need to be ready to send the next batch of signals. HHMI Investigator Edwin Chapman and his team at the University of Wisconsin recently showed that two calcium-binding proteins, calmodulin and synaptotagmin 7, work together to ensure that neurons have adequate vesicles for communication.

One route of restoring synaptic vesicle supplies depends on calcium and calmodulin. Chapman’s team discovered that, in response to Ca2+, synaptotagmin 7 binds to calmodulin, and this complex initiates the vesicle replenishment pathway. The study, published in eLife on February 25, 2014, clarifies a number of controversies about the function of synaptotagmin 7 in the nervous system.

Now that Chapman knows what’s involved in the pathway, he wants to figure out how the components work together to replenish the vesicles. “Do they clear out release sites so incoming vesicles can dock and fuse? Do they refill the releasable pool of vesicles? Or do they do both?” he wonders. “At the moment, this remains a mystery.”