



HIV ERADICATION: TIME TO TALK ABOUT A CURE

Since the earliest years of the epidemic, a cure has been the elusive “holy grail” of HIV/AIDS research. Several false starts and failed attempts gave rise to pessimism, and as efforts focused on improving antiretroviral therapy (ART) and managing its complications, the idea of HIV eradication faded into the background.

Liz Highleyman

But in recent years, antiretroviral drugs have reached the limit of their effectiveness. The cost of providing universal access has become unsustainable, and accumulating evidence underscores the detrimental effects of persistent HIV infection even while plasma viral load is low and CD4 cell count is high.

Scientists today are exploring a variety of strategies in the hope of either completely eradicating HIV from the body (a sterilizing cure) or reducing it to such a low level that the immune system can maintain control without antiretroviral drugs (a functional cure). Among these are agents that flush HIV out of latent reservoirs, drugs that keep hidden virus permanently inactive, immune-strengthening therapies, and gene therapy that protects cells from infection. Most experts think a cure will likely require a combination approach.

With ART now keeping HIV suppressed over the long term, the reluctance to talk about a cure has evaporated. Researchers and advocates worldwide are once again asking: Is lifelong treatment the best we can do?

THE QUEST FOR A CURE

In the early years of AIDS, people with HIV held out hope for a cure, but it soon became apparent that the virus is wily and tenacious, infecting and killing the very CD4 T-cells needed to mount an effective immune response. What's more, HIV integrates its genetic material into human cells, turning them into virus-producing factories. It also hides in sequestered body compartments where antiretroviral drugs may not reach, such as the lymph nodes, brain, and genital tract.

With the advent of effective combination ART in the mid-1990s, some researchers suggested that given enough time, antiretroviral drugs might eventually wipe out all HIV in the body.

At the XI International AIDS Conference in Vancouver in 1996, David Ho from the Aaron Diamond AIDS Research Center (who would soon be named *Time* magazine's Man of the Year) proposed that a "hit early, hit hard" strategy using a potent combination regimen could potentially eradicate virus-infected T-cells—and with them, the virus—within two to three years.

Around the same time, however, Robert Siliciano and his team at Johns Hopkins were conducting research that would yield a more sobering finding: In the May 8, 1997, issue of *Nature*, they reported that HIV can hide in a "reservoir" of long-lived resting CD4 T-cells. Because it is not actively replicating, this virus is invisible to the immune system and out of reach of antiretroviral drugs.

HIV's genetic blueprint, known as proviral DNA, can lie dormant for years or even decades within a host cell's chromosomes, ready to produce new virus when the cell is activated.

This viral reservoir decreases slowly in people on ART as resting cells die, but researchers estimated that complete elimination could take 70 years.

In 1997 Tae-Wook Chun and Anthony Fauci from the National Institutes of Allergy and Infectious Diseases (NIAID) reported that they could still detect integrated HIV DNA in resting CD4 cells from a small cohort of patients who started treatment early and had suppressed plasma viral load after a year on combination ART. A decade later, based on the half-life of latently infected T-cells, Chun's group estimated that early treatment might eliminate all virus in these cells in about 7.7 years.

Yet as people stayed on ART longer, it became clear that residual HIV still could be found after three, seven, and eventually ten years on suppressive therapy. Even when plasma viral load is "undetectable," ultrasensitive tests show that HIV often still persists at low levels in the blood and almost always lurks in cellular and anatomic reservoirs. Because these reservoirs can start releasing HIV at any time, even people with viral loads below 50 copies/mL must remain on ART to prevent replication of escaping virus.

This realization that HIV persists despite the best available antiretroviral drugs—along with the disappointment following over-exuberant media hype about eradication—put a damper on talk of a cure for the next decade.

CRACKING CONSENSUS

In the lead-up to the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention in the summer of 2007, the consensus around the futility of a cure was starting to crack. The approval

of two novel classes of antiretroviral drugs—integrase inhibitors and CCR5 antagonists—offered for the first time in years the ability to target HIV at more stages of its life cycle.

Although NIAID director Fauci told reporters at that meeting, "we haven't even come close to truly eradicating [HIV] in anyone, and I think we should just stop talking about it," by the following year he had changed his tune. "I am cautiously optimistic that we will be able to cure some patients under certain circumstances," he wrote in a CNN article summarizing his talk at the XVII International AIDS Conference in Mexico City.

The prospect of a cure really came into its own in the summer of 2010. At the XVIII International AIDS Conference in Vienna, Sharon Lewin from Monash University gave an opening lecture highlighting the issue, garnering international media attention.

"We should not and cannot continue to accept that HIV is a chronic illness that commits patients to lifelong treatment," Lewin said. "In the absence of an effective vaccine, we must seriously pursue the possibility of cure."

Preceding the conference, the International AIDS Society sponsored a workshop titled "Towards a Cure: HIV Reservoirs and Strategies to Control Them," which brought together 200 researchers and advocates to discuss the latest advances in the field. A related satellite session at the 2011 IAS Conference in Rome will look at "Controversies in HIV Cure Research."

At the 18th Conference on Retroviruses and Opportunistic Infections (CROI 2011), researchers presented the first data from a human trial of an experimental gene therapy approach that deletes CCR5 receptors from T-cells in an attempt to halt HIV entry.

At the same meeting, the IAS launched an international working group to develop a consensus on the state of HIV reservoir science, define research priorities for tackling persistent virus, and advocate for increased

Sterilizing cure: eliminating all traces of HIV from the body, including cellular reservoirs such as resting CD4 T-cells and anatomic reservoirs such as the brain and gut.

Functional cure: remission, or enabling people to live long-term with no active HIV replication or disease progression in the absence of ongoing antiretroviral therapy.

funding. The group will meet again in Rome and plans to release a formal strategy report at the 2012 International AIDS Conference in Washington, DC.

“In the field of HIV/AIDS, there are two main priorities: to try to get a vaccine and to try to get a cure,” said working group cochair and IAS president-elect Françoise Barré-Sinoussi during an interview in Boston. “The HIV epidemic is still here and is still increasing in some populations. That means the game is not over and we have to find new strategies for the future.”

WHY WE NEED A CURE

Despite remarkable advances in anti-retroviral treatment, people with HIV still face a lifetime of therapy with its attendant known and unknown long-term toxicities. Lifelong adherence is a challenge for many people, and treatment can eventually fail even in the most conscientious patients.

Recent research by Steven Deeks from the University of California at San Francisco (UCSF) and others shows that persistent immune activation and inflammation due to chronic HIV infection can wreak havoc throughout the body, even when plasma viral load is undetectable. There is a growing consensus that persistent virus contributes to the elevated risk of cardiovascular disease, cancer, and the appearance of accelerated aging in people with HIV. (See “Inflammation, Immune Activation, and HIV,” *BETA*, Spring/Summer 2010.)

What’s more, despite considerable progress toward universal access to antiretroviral drugs, providing long-term ART to millions of people worldwide through public and private aid efforts is unlikely to be sustainable. For every two people who start therapy today, it is estimated that three more are newly infected. “We will not be able to treat ourselves out of the epidemic,” Deeks told *BETA*. “The global need for treatment will always be far greater than our capacity to deliver.”

Neither can prevention efforts alone put an end to HIV. “Let’s dream a little fantasy and say that tomorrow we wave our magic wand and we can prevent all of the new infections,” said Rowena Johnston, vice president and director of research for amfAR (the Foundation for AIDS Research), at a recent public forum presented by San Francisco AIDS Foundation. “We still have tens of millions of people with HIV *right now* for whom those prevention efforts come too late.”

And if “treatment *is* prevention”—the new favorite slogan of many advocates—a cure would be the ultimate prevention strategy.

Over the past 30 years, HIV medicine has evolved from simply keeping people alive, to maintaining undetectable viral load as long as possible, to dealing with non-AIDS conditions in an aging population. Today the question has become: Can we do better?

BARRIERS TO ERADICATION

Ultrasensitive tests reveal very low levels of plasma HIV RNA (as little as 1 copy/mL) in most people with “undetectable” viral load. Replication-competent HIV can still be isolated from resting CD4 T-cells from people with the longest duration of combination ART use—now around 15 years—and viral rebound almost always occurs soon after treatment interruption.

SOURCES OF RESIDUAL VIRUS

Researchers continue to debate the source of persistent plasma HIV RNA in people on suppressive ART. Does it arise from ongoing low-level replication that antiretroviral drugs have not managed to shut down? Or is it due to residual virus leaking out of long-term reservoirs such as resting CD4 memory T-cells and sequestered sites such as the brain?

“This is not just nerdy, but a real clinical question,” Frank Maldarelli from the National Cancer Institute said at the 2010 IAS Reservoirs workshop. “If there is still ongoing replication,

we need better ART. If persistence is due to long-lived reservoirs, we need other strategies.”

Several research teams have reported that residual HIV in people on ART does not show much evidence of mutation—as would be expected with uncontrolled viral replication—indicating that it likely originates from reservoirs rather than from low-level continuing replication.

But others find evidence that HIV replication may still be occurring despite ART. In the April 2010 issue of *Nature Medicine*, Maria Buzón and colleagues from Spain reported that adding the integrase inhibitor raltegravir (Isentress) to a suppressive ART regimen led to accumulation of bits of viral DNA known as 2-LTR circles, suggesting that HIV is still copying its genetic material but cannot insert it into host cell chromosomes. Using novel assays, Una O’Doherty’s group at the University of Pennsylvania also detected unintegrated HIV DNA, suggesting continued viral replication.

Most experts now agree that while low-level ongoing replication is likely a factor in some individuals—especially replication in the gut, brain, and other anatomic reservoirs—persistent HIV is largely attributable to virus escaping from a reservoir of latently infected resting immune cells.

ACTIVATED AND LATENT CD4 CELLS

A brief look at the HIV life cycle helps explain why eradicating persistent HIV is such a daunting challenge.

HIV uses surface receptors to enter human cells, primarily the CD4 or “helper” T-cells that coordinate the overall immune response. These cells are named for their CD4 receptor, which HIV uses—along with either the CCR5 or CXCR4 coreceptor—to gain entry. Some CD4 cells circulate in the blood, but most reside in lymphoid tissues such as the lymph nodes and lining of the gut.

HIV primarily infects activated CD4 T-cells, or those currently “on duty.” Once inside a cell, retroviruses like HIV use their reverse transcriptase enzyme to copy their genetic material from RNA to DNA. Next, the integrase enzyme inserts these new DNA copies into the host cell’s chromosomes. Utilizing the cell’s own machinery, this proviral DNA blueprint is used to produce proteins which are assembled into new virus particles that burst out of the cell’s membrane and go on to infect other cells.

Infected activated CD4 T-cells soon exhaust themselves producing new virus and die, or they may be eliminated by CD8 “killer” T-cells. Initially the body can produce enough replacement cells, but eventually HIV gets the upper hand and the CD4 cell count begins to fall.

HIV may also integrate its genetic material into activated CD4 cells that then go into a resting state, and possibly into cells that are already dormant. A reservoir of these latently infected T-cells is established during the earliest stage of HIV disease.

As long as the host cell remains at rest, proviral DNA stays silent; in Siliciano’s words, this integrated genetic material allows the virus to “persist as information.” Sequestered provirus in resting cells is hidden from the immune system and invulnerable to currently available antiretroviral drugs. But eventually the host cell may be activated—for example, when it encounters a pathogen it recognizes—which turns on the viral DNA and renews production of infectious virus.

When first produced in the bone marrow, CD4 T-cells are naive, meaning they have the ability to respond to new threats. Once a T-cell learns to recognize and respond to a specific threat, it becomes antigen-experienced, or “committed.”

When an experienced effector T-cell recognizes its target, it proliferates and goes into action. These activated cells burn themselves out quickly,

typically within a day or so. Normally the body produces enough T-cells to replace those that are lost, thus maintaining homeostasis, or a steady state.

But a subset of CD4 T-cells lives much longer. After mounting an immune response, some antigen-experienced cells—called memory T-cells—go into a resting state. These long-lived memory cells, with a life span of years or decades, act as sentinels, enabling the immune system to recognize and respond more rapidly to threats encountered in the past.

The absolute number of resting memory CD4 T-cells harboring replication-competent virus is small—on average about one in a million resting CD4 cells, or as few as one in ten million in a person on long-term suppressive ART—but this is enough to reignite disease progression if treatment is stopped.

There is further specialization within the memory CD4 T-cell population. Nicolas Chomont from the Vaccine and Gene Therapy Institute (VGTI) and others have shown that central memory T-cells (the longest-lived type) and transitional memory cells are the main reservoirs of latent HIV. Central memory cells eventually die off, but proviral DNA in transitional memory cells may be copied into daughter cells as they undergo homeostatic proliferation (ongoing division to maintain a steady level).

The VGTI team found that mostly central memory T-cells make up the latent HIV reservoir in people who start ART early and respond well with large CD4 cell gains, while people with poor CD4 cell recovery have more latently infected transitional memory cells. Since these two cell types enable HIV persistence in different ways, the researchers concluded that complete viral eradication will require a combination approach.

OTHER RESERVOIRS

Researchers have long debated whether hidden HIV resides in other

cellular reservoirs besides resting CD4 T-cells. Proposed candidates include naive CD4 T-cells, monocytes and macrophages, dendritic cells, and stem cells in the bone marrow.

Viral dynamics, or how HIV levels change after starting treatment, gives clues about the nature of these reservoirs. In a keynote lecture at CROI 2009, Siliciano argued that decay patterns and gene sequencing indicate that residual virus is coming from a second, unknown cellular reservoir in addition to memory CD4 T-cells.

Macrophages and their precursors, monocytes, carry receptors that HIV can use for entry. Siliciano speculated that his proposed second reservoir might be a progenitor cell, or stem cell, further back in the monocyte/macrophage line.

In the April 2010 issue of *Nature Medicine*, Christoph Carter from the University of Michigan at Ann Arbor and colleagues reported that latent HIV can hide in CD34 hematopoietic stem cells, which give rise to all types of blood cells. When these stem cells were forced to differentiate in the laboratory, proviral DNA was activated and began producing new virus.

In addition to cellular reservoirs, HIV also hides in areas of the body that act as “sanctuary sites.” Within days after infection the virus establishes itself in anatomic reservoirs such as the central nervous system and the gut. In fact, gut-associated lymphoid tissue (GALT) in the lining of the intestines is the largest source of vulnerable CD4 T-cells.

In the brain, HIV primarily targets specialized macrophages called microglia; it does most of its damage to brain tissue by triggering inflammation. (See “HIV and the Brain,” *BETA*, Summer/Fall 2009.)

At the IAS Reservoirs workshop, Melissa Churchill from Monash University reported that autopsies of brains from people with HIV revealed proviral DNA in astrocytes (a type of brain support cell), with more

pronounced infection in people with HIV-associated dementia.

VIRAL LATENCY

How does HIV manage to remain latent in resting T-cells? This process is a topic of intensive investigation, as it offers clues about potential strategies for flushing the virus out of these cells—a key step in eradication.

The study of how genes are turned on or off is known as epigenetics. Every cell contains the complete human genome in its chromosomes, but a variety of mechanisms regulate which specific genes are used to manufacture proteins. “The fingernail gene in a tooth cell is turned off forever,” eradication researcher David Margolis from the University of North Carolina explained at a February 2010 forum sponsored by the AIDS Policy Project, an advocacy group promoting cure-related research.

Multiple mechanisms have been implicated in epigenetic silencing, or turning off proviral DNA in resting CD4 T-cells, as well as reversal of this process when the cell is activated.

HIV has promoter and enhancer elements at one end of its proviral DNA that regulate viral transcription (see sidebar below for definitions of terms). In a latent state, these regulatory elements are hidden. When a resting cell is activated, the proviral HIV

blueprint transcribes just a few genes at first, producing a viral protein called Tat; once a critical amount of Tat is made, replication can accelerate.

Host cell chemical signals help to either maintain HIV in a latent state or cause it to awaken and begin producing new virus. Some of the same factors that trigger human gene transcription do the same for proviral DNA, including nuclear factor kappa-B (NF- κ B), nuclear factor of activated T-cells (NF-AT), and positive transcription elongation factor b (P-TEF). Tat works by recruiting these factors to the HIV promoter element, leading to activation of proviral genes.

In a cell’s nucleus, DNA is coiled around structures called histones, allowing long chains of genetic instructions—about two meters in humans—to fit into a tiny space. A unit of DNA wrapped around a histone is called a nucleosome, multiple nucleosomes plus accessory proteins make up chromatin, and chromatin is packaged into chromosomes.

Acetylation, methylation, and phosphorylation are chemical changes that determine whether chromatin is condensed and unusable, or expanded so it can be used to build new proteins. Histone deacetylases (HDACs) are enzymes that keep DNA tightly bound to histones and therefore inaccessible. HDACs play a key

role in maintaining proviral latency; as discussed below, drugs known as HDAC inhibitors reverse this process, enabling expression of proviral DNA and production of new virus.

Activating latent cells to flush out HIV and preventing cell activation to keep the virus permanently silenced are both potential approaches to a cure. But much remains to be learned about HIV reservoirs, how the virus establishes latency and reawakens, and how human host factors influence these processes.

“The virus is telling us how much we don’t know about the human immune system,” Maureen Goode now from the University of Florida explained in an IFARA interview at the Vienna AIDS conference. “If we understood everything about human immunology and all the different cell types and how they behave, [a cure] would basically be a no-brainer.”

APPROACHES TO ERADICATION

Researchers are exploring many approaches for eradicating HIV or achieving a functional cure, most of which can be categorized into a several broad areas:

- Starting ART very early before viral reservoirs are fully established
- Intensifying antiretroviral therapy to stop residual HIV replication
- Activating resting T-cells to purge or flush out latent virus
- Maintaining latency to keep proviral DNA permanently silenced
- Eliminating or disabling HIV-containing resting cells
- Protecting uninfected cells against viral entry
- Strengthening the immune system’s response to HIV

ART DURING PRIMARY INFECTION

One of the earliest proposed approaches for curing HIV is starting antiretroviral treatment very early, during primary or acute infection.

As noted above, HIV establishes itself in resting CD4 cells and anatom-

Gene expression: the entire process by which genes are used to produce proteins.

Transcription: production of a complementary messenger RNA (mRNA) strand of nucleotides from an original DNA template, using the RNA polymerase enzyme.

Reverse transcription: production of a complementary DNA strand from an original RNA template using the reverse transcriptase enzyme, as done by retroviruses.

Elongation: addition of more nucleotide building blocks to lengthen a newly formed strand of RNA.

Translation: the process by which cell structures called ribosomes build proteins from amino acids using messenger RNA as a template.

ic reservoirs within days after initial infection. Some researchers hypothesize that if a person starts potent combination ART during the first days, weeks, or even months after infection—before these latent reservoirs are fully established—it may be easier to eradicate the virus.

Chun and Fauci's calculation that the reservoir of latently infected resting CD4 T-cells might be completely eliminated in 7.7 years was based on a small cohort of patients who began ART within the first six months after initial HIV infection. After starting treatment, the number of latently infected CD4 cells decreased between ten-fold and 100-fold, down to approximately ten per billion.

But in an editorial accompanying Chun's 2007 report, Margolis and Nancie Archin expressed skepticism. A close examination of viral decay patterns, they explained, suggests that reservoir size may never fall below one latently infected CD4 cell per billion, even after years of therapy. Given that the average adult has approximately 110 billion resting CD4 T-cells, they concluded, "the few infected cells remaining may be enough to reignite infection."

Chun confirmed this concern at the IAS Reservoirs workshop and in the November 27, 2010, issue of *AIDS*, presenting further data from his early-therapy cohort, who by now have maintained undetectable plasma HIV RNA on ART for up to a decade.

As expected, nine patients who started treatment during primary infection had significantly less proviral DNA in their CD4 T-cells than people treated later. But more intensive testing using a high-input co-culture assay that can detect virus in a larger population of cells revealed that eight early-treated patients with "undetectable" plasma viral load still harbored a small amount of hidden infectious HIV.

One individual had a very small cellular HIV reservoir—estimated at one per 1.7 billion resting CD4

cells—and no detectable HIV DNA in a colon biopsy sample. Nevertheless, his plasma viral load rebounded within 50 days after stopping antiretroviral drugs.

Treatment during very early infection is impractical today because most people are not diagnosed so soon. While some individuals experience symptoms of acute retroviral infection, these are easily mistaken for the flu.

But routine HIV testing and measurement of viral RNA rather than antibodies could increase the likelihood of catching infection at an early stage. According to Fauci, starting ART even within the first several months after infection can help keep viral reservoirs low, improving prospects for a functional cure.

ART INTENSIFICATION

The hypothesis that residual viral load is attributable to ongoing low-level HIV replication has motivated studies to determine whether ART intensification might eliminate the virus. If three antiretroviral drugs can decrease HIV RNA levels almost to zero, it makes sense that adding more could potentially finish the job.

Numerous studies have explored adding extra drugs to a regimen that is already keeping viral load below 50 copies/mL. Some have used newer, more potent drugs from existing classes, while others have tested agents from novel classes, including entry inhibitors and integrase inhibitors.

At this year's CROI, Rajesh Gandhi and fellow investigators with the ACTG A5244 trial found that adding raltegravir to a suppressive ART regimen did not further reduce low-level plasma HIV RNA, DNA, or 2-LTR circles. "Intensification of antiretroviral therapy is unlikely to lead to HIV eradication," they concluded. "Strategies that directly target latently infected cells may be more likely to eradicate HIV."

Overall, intensification studies have not produced impressive results. Some researchers have found that raltegravir and the CCR5 antagonist

maraviroc (Selzentry) may reduce immune activation and inflammation. But so far there is no conclusive evidence that any combination of current antiretroviral drugs can eradicate HIV, leading Siliciano to conclude, "We have reached the theoretical limit of antiretroviral therapy."

But researchers have got yet given up on treatment intensification. The German New Era Study is looking at treatment-experienced patients with viral load suppressed for three years who add both maraviroc and raltegravir to their existing boosted protease inhibitor regimen. The EraMune trials are evaluating whether an intensified ART regimen with either interleukin 7 or a therapeutic vaccine can eliminate HIV from the body (see "Open Clinical Trials," page 53).

"This is the mother of all intensification trials," Romas Geleziunas, who works on cure research at Gilead Sciences, said at an AIDS Policy Project forum. "If it doesn't work, we have to put it to rest and move on."

But many researchers have already moved on, convinced that viral persistence in latent reservoirs—rather than ongoing replication—is the key barrier to achieving a cure.

CCR5 GENE THERAPY

The cure strategy that has received the most widespread attention—though still far from clinical application—is gene therapy to protect susceptible cells from HIV infection. If new virus emerging from latent reservoirs cannot find cells to enter, it will die off without causing disease progression. This approach would not completely eradicate HIV, but could enable a functional cure.

Gene therapy for HIV is not new, but early attempts altered the virus, not the host. In 2004, Ronald Mitsuasu and colleagues at the University of California at Los Angeles (UCLA) reported that a ribozyme or "molecular scissors" that disrupts the HIV *Tat* gene was successfully inserted

into human hematopoietic stem cells. A follow-up study with 74 HIV positive patients who interrupted ART showed that while the altered stem cells did not significantly reduce viral load, recipients had higher CD4 cell counts over two years.

Several years ago Carl June, Pablo Tebas, and colleagues from the University of Pennsylvania and VIRxSYS began testing a genetically engineered HIV shell containing antisense or complementary RNA (called VRX496) that blocks expression of the HIV envelope gene. At CROI 2010, they reported that patients who had the antisense RNA inserted into their CD4 T-cells before stopping ART had lower viral load set points; one participant maintained undetectable viral load for more than 14 weeks off treatment.

Today, the most extensively studied method involves disabling the gene responsible for expression of the CCR5 coreceptor in human cells. Recall that HIV uses the CD4 receptor along with one of two coreceptors—CCR5 or CXCR4—to enter cells. Individuals may harbor exclusively CCR5-tropic virus, exclusively CXCR4-tropic virus, or a mix of both types.

People with a natural genetic variation known as CCR5-delta-32 (deletion of part of the gene that encodes CCR5) are less likely to become infected with HIV and experience slower disease progression. Individuals with two copies of this variation (one inherited from each parent) may be “elite controllers” who maintain undetectable viral load with little or no disease progression in the absence of ART.

This gene therapy approach was largely inspired by the Berlin Patient, a man who appears to have been cured of HIV after receiving bone marrow transplants for leukemia from a donor with the double CCR5-delta-32 gene variation (see sidebar, page 20).

But bone marrow transplants are risky due to the strong chemotherapy or radiation needed to eliminate the patient’s original immune cells,

with a mortality rate of about 25%. Furthermore, the CCR5-delta-32 mutation is rare—occurring in only about 1% of Northern Europeans and even fewer people of African or Asian descent—so finding suitable donors would be difficult. And the cost per procedure can be upwards of \$200,000. Gene therapy aims to mimic the CCR5-delta-32 effect without these drawbacks.

Zinc finger gene therapy technology developed by Sangamo BioSciences is furthest along in development. This technique uses a zinc finger nuclease (ZFN), a synthetic protein carried by an adenovirus vector that can cut DNA strands at a specific location. The nuclease causes a double-strand DNA break in the CCR5 gene, and the ensuing repair process permanently disrupts the gene. What’s more, the altered version can be passed along to daughter cells.

Laboratory studies have shown that ZNF-modified CD4 T-cells are protected against infection with CCR5-tropic HIV. In preclinical studies, researchers could reproducibly achieve up to 50% modified CD4 cells in mice with a humanized immune system.

At CROI 2011, Jay Lalezari from Quest Clinical Research and Carl June presented findings from the first pilot studies of the Sangamo zinc finger technique in HIV positive people, assessing whether autologous (self-donated) CD4 T-cells with deleted CCR5 (dubbed SB-728-T) would proliferate, persist, and behave like normal T-cells in the body.

Lalezari’s ongoing study includes nine HIV positive men infected for 20 to 30 years, with long-term viral load suppression on ART but poor CD4 cell recovery. Three successive cohorts received 10, 20, and 30 billion altered cells. June’s study includes six patients with low and six patients with high CD4 counts, all with undetectable viral load. Participants in the latter group had the option of undergoing ART interruption.

Participants in both studies first had blood withdrawn in a procedure called apheresis; CD4 T-cells were filtered out and the rest of the blood was returned to the body. The extracted cells were activated, expanded, and genetically altered using the ZFN technique. The modified cells were then reinfused back into the patients and allowed to proliferate.

The apheresis and reinfusion process was safe and generally well tolerated. Some participants experienced temporary flu-like symptoms, but there were no serious adverse events or abnormal laboratory results.

Due to safety concerns, only a portion of CD4 cells were removed and replaced. About 25% of harvested cells were successfully modified with the ZFN technique. The altered CD4 cells engrafted, or took up residence, in all participants.

The SB-728 modified CD4 T-cells persisted and proliferated normally in all but one patient. June reported an overall three-fold expansion of cells, with one person having a 40-fold increase. Rectal tissue biopsies revealed that the CCR5-deleted cells migrated to the gut lining like normal T-cells and were still present six months after infusion.

Most study participants experienced significant and sustained CD4 cell gains, averaging about 200 cells/mm³; one person saw an increase of 2,200 cells/mm³. Most also experienced normalization of their CD4 to CD8 cell ratio. One participant, AIDS activist Matt Sharp, reported that his sustained CD4 cell increase allowed him to stop taking prophylactic medications to prevent opportunistic infections.

“The trajectory of any kind of research is that you want to find a cure,” Sharp told *BETA*. “I want to get rid of HIV so my immune system can be restored, so I don’t have inflammation and resulting problems related to aging, and so I can live out my life without worrying about taking a handful of pills every day.”

THE BERLIN PATIENTS

In the history of AIDS, two men—both dubbed “the Berlin Patient”—will be remembered as harbingers in the quest for a cure.

The first Berlin Patient was a young German man who in 1996 sought care due to flu-like symptoms about three weeks after having unprotected sex. His doctor, Heiko Jessen, started him on ART and hydroxyurea, a cancer drug.

Hydroxyurea expert Franco Lori described the case at an AIDS conference in Hamburg in 1997. After starting combination therapy, the man rapidly reached an “undetectable” viral load according to an older test with a lower limit of 500 copies/mL. When he stopped his drugs a few months later due to a bout of hepatitis A, his HIV viral load stayed undetectable. About five weeks later, he decided to permanently discontinue therapy and his virus remained suppressed.

This Berlin Patient was the first individual known to have achieved “remission” of HIV, and the case made headlines around the world, including a profile in the *New York Times Magazine*. Lori’s team presented further details at CROI 1999 and in the May 27, 1999, *New England Journal of Medicine*. By that time, Berlin Patient #1 had been off treatment for about two years, still with no plasma viral rebound. But traces of HIV RNA were detected in his lymph nodes, and replication-competent virus was isolated from a small number of resting CD4 T-cells after Robert Siliciano developed a sensitive test.

Although his HIV was not eradicated, the man’s immune system managed to control the virus, demonstrating that a functional cure is within the realm of possibility. “I’ve never met him, and I don’t even know his name, but I’ve followed his case,” a member of an HIV positive support group told journalist Mark Schoofs. “He is what we want to be.”

The second Berlin Patient came to the world’s attention a decade later. An American man living in Germany, he underwent treatment for acute myeloid leukemia at Berlin’s Charité Medical University in 2006. At that time, he had been HIV positive for more than ten years and on ART for four years, and had undetectable viral load. But he had a history of high viral load and disease progression, so was not a natural elite controller.

After initial chemotherapy failed, the next step was a bone marrow transplant. Strong chemotherapy was used to kill off white blood cells, which eliminates the cancer but leaves the patient without a functioning immune system. The man then received a bone marrow transplant containing hematopoietic stem cells; the donated stem cells essentially build a new immune system.

The man’s doctor, Gero Hütter—a hematologist with no particular experience in HIV—had read that individuals with the CCR5-delta-32 genetic variation are protected against HIV infection. Against all odds, he found a bone marrow donor who was both a genetic match and carried two copies of the uncommon variation, meaning the donor’s cells did not express CCR5 receptors.

Berlin Patient #2 stopped ART the day before his first bone marrow transplant in 2007 and afterward received immunosuppressant drugs to prevent the donor cells from attacking his body. The transplant was successful and, as hypothesized, the newly reconstituted CD4 T-cells lacked CCR5 receptors. But almost a year later, the man had a relapse of leukemia. The same donor was persuaded to part with more bone marrow, and the patient received a second transplant after chemotherapy and whole-body radiation.

The man stayed off ART, and since two months after the first procedure has maintained undetectable plasma HIV RNA and undetectable proviral DNA in resting CD4 T-cells. Hütter presented this Berlin success story at CROI 2008 and in the February 12, 2009, *New England Journal of Medicine*. The case sparked interest from both HIV researchers and the public at large after an in-depth article by Schoofs in the *Wall Street Journal*.

In an update at the IAS Reservoirs workshop and in the March 10, 2011, issue of *Blood*, Hütter’s team reported that four years after the first transplant and still off ART, the man remains in remission from leukemia and shows no signs of HIV. Using the best available technology, Siliciano and others have found no HIV RNA or DNA in his blood plasma, lymph nodes, rectal mucosa, cerebrospinal fluid, brain tissue, or resting CD4 T-cell samples. What’s more, his CD4 T-cell count has increased to a normal level.

A few months after the Vienna meeting, this Berlin Patient revealed his identity as Timothy Brown, now in overall good health and living in San Francisco. While it is not possible to prove that Brown has no remaining HIV anywhere in his body—or whether its disappearance is due to the CCR5-delta-32 stem cell transplant, strong chemotherapy, a graft-vs-host reaction, the anti-inflammatory effect of immunosuppressant drugs, or some other unknown factor—he appears to have achieved a sustained functional cure.

“I am hoping for lots of effort and money to be directed toward a cure that may be attainable for everyone,” Brown told *BETA*. “I am really very much hoping that my friends and all others living with HIV will have access to a cure very soon.”

At the same meeting, June described two people treated with SB-728 modified T-cells who attempted treatment interruptions while their plasma HIV RNA was undetectable. Both maintained stable CD4 counts and CCR5-tropic virus; viral load took longer than the usual two to four weeks to return to baseline, with one participant experiencing a ten-week delay.

The next step is to test the procedure in HIV positive people with replicating virus to see if the CCR5-deleted CD4 cells can reduce viral load and confer a clinical benefit.

Lalezari and June are currently enrolling more participants into their trials, including untreated people with CD4 counts of at least 500 cells/mm³ and some “salvage” patients with highly resistant HIV who are not responding to current therapy (see “Open Clinical Trials, page 53).

Lalezari said these results represent a promising proof of concept, but cautioned that it is too soon to talk about this method as a cure for HIV. Ultimately, individuals who are responding well to ART will have to stop antiretroviral drugs—or untreated people will have to stay off them—in larger clinical trials to see if the method enables a sustained functional cure.

CXCR4 AND STEM CELLS

An obvious follow-up question: What about CXCR4-tropic virus? Since HIV can use either CCR5 or CXCR4 to enter CD4 T-cells, disruption of both coreceptors would likely be necessary to fully protect cells from infection. Fortunately, the Sangamo zinc finger technique can cause a break in the CXCR4 gene as well, resulting in T-cells that lack CXCR4 coreceptors.

At CROI, Craig Wilen from the University of Pennsylvania presented the first data on gene therapy to interfere with CXCR4 expression. Laboratory studies found that the zinc finger procedure did not impair CD4 T-cell proliferation. Altered cells exposed to HIV were protected from infection

and showed a significant survival advantage. In mice with a humanized immune system, altered CD4 cells were protected from infection by CXCR4-tropic HIV.

If gene therapy works as intended, modified T-cells that resist HIV infection would have a survival advantage over normal cells, so over time a growing proportion of cells would lack CCR5 and/or CXCR4 coreceptors and therefore be resistant to infection. But T-cells naturally die over time, so zinc finger alteration might have to be repeated periodically.

A similar approach, however, might confer longer—perhaps even lifelong—protection. In the August 2010 issue of *Nature Biotechnology*, Nathalia Holt and Paula Cannon from the University of Southern California and colleagues reported that the Sangamo zinc finger technique can disrupt the CCR5 gene in CD34 hematopoietic stem cells from humanized mice. Since these stem cells give rise to all types of blood cells, the resulting CD4 T-cells lacked the CCR5 coreceptor and therefore were protected against HIV infection.

Cannon’s group plans to study the technique in HIV positive people with lymphoma. John Zaia and colleagues at City of Hope Comprehensive Cancer Center have already used gene therapy to alter hematopoietic stem cells in HIV positive lymphoma patients. The rationale is that risky approaches can be ethically tested in people who already require stem cell transplants due to life-threatening cancer.

Zaia’s method uses three different techniques to make cells resistant to HIV: ribozyme “molecular scissors” to disable the CCR5 gene, a TAR decoy, and a short hairpin small interfering RNA (siRNA) that interferes with HIV Tat and Rev proteins.

As reported in the June 16, 2010, issue of *Science Translational Medicine*, Zaia’s team administered modified stem cells to four HIV positive people undergoing chemotherapy for lymphoma. The altered stem cells suc-

cessfully engrafted, proliferated, and differentiated like normal stem cells, and were still present after two years. The researchers will next test the technique in lymphoma patients who have their normal stem cells destroyed by chemotherapy or radiation.

“[I]f one-shot, modified hematopoietic stem cell-based gene therapy can be made efficacious and accessible in the context of HIV disease, similar approaches will likely be applicable to a host of other chronic diseases,” Steven Deeks and Joseph McCune wrote in an editorial accompanying Cannon’s *Nature Biotechnology* article. “In the same way that problems associated with the reliance on fossil fuels have stimulated the development of alternative strategies of energy delivery, so too may the ongoing crisis in the HIV epidemic spark novel approaches to the provision of healthcare in the future.”

PURGING LATENT HIV

Because latent HIV in resting T-cells is both invisible to the immune system and invulnerable to antiretroviral drugs, researchers are studying various methods of activating quiescent cells in order to awaken hidden proviral DNA, with the goal of purging or flushing out the viral reservoir.

According to David Margolis, this may be accomplished either by directly activating resting cells and their resident HIV, or by disabling mechanisms that keep them inactive—that is, by “giving them a push” or “taking the brakes off.”

Encouraging production of more HIV seems counterintuitive, but the idea is that people would stay on potent ART as a “safety net” to disable new virus as it emerges from activated cells. Eventually all reservoir cells would release their hidden HIV, and once that final batch of virus is killed off, there would be no source of renewed infection after stopping therapy.

One early approach involved activating all resting memory CD4 cells in

the body using interleukin 2 (IL-2) or other cytokines. This is risky, however, because activating too many T-cells at once can set off a potentially deadly systemic inflammatory response known as a cytokine storm. The global approach has the added disadvantage of providing many more activated CD4 cells for HIV to attack.

In the late 1990s, researchers at the Academic Medical Center in Amsterdam tested OKT3, a monoclonal antibody that activates T-cells, in three HIV patients receiving stable ART and IL-2. CD4 and CD8 T-cell activation increased to almost 100%, but this was followed by profound and long-lasting CD4 cell depletion. Participants experienced severe side effects and one developed kidney failure.

Other studies of IL-2 have produced mixed results. In 1999, Chun and Fauci's team reported that patients who received intermittent IL-2 along with ART had smaller reservoirs of resting CD4 cells harboring replication-competent HIV. But the German COSMIC study showed that adding IL-2 to ART had no beneficial effect on proviral DNA levels. Studies of cytokine combinations (including, for example, IL-2, IL-6, IL-7, tumor necrosis factor-alpha, and interferon-gamma) are likewise conflicting and some have resulted in more harm than good.

Recall that HIV gene expression requires complex interactions between viral regulatory proteins like Tat and cellular transcription factors such as NF-kB and P-TEFb, suggesting a plethora of treatment targets. Interfering with such factors could cause the integrated viral blueprint to start producing new virus, but it may also cause excessive immune activation or other adverse outcomes.

Clearly, a more promising strategy would be to activate only the small pool of resting CD4 T-cells that carry HIV proviral DNA, but this has proven quite difficult. Another trick is finding agents that selectively trigger

or mimic some—but not all—of these factors' effects.

Sandrina Da Fonseca and colleagues from VGTI showed that CD4 T-cells containing proviral DNA express more of a surface antigen known as programmed death 1, or PD-1. Interactions between PD-1 and its receptor, PD-L1, help maintain these cells in a resting state and keep integrated virus latent, they reported at CROI 2011. Conversely, agents that block this interaction can spur HIV reactivation and release.

A class of agents known as protein kinase C activators enhances transcription of latent HIV without triggering activation of uninfected cells. Screening a large library of compounds, Frank Wolschendorf and colleagues found one, dubbed HIV-1-reactivating protein factor, which triggers a brief pulse of NF-kB that activates Tat and sets off viral production—described as “hit and run stimulation”—but not enough to release inflammatory cytokines.

Prostratin, derived from the Somoan mamala tree, triggers NF-kB activity without causing overall T-cell activation, though it has other toxicities. In the laboratory, it promotes proviral HIV expression in latently infected CD4 cells and also works as an entry inhibitor by reducing expression of CD4 and CXCR4 receptors. Spanish researchers have described a related compound known as SJ23B, derived from a euphorbia plant, that has ten times greater potency than prostratin.

Using a novel laboratory model to screen more than 2,000 small molecules, Siliciano's group at Johns Hopkins identified 5-hydroxynaphthalene-1,4-dione (5HN), a compound from the black walnut tree that activates latent HIV by producing reactive oxygen species and triggering NF-kB; however, it also may be too toxic for generally healthy HIV patients.

Auranofin (Gar1041; brand name Ridaura), a gold-based compound used to treat rheumatoid arthritis, also triggers NF-kB via reactive oxygen

species. Andrea Savarino from Istituto Superiore di Sanita and colleagues found that monkeys treated with ART plus auranofin showed decreased SIV (a simian relative of HIV) DNA and were able to maintain low viral loads and high CD4 counts without ART.

P-TEFb, required for elongation of RNA strands transcribed from both cellular and proviral DNA, is another potential target. Hexamethyl bisacetamide (HMBA) releases P-TEFb from its inactive state so it can trigger the HIV promoter to start viral gene expression even in the absence of Tat. Margolis's team showed that HMBA stimulated HIV production in resting CD4 T-cells, but it too has toxicity issues.

HDAC INHIBITORS

One way to “take the brakes off” is chromatin remodeling, or changing how HIV DNA binds to histones in host cell chromosomes. As explained above, a chemical reaction called acetylation keeps DNA accessible, while a complementary reaction, methylation, has the opposite effect. Histone deacetylase enzymes keep DNA tightly bound and unusable; HDAC inhibitors and methylation inhibitors release DNA so it can be used to direct virus production.

Valproic acid (Depakote), a weak and nonselective HDAC inhibitor used to treat epilepsy and bipolar disorder, has been extensively studied as a way to flush HIV out of resting T-cells. After seeing promising results in the laboratory, Margolis and his team conducted a pilot study in which they gave 500–750 mg twice-daily valproic acid to four HIV positive people on ART who first added enfuvirtide (Fuzeon) to provide a stronger safety net.

In 2005, the researchers reported that after three months, the number of latently infected resting CD4 T-cells decreased by about 70%–80% in three of the four patients. These findings prompted another round of headlines about a possible AIDS cure, but again the excitement proved short-lived.

Three years later, in the June 19, 2008, issue of *AIDS*, Margolis and colleagues reported disappointing findings from a larger follow-up study. Here, 11 HIV positive people with stable viral suppression added 1,000 mg valproic acid to their standard ART regimen. Four participants (36%) showed a reduction in latently infected CD4 cells, including three who also experienced further reductions in viral load; the rest, however, had no significant change.

Likewise, Siliciano's team reported that in a study of nine HIV positive people with long-term viral suppression on ART who happened to be taking valproic acid for neurological or psychiatric conditions, levels of latently infected resting CD4 cells did not differ from those of HIV patients not using the drug, nor did they decrease over time.

These studies indicate that valproic acid is not potent enough—or perhaps does not target the right forms of HDAC—to appreciably reduce the size of the latent HIV reservoir; they do, however, offer proof of concept that this approach may have some benefit.

Margolis and several other researchers have shown that certain HDAC types or isoforms work better than others. Class I HDAC inhibitors (which include HDAC-1, -2, and -3) do a better job regulating HIV expression than Class II HDACs. Within Class I, agents that inhibit only HDAC-1 and -2 appear to be less potent HIV activators than those targeting HDAC-1, -2, and -3, suggesting that HDAC-3 plays an important role in maintaining viral latency.

Building on these findings, researchers have studied a number of selective HDAC inhibitors in the laboratory, looking for those that can activate latent HIV with minimal side effects. Agents that have shown some promise include apicidin, entinostat, metacept-1 and -3, oxamflatin, scriptaid, and trichostatin A, most

of which were initially developed as cancer chemotherapies.

One potential candidate, vorinostat or suberoylanilide hydroxamic acid (SAHA; marketed by Merck as Zolinza), is approved in the U.S. for treatment of cutaneous T-cell lymphoma. Margolis and others have shown in laboratory studies that vorinostat triggers HIV production from resting CD4 T-cells from people on ART. A small clinical trial of vorinostat in people with HIV recently started enrolling, but the drug may be too toxic for routine use.

Fortunately, some newer HDAC inhibitors—including givinostat (ITF2357) and the related compounds CG05 and CG06—appear to more specifically target HIV activation and may cause fewer side effects. Shay Matalon from the University of Colorado and colleagues, for example, reported last year that givinostat increased HIV gene expression by up to 15-fold in a laboratory study, compared with less than two-fold using valproic acid.

Most experts think these types of therapies will work best in combination, targeting multiple steps of the cell activation and proviral gene expression process. Sophie Reuse and colleagues from Belgium, for example, reported at the IAS Reservoirs workshop that a combination of clinically available HDAC inhibitors plus prostratin synergistically activated the HIV promoter element, leading to enhanced viral gene expression.

In the June 2009 issue of *Retrovirology*, Savarino's group described a "shock and kill" approach using Class I HDAC inhibitors plus the pro-oxidant agent buthionine sulfoximine (BSO). The HDAC inhibitors they tested activated latent HIV in cell cultures, but only at toxic doses; adding BSO enabled the HDAC inhibitors to work at lower, more tolerable doses. The researchers later reported that a similar HDAC inhibitor/pro-oxidant combination—vorinostat plus auranofin—prevented SIV disease progression in monkeys.

In the April 5, 2011, issue of *PLoS ONE*, Michael Kovochich and colleagues from UCLA described an approach using nanotechnology to deliver drugs more precisely to desired targets. A nanoparticle incorporating the protein kinase C activator bryostatins-2 activated resting T-cells and stimulated latent virus production *in vitro* and in humanized mice. Adding the HDAC inhibitor sodium butyrate enhanced activation, and the particles could also be loaded with the anti-retroviral drug nelfinavir (Viracept) to simultaneously activate latent virus and inhibit its replication.

OTHER APPROACHES

The resting cell activation approach aims to purge latent HIV from reservoirs, but the opposite strategy—keeping integrated viral DNA permanently silenced—could be another way to achieve a functional cure.

Methylation, the complementary process that keeps DNA tightly bound to histones, has not been as well studied as acetylation. Methylation inhibitors such as decitabine (Dacogen) work like HDAC inhibitors. Conversely, agents that promote methylation might prevent proviral DNA from ever being used to produce new virus. Some researchers think it may be necessary to manipulate both acetylation and methylation to control latent HIV expression.

Once HIV has succeeded in copying its genetic material and producing component proteins, other approaches have been explored for preventing virus assembly and release. A human protein called tetherin, for example, prevents the release of new virus particles from CD4 cells, thereby limiting infection of additional cells.

Disabling or killing cells that harbor proviral DNA is another potential strategy for preventing latent HIV from ever producing new virus.

Minocycline, an inexpensive and generally well-tolerated broad-spectrum antibiotic, appears to target and

disable resting CD4 T-cells, preventing their reactivation and release of hidden virus. After observing that minocycline reduced cerebrospinal fluid viral load in monkeys with SIV, Siliciano's group looked at resting CD4 T-cells obtained from HIV positive people on ART, treating half with minocycline and leaving half untreated.

As described in the April 15, 2010, *Journal of Infectious Diseases*, they found that minocycline selectively interrupts signaling pathways critical for T-cell activation. Because HIV gene expression generally does not occur in inactive T-cells, the cell cultures exposed to minocycline had lower virus levels than untreated cells.

Other researchers have tried killing HIV-infected resting cells outright. Theoretically, since only one in a million resting CD4 T-cells contains the virus, this should not deplete their numbers enough to cause harm. But again, the challenge is determining which ones to kill.

Scientists have proposed using antibodies or harmless retroviruses to deliver toxins to HIV-infected resting cells. As early as 1999, Cynthia McCoy and colleagues from the University of Texas reported that genetically engineered immunotoxins targeting the CD45RO marker on memory CD4 T-cells killed HIV-containing memory cells while sparing naive CD4 T-cells and certain other memory cells with different marker configurations.

Abraham Loyter and colleagues at Hebrew University recently reported that a combination of peptides plus saquinavir (Invirase) increased integration of HIV DNA into host cells to such an extent that they underwent apoptosis, or cell suicide. In a laboratory study this lethal mix led to death of infected T-cells and "total extermination" of the virus, but it did not appear to have an effect on uninfected cells, the researchers said.

Other research aims to boost the immune system's response to HIV. Dozens of therapeutic vaccine

candidates have been tested, but despite some promising activity in laboratory and animal studies, none have been shown to consistently and significantly decrease—much less eradicate—HIV over the long term in clinical trials.

Investigators have also explored many other immune-based therapies, including gene therapy to make CD8 T-cells respond more strongly to the virus, but with every method tested so far HIV comes back after ART is stopped.

Finally, reducing the harm caused by HIV could be another way to implement a functional cure. A growing body of evidence indicates that persistent immune activation and inflammation are responsible for much of the damage related to chronic HIV infection. If researchers could find a way to shut down this response, the virus might be rendered harmless, in the same way some monkey species harbor persistent replicating SIV without disease progression.

Given the tremendous complexity of HIV infection and the immune system's response, most experts predict that a cure will most likely come from a combination approach—for example, cytokines to activate resting CD4 T-cells, agents such as HDAC inhibitors that turn on viral gene expression, a potent ART regimen to kill virus as it is released from reservoirs, gene therapy to make T-cells resistant to viral entry, and therapeutic vaccines to help the immune system fight any last traces of virus.

"Multiple and perhaps interacting processes" are involved in viral persistence and "all HIV patients are not the same," Daria Hazuda from Merck concluded at the IAS Reservoirs workshop. "Maybe different approaches will be required for different patients."

"Over the next five years, we're going to see development of a variety of interventions that have a partial effect, and once we identify these various partially effective interventions, the next step will be to combine them

to create a cure; the first part is easier, the second part will be harder," Steven Deeks predicted.

RESEARCH HURDLES

Though they have many promising leads to pursue, researchers face a number of challenges as they search for a cure for HIV.

The Berlin Patient's story underlines one such issue: How will we know if someone has completely and permanently gotten rid of HIV? State-of-the-art viral load tests can now measure plasma HIV levels down to a single copy per milliliter. But it is much harder to detect latent HIV in resting CD4 T-cells.

Given the usual estimate that roughly one in a million resting CD4 cells harbors HIV—and the fact that the vast majority of these cells reside in tissues such as the gut—it takes liters of blood to collect just a few. If treatment reduces the number of virus-containing cells by 100-fold, it may not be possible to find them at all using today's technology.

Theoretically, it is conceivable that some people have eradicated HIV without treatment, and therefore never got tested and never came to the attention of researchers. This seems unlikely, however, given that no one who has been followed from the time of acute infection has been known to clear the virus.

But what about people who appear to eliminate HIV using the new therapies under development? The IAS Reservoirs workshop last summer featured a debate about the Berlin case, with some skeptics asking Gero Hütter how he could be sure his patient had no residual HIV anywhere in his body.

As long as the Berlin Patient experiences no disease progression, it may not matter whether he harbors hidden HIV—a long-term functional cure is still a major accomplishment. But determining whether HIV is really gone becomes critical when decid-

ing whether and when to discontinue antiretroviral treatment.

How long should viral load remain undetectable before considering ART interruption? What should be the threshold for deciding that HIV has bounced back enough to call an experimental approach a failure? How often should people be tested to be confident they are not experiencing viral “blips”? How often do they need invasive tests such as rectal biopsies or spinal taps? And how long do people have to remain apparently virus-free without ART before declaring that they are indeed cured?

With regard to drug development, investigators are devising novel screening methods to test large numbers of compounds, looking for those that might have an effect on the viral life cycle. Pharmaceutical companies, including Bristol-Myers Squibb, Gilead, Merck, and Tibotec/Janssen have ongoing programs to search for candidates that might play a role in curing HIV. According to Hazuda, Merck has already screened tens of thousands of compounds, including HDAC inhibitors, and found several dozen that warrant further testing.

Many compounds under study for HIV have already been tested in animals and humans for other indications, and some have been approved by the U.S. Food and Drug Administration (FDA), mostly as cancer chemotherapies. One agent that strongly activated latent HIV in a recent lab study and is now entering clinical trials—disulfiram (Antabuse)—is used to manage alcohol abuse, illustrating the benefit of casting a wide net.

The fact that some promising compounds are already approved will likely shorten the period of preclinical research before they can enter clinical trials for people with HIV, but other hurdles lie ahead.

Given the excellent safety and effectiveness of modern antiretroviral drugs, cure candidates have a higher bar to clear. Interfering with chromatin

remodeling, transcription factors, and other elements of the gene expression process could have harmful effects on human cells, such as triggering the development of cancer.

While the FDA allows use of potentially dangerous drugs for life-threatening conditions—largely thanks to the work of AIDS activists in the 1980s and 1990s—many people feel HIV infection no longer falls into that category.

The regulatory process and clinical trial system generally do not allow testing potentially harmful therapies in healthy people. While it may be acceptable to give a toxic or oncogenic (cancer-causing) drug to a person with no other good treatment options, the risk may be too high for HIV positive people who are keeping their virus suppressed on ART and have no signs of disease progression.

“We can do more to patients with cancer or late-stage AIDS because it will save their lives, but for a person with suppressed HIV, I can’t go and give them something that gives them cancer,” explained Margolis, whose study of vorinostat for HIV was initially rejected by the FDA.

Yet some people with HIV are eager to participate in this type of study, both for the sake of their own long-term health and freedom from lifelong daily therapy, and to advance the science to help other people in the future. Furthermore, a certain degree of toxicity may be acceptable if a therapy only has to be used occasionally—ideally, only once—or only for a short period.

“I realize that this experiment may fail, but scientists will learn from the information and take it to the next step,” said gene therapy trial participant Matt Sharp. “Despite the unknowns of entering a Phase I gene modification trial, I recognize that much of the success of my HIV treatment history has happened because I chose to take risks along the way.”

AIDS advocates, researchers, pharmaceutical industry representatives, and FDA officials are currently

hashing out procedures to enable well-informed HIV positive volunteers to take part in studies of high-risk approaches that could potentially have a very high return.

SHOW US THE MONEY

Along with regulatory issues, another major barrier to HIV cure research is inadequate funding.

A recent AIDS Policy Project report estimated that in 2009 the U.S. federal government spent less than 3% of its annual \$1.5 billion HIV/AIDS research budget on work that could lead to a cure; the advocates believe the amount should be upwards of \$200 million.

NIAID director Fauci disputed this figure, claiming it does not capture everything that might contribute to a cure. But it is clear that the amount is dwarfed by spending on HIV vaccine research, which even after 20 years has yet to demonstrate much promise in human clinical trials.

“[S]hortly after HIV was found to be the cause of AIDS, some researchers claimed, ‘It will be impossible to treat this disease at all,’” Project Inform founder Martin Delaney recalled in a 2008 essay. “Little more than 20 years later, scientists claim that people with HIV and access to treatment could expect to live a normal life span. A cure is not only possible; it is the next step in HIV research.”

Delaney, Douglas Richman, and coauthors published an article in the March 6, 2009, issue of *Science*—not long before Delaney’s death from liver cancer—calling for a collaborative effort to pool resources to advance cure-related research.

In June 2010, NIAID and the National Institute of Mental Health established such a project, dubbed the Martin Delaney Collaboratory Towards an HIV-1 Cure. The initiative aims to fund projects that will expand knowledge about HIV latency and persistence to inform eradication strategies; applicants are required to include a

translational component to bridge basic science and clinical care.

But while NIAID has indicated that HIV eradication is one of its “highest priorities,” the agency allocated only a relatively paltry \$8.5 million to the effort, prompting advocates to accuse the government of failing to put its money where its mouth is.

Other sources have taken up some of the slack. The stem cell gene therapy research conducted by Zaia and Cannon, for example, has largely been funded by a \$14.5 million grant from the California Institute for Regenerative Medicine (CIRM), the result of a 2004 ballot initiative to support stem cell research.

In May 2010, amfAR announced the first grants from its amfAR Research Consortium on HIV Eradication (ARCHE), established to advance research on strategies for viral eradication and a functional cure. And as part of its Grand Challenges in Global Health initiative, the Bill and Melinda Gates Foundation included the design of new approaches to cure HIV infection as one of its key challenges.

Visionaries in the pharmaceutical industry are also looking toward a cure, despite the concern of some activists that companies will be loathe to support approaches that might threaten their steady stream of income from lifelong ART.

In order to avoid wasting money, time, and effort—and to put the fewest possible patients at risk—researchers must be able to collaborate at all stages. This includes sharing information about unsuccessful candidates so that other investigators can avoid going down the same futile paths.

“We hope to make breakthroughs with this first generation of ideas, but it is also certainly conceivable that our first ideas might not work,” said Gilead’s Geleziunas. “However, science is often an iterative process, and this first generation of ideas might produce novel insights which will lead us to better ideas.”

In short, getting new therapies from bench to bedside requires resources at all levels. But money issues will not disappear once a cure is developed. How can we justify the cost of cure research and implementation, many ask, when millions of people worldwide do not even have access to today’s standard-of-care ART?

No one expects that a cure for HIV will be cheap. But even a high-tech approach like gene therapy—if it only needs to be done once or at most a few times—might prove cost effective compared with decades of antiretroviral treatment, monitoring, and management of ART-related complications.

In her Vienna plenary talk, Sharon Lewin estimated that treating most HIV positive people in low- and middle-income countries at the old World Health Organization CD4 threshold of 200 cells/mm³—to say nothing of raising the threshold to 350 or 500 cells/mm³—could consume half the U.S. foreign aid budget within a decade.

A basic antiretroviral regimen typically costs around \$20,000 per year in the U.S. (though it runs much less in resource-limited countries that take advantage of generic drugs and special deals with industry). At that price, even the estimated \$200,000 cost of a Berlin Patient-style stem cell transplant pales in comparison with perhaps \$1 million for a lifetime of ART.

But if history is any guide, even procedures as intensive and costly as stem cell gene therapy will become less expensive over time as techniques are automated and scaled up. And therapy that is administered once or only a few times might also help overcome the shortage of medical personnel and infrastructure needed to deliver lifelong daily treatment in resource-limited settings.

“A cure will require funding commitments, strong community engagement, rigorous and innovative scientific endeavor and, above all, further collaborative multidisciplinary science with a better connection between

basic and clinical research—in short, all the same ingredients that got us where we are today with global antiretroviral treatment,” Barré-Sinoussi wrote in a New York Times editorial marking the 30th anniversary of the first report of AIDS.

HOPE FOR THE FUTURE

In summary, research to date on HIV eradication and the likely more achievable goal of a functional cure has spotlighted several promising proofs of concept, but none of these approaches are ready for widespread clinical application.

In their 2009 *Science* review, Richman and coauthors wrote, “We propose that a drug-free remission should be the new goal of HIV therapeutics.”

“This is exactly what patients want,” said Alain Lefeuvre, Chief of Infectious Diseases at Toulon General Hospital and chair of the annual International Workshop on HIV Persistence, Reservoirs and Eradication Strategies. “They want drug-free time.”

Some experts question whether remission without complete viral eradication is really enough, given recent findings about the detrimental effects of chronic inflammation among people with undetectable viral replication on ART and eventual disease progression even among elite controllers.

“[P]erhaps the only way for an HIV-infected person to achieve normal health is through a cure,” Steven Deeks suggested.

Many agree, however, that until a cure comes along, state-of-the-art antiretroviral therapy is the best way to prepare to take advantage of it. “My belief is that getting on treatment early, staying on treatment, and keeping the virus undetectable [will make patients] most likely to be successful with future strategies for a cure,” predicted UCSF Positive Health Program medical director Bradley Hare.

As for when this might happen, experts are hesitant to give a timeline, mindful of the inaccuracy of earlier

predictions. While a preventive HIV vaccine has taken much longer than government officials predicted in the 1980s, effective ART came about faster than many expected. Fauci has suggested that prolonged treatment breaks may be possible for some patients within five years; Margolis and Deeks both put the timeframe for a functional cure at around ten years, but most expect complete HIV eradication to take longer.

“It’s astonishing that people are having intelligent conversations about HIV that includes the word ‘cure,’” said Jay Lalezari. “The three-pronged approach is gene therapy to manipulate the host, immune-based therapy to manipulate the immune system, and drug therapy to force HIV out of its hiding place in tissue reservoirs. Whether a cure is going to come from one or some combination of all three, I do think it’s possible that in our lifetime we will be curing HIV.”

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